EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

21st ENGL PLENARY MEETING

4-5 June 2014, Ispra, Italy

Meeting Report

1 Welcome

The Chair welcomed the participants and reviewed the points of the agenda.

1.1 Approval of the Agenda

The agenda (Annex 1) was approved without amendments.

1.2 Approval Report 20th ENGL plenary

The report was adopted without changes. It will be made available on the EU-RL GMFF web page, subsite ENGL.

1.3 Outcome of the 26th ENGL SC meeting (March 2014)

The Secretariat summarised the outcome of the meeting. The report was adopted without changes. It will be made available on the EU-RL GMFF web page, subsite ENGL.

1.4 Dynamic Action List (DAL) of 20th ENGL plenary

The Secretariat presented the dynamic actions list. Most items have been covered, only few still need follow-up

1.5 Update from SANCO

SANCO informed that 22 RASFF (Rapid Alert System on Food and Feed) notifications on Bt63 rice in choline chloride feed additive have been received since 30 January 2014.

The EU-RL GMFF tested about twenty samples of feed additives and relative pre-mixes provided by some Member States and prepared a technical note on DNA extraction and detection of rice and Bt63 in the feed additive and in pre-mixes (available at: http://gmo-crl.jrc.ec.europa.eu/doc/EURL-EM-01-14-VR.pdf).

In a recent meeting with the European Commission (EC), Chinese authorities were requested to take corrective measures, to consider the removal of rice from the choline chloride corn cob formulation and other feed additives and to perform systematic checks on the products exported to the EU. If the measures will be unsuccessful the possibility would be considered of extending the scope of Decision 2013/287/UE.

SANCO also explained the RASFF procedure and the classification of notifications distinguishing a "RASFF alert", posing an immediate health risk, from "RASFF information" when a risk has been identified but no rapid action needs to be taken. "RASFF news" are used when interesting information need to be distributed to control authorities. Only non-compliances involved in health risks should be reported to the national RASFF contact point. The EC has published a Standard Operating Procedure (SOP) on how to classify different type of notifications and detailing the criteria to determine when a rapid alert or information notification to the RASFF is required.

1.6 Commission Regulation (EU) No 691/2013 amending regulation (EC) No 152/2009 regarding methods of sampling and analysis: discussion on Annex 2, point 3: Number of determinations

A question was raised on how the number of analytical determinations to be done (at least two according to annex II of regulation 152/2009) has an impact on the reduction of the aggregate sample to several (final) laboratory samples and on the number of analytical determinations that should be carried out. It was agreed that since two test portions are usually analysed, this corresponds to the two determinations that are required. The analysis is to be performed on one laboratory sample and, if needed, the counter analysis is to be performed on the second laboratory sample.

1.7 Mapping non-GMO ENGL activities

The Chairman noted that other aspects beside GMOs are gaining public relevance, such as genetic testing, food allergens or identification of ingredients origin. The JRC was requested to explore other areas where the network expertise could be used.

The Secretariat carries out a survey to map the GMO and non-GMO activities of the ENGL members via a web-form that is available on the ENGLnet. ENGL members are invited to complete it. Preliminary results of this survey were presented.

All the respondents (total of 19 at the time of the meeting) indicated to perform GMO detection in food/feed, seeds and environmental samples and also different related research activities, such as method development, transgene stability, and new detection techniques for non-authorised GMOs. Nine of these laboratories currently perform detection of food pathogens; eight do identification of animal species in food/feed; seven work on food allergens; four are involved in testing for food authenticity and adulteration; four labs test for plant pathogens; four perform plant varieties characterisation; and two do analyses of bacteria in water.

Taking account of these results and the interest of the participants 3 topics for the break-out groups were agreed and rapporteurs and moderators identified. The participants proposed also to discuss mutations on species-specific target sequences having an impact on GMO quantifications and **the** Chair suggested discussing this within the species identification break-out group.

2. Progress reports ENGL working groups

2.1 WG SPP (Sample Preparation Procedure)

The WG leader announced that the version 12.2 had been submitted for review to the ENGL from the 5th to the 23rd of May 2014. Most comments received were editorial and were integrated in the last version. All points were addressed even though not completely for some complex issues.

The Chairman suggested to adopt the document was adopted and the Chair thanked the WG-members for their contribution. The guidelines will be finalised (edited) and published on the EU-RL GMFF website (ENGL page).

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

During the last group meeting new criteria were established for selecting methods for validation. The AG agreed to give high priority to methods that close an analytical gap, (in particular for

screening for un-authorised GMOs), offer an added value, and comply with the ENGL acceptance criteria.

The five methods previously selected were reviewed according to these criteria and new gaps in GMO analysis were identified. The T35 sequence of the pCAMBIA vector was identified in 30% of examined publications on un-authorised GMOs and recognised by the advisory group as a possible analytical gap. The participants agreed to validate the identified method targeting the element, given that it would offer an added value and seems to comply with the ENGL acceptance criteria.

A new template for submission of method proposals for validation has been published on the web page of the GMOMETHODS database (http://gmo-crl.jrc.ec.europa.eu/gmomethods/) and ENGL members are invited to use it for informing the AG about promising methods that meet, in the assessment of the ENGL member, the above mentioned criteria.

2.3 WG DIR (Detection Interpretation Reporting)

The chair of the WG reviewed the current structure of the report and informed that the draft document has been uploaded on the WG workspace. An agreement still needs to be reached on some issues. It was announced that for mid-July 2014 members of the group will prepare a final draft to be distributed to the ENGL for comments. In Sep 2014 the WG will submit the final document to the Steering Committee and tentatively publish it by the end of 2014.

2.4 WG MPR (Method Performance Requirements)

The final draft was presented for comments to the 26th SC in March 2014, then to the ENGL and EuropaBio (EU biotech industry association) in April 2014 and finalised for the 21st ENGL plenary in June 2014.

Main concerns expressed regarded the clarity of the modularity concept and the new criteria adopted for evaluating amplification efficiency.

The Chairman proposed to leave the original criteria and insert a note to take into account that when DNA is extracted from difficult matrixes it may not always be possible that the PCR efficiency on the extracted DNA remains within the acceptance criteria.

The document, amended as specified above, will be published on the EU-RL GMFF website.

2.5 WG-IGSE (Identification of stacked GM events)

The final document was presented. Ten approaches are covered in the report falling into three categories: marker assisted identification (which involves the identification of specific metabolic or genomic markers), single-cell analyses (which requires isolation of intact cells or nuclei); and statistical approaches, providing indication of how well the presence of a stacked event may explain the observed results.

The report was approved and is available at http://gmo-crl.jrc.ec.europa.eu/ENGL/docs/WG-IGSE-Report.pdf. The WG remains active for monitoring advances in technology.

The Chairman acknowledged the rapidity of delivery of the WG and congratulated the Chair and the WG members.

2.6 WG-ST (seed testing)

The WG Chair reported that during the first meeting of the WG the outline of the document was agreed, and the next tasks defined and assigned to the WG-members. A drafting team was established that will meet in June to prepare a consolidated draft to be discussed and finalised at the second meeting of the WG planned for 9-10 September 2014. The final draft of the WG report should be presented at the ENGL Steering Committee in September.

3. Break-up groups

The three groups (DNA extraction, species detection and digital PCR) met in separate meeting rooms.

4. Report of break-out groups discussions

The designated rapporteurs provided a summary of the three break-out groups discussions.

4.1 DNA extraction

The group identified a need to map the matrixes that laboratories have to process and especially those DNA extraction methods that are successfully used for more problematic matrices. The group proposed designing a web platform for sharing knowledge and solutions among laboratories and to provide the DNA-extraction protocols that are successfully used

The Chairman commented that the platform could be created and noted that a moderator would be needed for organising the information. He further commented that the objective of the platform should be defined and proposed to create a Task force or a WG to implement it.

4.2 Species detection

The group members have experience in detection of allergens and dietary products, meat authenticity testing and microbial detection. Some challenges in these areas were identified, such as the relative quantification of species in meat products or the lack of reference materials for allergens. The group proposed to extend the scope of the GMOMETHODS database to allergens and species detection in meat and expanding the barcoding sequence database. The sequence core facility could be broadened and pre-spotted plates for microorganisms could be developed. It was suggested to organise a workshop on the subject. The completion of the survey on activities mapping within the ENGL is considered important.

4.3 Digital PCR

Among the laboratories that had already purchased a dPCR-machine, six acquired a Bio-Rad digital droplet while three bought a chip-based machine. In many cases, the purchase was very recent. Some other labs are in the process of deciding about purchasing a dPCR.

In the labs that were represented at the group, digital PCR (dPCR) is mainly used for GMO quantification and experiments are in progress to test if this technology can overcome PCR inhibition effects and correctly quantify the GMO content in real samples. The preliminary results are promising, indicating a performance similar to that of qPCR. Other expected uses include virus/pathogen/allergen detection and species identification (for example in meat samples).

Preliminary results with dPCR showed that the application of qPCR validated methods does not appear to be problematic (although some adjustments may be needed). In general, droplet digital PCR (ddPCR) is preferred to chips-based dPCR; critical steps identified are the generation of the droplets and the loading of the chip. Other critical elements included the amount of DNA and the genome size.

The experience gained so far suggests that different parameters of the dPCR technology, such as the LOD, still need to be investigated and the group agreed that it is premature establishing an ENGL mandate for a working group on dPCR.

The laboratories were encouraged to generate data and share experiences and results. It was noted that 14 ENGL members are already using a digital PCR machine even though none of them are currently using it for routine testing.

It was agreed that the secretariat would try to organise an ad-hoc group meeting of ENGL members that already work with dPCR, followed by reporting on the practical experience at the next ENGL plenary.

5. ENGL matters

5.1 Open agenda point for discussion on NRLs Regulation (EC) No 120/2014, amending Regulation 1981/2006

The Chairman noted a decrease in participation of NRLs nominated under Regulation (EC) No 120/2014 to the comparative tests organised by the EU-RL GMFF. The participation of these NRLs is not compulsory but highly recommended and necessary to assure high quality for laboratories participating to the validation studies organized by the EU-RL GMFF. The Chairman indicated that the EU-RL GMFF will assign a negative priority to laboratories not participating to the CT rounds which would reduce the probability that they would be invited to validation ring trials.

6. Scientific and technical session 1

6.1 Developments in bio-economy: possible role of the ENGL (Dr. G. Van den Eede, Adviser for Bio-Economy, JRC)

A core concept in bio-economy is using waste as a resource; the current agricultural yield may not be sufficient if biomass is promoted for production of energy. Within that context, biotechnology can increase the biomass crop production with the 2nd generation of GMOs.

For the coming years the following issues will be at the centre of policy discussions:

a) Plant breeding techniques
b) Molecular farming for non-feed energy applications where crops could be used for synthesizing molecular components. These plants are not covered by legislation since they are not released on the market and their products are not going to be considered for food/feed use.
c) Fuel production for supporting energy independency.

The Chairman noted that the ENGL role could become very important in the context of these new technologies. He mentioned that the European Commission is reviewing the new breeding techniques to verify whether they fall under the remit of the EU GMO legislation.

6.2 Update on GMOs approved in China and current research activities (Prof. D. Zhang, Shanghai Jiao Tong University)

GM crops commercialised in China are tomato, cotton, sweet pepper, papaya, maize and rice. Field trials for Bt insect-resistant rice events are being organised to establish the environmental safety of the related lines.

Research activities underway include:

- a simple DNA extraction device requiring no laboratory equipment where DNA could be extracted in 15 minutes using a silica gel membrane filtration column and microcrystalline wax encapsulated detection beads containing SYBR green fluorescent dye;

- a high-throughput method for analysing multiple DNA targets with micro droplet PCR by applying two different rounds of amplification with primers specific for common sequences and individual sequences; a total of 91 targets including 18 universal elements could be covered with this new approach;

- the use of NGS technology for characterising GMOs;

- high throughput metabolites profiling to compare GMOs with the corresponding natural variations among non-GM cultivars.

6.3 Allergens detection (Dr. M. De Loose, BE)

In the EU there is a legal base for labelling ingredients that may cause allergic reactions to certain consumers; the official list includes 14 allergens.

Similarities exist with the EU GMO legislation but some elements are missing, e.g. certified reference materials and thresholds. Indeed no guidance on which method to use exists even though allergens may pose life threatening situations. It is difficult to validate methods for the lack of CRMs. Moreover, results may be negative but the product may still produce an allergic reaction. The choice of the method requires the identification and selection of the target analytes. The polyclonal antibodies used in the tests may be very sensitive but not specific or may be induced against an epitope that is not causing an allergic response.

90% of labels are not very informative, resulting in a limitation of freedom of choice for allergic citizens.

Dr. De Loose recommended establishing an EU-RL to supervise and organise the control system, thus providing more reliable nutritional labels and better harmonisation of analytical approaches. The speaker concluded by suggesting a discussion on how the ENGL expertise may be used in this field.

6.4 Update on GM papaya (RIKILT and JRC)

RIKILT (NL) reported the detection of GM papaya in capsule and pills of health products. Since the samples resulted negative for 55-1/63-1 events, an alternative analytical strategy was followed for identifying the GM event actually responsible for the contamination. Primers targeting a common region of the PRSV-CP gene, presenting sequences specific for the area of cultivation of the corresponding papaya were designed. Analysis of the amplified fragment and of the border sequences indicated that the GM contamination may be originating from China or Taiwan.

The EU-RL GMFF provided an update on GM-papaya sequence characterisation. The work continues to characterise GM papayas commercialised in USA, China and Taiwan. Collaboration in this field is going on with German and Belgian national laboratories.

7. Scientific and technical session 2

7.1 Preliminary results of the pilot project on pre-spotted plates (JRC)

Preliminary results (62 out of 180 samples) were presented. From these results, the PSP approach seems to be advantageous in terms of time required for the analysis for the screening and identification steps. 86% of the samples resulted correctly identified while for the remaining 13% of the samples, 7.7% needed clarification, 3.3% required further explanation and only 2.2% resulted in a non-detectable event (false negative). These preliminary results are therefore very encouraging, especially for the screening step.

7.2 Update on CEN/ISO (Dr. L. Grohmann, DE)

CEN activities

CEN TC 275 WG 11 approved the final draft of the CEN technical specification N16707 on PCR-based screening strategies, which includes the matrix approach. The standard will be published in mid-2014. A meeting will be organised in July in Berlin to discuss the ISO draft standard on validation of qualitative methods (under development by ISO TC 34 SC16).

ISO activities

ISO TC34 SC16 is active on various projects. One is the development of a standard on validation of qualitative (binary) methods, based on the approach called "probability of detection (POD)". This approach aims at combining the sensitivity, specificity, false positive and false negative values in

only one parameter to easily compare the validation performance of different methods. Discussions on the statistical model and on the applicability of the POD approach particularly to PCR methods are ongoing.

7.3 GMOval: update (JRC)

The project aims at the validation of four screening methods developed within the previous GMOseek project. The data generated may be also useful to verify the real applicability of the POD model for qualitative PCR methods. All four methods will be validated trough international ring-trials by fall 2014.

8. AOB and DAL ENGL 21st

The DAL resulting from the 21st ENGL plenary meeting was presented and agreed (annex 2).

All presentations can be found at:
https://englnet.jrc.ec.europa.eu/21stENGLplenary/default.aspx?InstanceID=1

Annex 1: agenda

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JOINT RESEARCH CENTRE
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Molecular Biology and Genomics Unit

21st ENGL PLENARY MEETING

4-5 June 2014, Barza, Italy

Final Draft Agenda

Day 1: 4th June 2014

	12:45	*Lunch*	
AP	Time	Topic	Documents
1	14:30	▪ Welcome	
1.1		▪ Approval of the Agenda	Draft Agenda
1.2		▪ Approval Report 20th ENGL plenary	
1.3		▪ Outcome of the 26th ENGL SC meeting (March 2014)	Report SC26
1.4		▪ Dynamic Action List (DAL) of 20th ENGL plenary	DAL-ENGL20
1.5		▪ Update from SANCO	
1.6		▪ Commission Regulation (EU) No 691/2013 amending regulation (EC) No 152/2009 regarding methods of sampling and analysis: discussion on Annex 2, point 3: Number of determinations	
1.7		▪ Mapping non-GMO ENGL activities	Presentation
	15:45	*Coffee Break*	
2	16:15	Progress reports ENGL working groups:	
2.1		▪ WG SPP (Sample Preparation Procedure)	Final document
2.2		▪ AG SMV (Advisory Group on Selection of Methods for Validation)	Update
2.3		▪ WG DIR (Detection Interpretation Reporting)	Final document
2.4		▪ WG MPR (Method Performance Requirements)	Final document
2.5		▪ WG-IGSE (Identification of stacked GM events)	Final document
2.6		▪ WG-ST (seed testing)	Update
	17:30	*End of day 1*	
	19:30	**Social dinner at il Melograno Angera**	

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Day 2: 5th June 2014

AP	Time	Topic	Documents
3	09:30	▪ Break-up groups	
3.1		1) DNA extraction	AP 3.1 Mandate
3.2		2) Species detection	AP 3.2 Mandate
3.3		3) TBD	
	10:45	*Coffee Break*	
4	11:15	Report of break-up groups discussions	
5	12:15	ENGL matters	
5.1		▪ Open agenda point for discussion on NRLs Regulation (EC) No 120/2014, amending Regulation 1981/2006	
	12:45	*Buffet lunch*	
6	14:00	Scientific and technical session 1:	
6.1		▪ Developments in bio-economy: possible role of the ENGL (Dr. G. Van den Eede, Adviser for Bio-Economy, JRC)	Presentation
6.2		▪ Update on GMOs approved in China and current research activities (Prof. D. Zhang, Shanghai Jiao Tong University)	Presentation
6.3		▪ Allergens detection (Dr. M. De Loose, BE)	Presentation
6.4		▪ Update on GM papaya (RIKILT and JRC)	Presentation
	15:30	*Coffee Break*	
7	16:00	Scientific and technical session 2:	
7.1		▪ Preliminary results of the pilot project on pre-spotted plates (JRC)	Presentation
7.2		▪ Update on CEN/ISO (Dr. L. Grohmann, DE)	Presentation
7.3		▪ GMOval: update (JRC)	Presentation
8	16:50	AOB DAL ENGL 21th	
	17:00	*End of meeting*	

Annex 2: DAL (Dynamic Action List) 21st ENGL Plenary

21th ENGL PLENARY ACTION LIST 6/5/2014				
ACTIONS	**Resp.**	**Timeline**	**Status**	**Comments**
ENGL CONSORTIUM AGREEMENT				
Make available on ENGLNet report and presentations of 21th ENGL Plenary	SEC	Jun-14	Open	
Send invitation and agenda of the 22th plenary ENGL	SEC	Sep-14	Open	
ENGL WORKING GROUPS				
WG Detection Interpretation Reporting (DIR)				
Send final draft to ENGL for comments	SEC	Jul-04	Open	
Send final draft to SC for adoption	SEC	Aug-14	Open	at 27th SC meeting
WG Method Performance Requirements (MPR)				
Finalise the doc upon editorial check + correct layout	SEC	Jul-14	Open	PCR efficiency remains as in the current version; add sentence/note in purity DNA
WG Sample Preparation Procedure (SPP)				
Publish the final document	SEC	Jun-04	Open	
AG Method Selection for Validation				
Organise ring trial pCanbia method	chair + EURL	Sep-14	Open	
WG on identification of stacks (IGSE)				
Publish the final version	SEC	Jun-04	Open	
OTHERS				
Setup working group on DNA extraction (mapping-platform sharing info)	EURL	Jun-14	Open	
Collate info on issues with CRMs	EURL	Jun-14	Open	
Organise workshop on species detection/allergens	SEC	Nov-14	Open	
Group meeting to share data on dPCR prior to plenary	SEC	Nov-14	Open	Decide when could be done; do a survey: who has the dPCR, wich one, interest in group meeting