EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

24th ENGL PLENARY MEETING

22-23 September 2015, Ispra, Italy

Meeting Report

Welcome by Directors IHCP and IRMM

The Director of the Institute for Health and Consumer Protection (IHCP) welcomed the participant. The Director of the Institute for Reference Material and Measurements (IRMM) presented the GMOs activities of the two Institutes located respectively at the JRC sites of Ispra and Geel.

She announced that in order to increase synergies and efficiency the JRC Director General decided to move the EURL GMFF activities under the control of IRMM. A road map has been designed for the year 2016.

She asked participants to reflect on the future of the ENGL and stressed that the activities will remain within the JRC. The IHCP Director added that the move will not have impact on the EU laboratories as a whole.

The JRC advisor for bio-economy, added that the existing expertise could be better used and focused on new areas where the high quality performance is needed such as i.e. species identification and diagnostics for human diseases. He remarked that the activities of the MBG Unit in Ispra will not disappear but on the contrary will be more effectively employed on new challenging areas.

It was commented that official control laboratories have so far greatly benefited from the JRC support. It was also stated that legislation on allergens presents the same type of problems faced by GMO laboratories some years ago and proposed including these new areas in the ENGL activities. The IRMM Director commented that the expertise could be enlarged to these subjects.

An ENGL member from Germany reviewed the ENGL activities and remarked that they have a global impact, underlining that ENGL has the leadership in the area and that it is recognised as a centre of excellence globally. It was warned that this decision could bring instability and it was suggested to discuss the issue within the ENGL Steering Committee. The IRMM Director stressed that the expertise on GMOs is also present at the IRMM site and welcomed the suggestion of discussing the transfer within the Steering Committee.

Participants also commented that Member States operate in sectors and warned that the enlargement of activities of the ENGL would fail if means were not found for motivating and financing them. They also expressed their concern on the possibility of changing the mandate of the ENGL. It was commented that it would be more practical to set up a new network with a new mandate.

Members asked to be reassured on the continuation of the support on GMO control activities.

The JRC advisor fro bio-economy assured that the core business was intended to remain and that the network will maintain its role in enforcing GMO legislation. The IRMM Director added that it will be discussed how to best address these issues and promised to make the transition as much as possible smooth and efficient.

Participants finally mentioned that within the ENGL, laboratories received substantial support also in the application of modern technology and wished to continue the collaboration. The IRMM Director assured that the know-how of the network will be made available by IRMM and that the collaboration with ENGL laboratories will continue.

Additional information provided by the Secretariat (after the meeting):

A working group, also attended by DG SANTE, analysed the suitable options for the transfer of the EURL GMFF under the control of IRMM; however, it became clear to the working group that the initial assumptions that triggered the early announcements were based on wrong assumptions (overlapping, possibility to

outsource, routine nature of activities, possibility to outsource EURL GMFF tasks). The Working Group also underlined the importance of the role of the ENGL and suggested that the mandate and the structure of the network remain unchanged. Also some Member States officially voiced their concern on the future of the ENGL and of the EURL GMFF at the October meeting of the PAFF Committee, Section Genetically Modified Food and Feed (report available and already distributed to all ENGL members by the Secretariat).

The JRC management has recently announced that no physical move of the EURL GMFF from IHCP-Ispra to IRMM- Geel will take place. The decision on other options (administrative relocation) is postponed.

Approval of the Agenda
The agenda (Annex 1) was approved without modifications

Approval Report 23rd ENGL plenary
The report of the last meeting was approved without changes.

Dynamic Action List (DAL) of 23rd ENGL plenary
The Secretary reviewed the open points of the list. No suitable speaker on new breeding techniques was found for the meeting. Participants reported their difficulties in assessing the method's trueness with AOCS certified reference material (as no mass fraction GM % is available) and complained about the quality of that material. The laboratories were advised to send complains to the producers in copy to the EURL GMFF if the material was found to be no fit for the purpose and were reminded that acceptance of CRM is specified in EU Regulation (641/2004) and should comply with ISO guidelines. The Chair added that the EURL GMFF had raised the issue at all levels concerned.

Outcome of the 29th ENGL SC meeting (June 2015)
The Secretary summarised the main outcome of the ENGL SC meeting.
He informed that long discussions took place on the possibility to hold one ENGL plenary per year. The proposal has the scope of freeing resources for other areas of interest, but no consensus was found and the discussion remained open.

Update from SANTE
The representative from DG SANTE was excused and could not provide an update.

Progress reports ENGL working group

AG SMV (Advisory Group on Selection of Methods for Validation)
The group, following a survey, selected a pentaplex PCR method for validation.
The pCambia T35S method selected in the previous year will be validated soon by the EURL GMFF.
Dr. Maria Pla (University of Girona, Spain) recently joined the WG.
Laboratories are encouraged to submit new proposal for method validation.

WG DIR (Detection Interpretation Reporting)
The final report in its final reviewing phase and it is expected to be published soon.

WG Seed Testing
The report has been provided to SANTE and will be discussed at the PAFF committee in December.

WG Update of Methods
The kick-off meeting of this WG took place in Ispra on 27-28 of May. The results of the survey launched by the EURL GMFF for identifying methods used by the laboratories and their implemented modifications will be valuable information the WG will take into account. The work is ongoing and the second meeting has taken place on 11-12 November 2015.

WG digital PCR
The kick-off meeting of this WG took place in Ispra at the end of June 2105. The outline of the WG report has been agreed and various contributions for the text have been collected. The WG plans to deliver a draft at the beginning of January 2016 and the finalised document for February 2016. The second WG meeting will take place in Ispra on 1-2 December 2015.

WG Unit of Measurement
The task of the WG is to define a practical recommendation on conversion of copy number into mass fractions. The kick-off meeting took place in Ispra at the end of June 2015. The first contributions were received in July 2015 and a consolidated draft is expected to be ready in December 2015. The adoption of the final document by the ENGL plenary is planned for April 2016. The second WG meeting has taken place on 16-17 November 2015 in Ispra.

Scientific and technical session 1

The application of isothermal nucleic acid amplification techniques in GMO analysis. (L. Yang, PRC)
Dr. Yang presented the advantages and disadvantages of two types of approaches for detecting nucleic acid transgenic sequences: non-isothermal and isothermal amplification. The latter could be very interesting for performing on-spot tests with a simplified procedure. Procedures and experimental applications of different types of isothermal amplification, the nucleic acid sequence-based amplification (NASBA), NASBA implemented microarray analysis (NAIMA), rolling circle amplification (RCA), recombinant polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP) were presented. Applications of LAMP amplification to GMO screening based on universal elements and GM maize and soybean event-specific identification were explained. Dr. Yang also presented a new DNA extraction devise and modification of LAMP assays including multiplex for on-spot quick monitoring of GM contents.

Multiplex quantification of individual GM events by droplet digital PCR (Dr. D. Dobnik, National Institute of Biology, Ljubljana)
Dr. Dobnik presented two new multiplex assays using ddPCR technology for detecting and quantifying seven EU authorised GM maize events. For each multiplex reaction two different fluorophores (FAM and HEX) were employed allowing distinguishing the amplification results of four different targets. The concentration of primers and probes is the most critical factor for obtaining a good separation of the signals. The performance of real-time PCR and ddPCR in the analysis of DNA sample mixes containing 7 individual GM maize lines were compared; ddPCR showed results comparable to qPCR with linearity, LOD and LOQ values compliant with the ENGL/EURL minimum performance parameters; however, some issues with specificity at GM concentrations higher than 300 copies/reaction were observed. Dr. Dobnik also reported on the development of a ddPCR multiplex calculator to optimise cluster positions in data analysis.

Event-specific multiplex PCR - screening for six GM soybean lines (L. Grohmann, DE)
Dr. Grohmann presented a new screening approach for detecting six GM soybean events which are not covered by the common element-specific PCR methods. The EUginius database and its related detection tools, as well as the JRC GMO matrix, are helping laboratories in the analysis of an increasing number of GMOs. However, a significant number of soybean and maize GM events containing unique genetic elements cannot be detected by the screening approaches commonly employed; thus a multiplex approach where event-specific singleplex for those GM soybean events are combined in the same assay has been developed. With this approach further identification of those events with event-specific singleplex methods is pursued only when a positive signal is observed from the multiplex reaction. The multiplex method was validated in a collaborative study where the false positive and negative rates, the probability of detection (POD) and the LOD were determined. The collaborative trial results indicate that the combination of event-specific methods is feasible. However, a general guidance is still needed for validation and verification of multi-target/multiplex PCR methods.

Scientific and technical session 2
Risk-based inspection of import flows (Martijn Slot, MSc, RIKILT Wageningen UR, NL)
Commercial shipments may contain unauthorised GMOs. RIKILT (NL) has developed a tool to minimise the number of random analyses and perform a more cost-effective risk-based sampling. This tool makes use of data on GMOs authorised worldwide to calculate the likelihood that unauthorised GMOs are present. Different parameters that may affect the presence of GMOs in the import are taken into account. RIKILT has developed a similar tool incorporating environmental aspects of uGMO crops in imports, taking into account the potential of GMOs to disperse into the environment. This model incorporates 105 entries with 25 crops in 31 countries.

Detection of GM fish (F. Debode, CRA-W)
Dr. Debode presented data on DNA extraction methods from salmon tissues. PCR detection methods for GM salmon (including the recently approved Aquabounty salmon) have been developed based on sequence information of the plasmids used for transformation. CRA-W also developed PCR methods for detecting ornamental fluorescent transgenic fish (glowfish). The detection of illegal imports of GM ornamental fish in some EU MS was reported.

Wheat reference genes: seeking a suitable taxon-specific module for GMO analysis (U. Marchesi, IZSLT, Italy)
Dr. Marchesi presented the results of a project funded by the Italian Ministry of Health for the varietal characterisation of *Triticum eastivum* and the selection of a suitable endogenous gene for GMO analysis. The methods developed by Matsuoka *et al*. and Ida *et al*., targeting the waxy and SSII wheat genes respectively were tested, using qPCR and ddPCR, on 16 wheat cultivars sown in Italy. The waxy and SSII gene targets were found to be stable and the two methods seemed equivalent in terms of performance.

They were completing the *in-house* validation of the real-time PCR modules and were planning to validate the ddPCR module which was very promising. The data supported the transferability of the taxon real-time PCR modules to the ddPCR systems.

Break-out Groups
Reports of break-out groups and discussion

1) DNA extraction (follow-up)
The group underlined the need of sharing information on the extraction methods used by the laboratories, the modifications implemented, problems encountered with solutions. The group observed that quality of DNA extracted may influence the correctness of the final result and that DNA quality controls, including evaluation of inhibition, could be quite different in the different laboratories. Members suggested conducting a survey to collect this kind of information. The Chair proposed preparing a table with that information to be provided to the SC and to decide later if a WG would be necessary. The EURL GMFF will be responsible for assembling the information in the table. The table will be provided as a web form.

2) Multi-target methods
The group shared different experiences in developing and using multi-target methods, including duplex methods, pentaplex for screening GM soybean events and ddPCR for detection of maize and soya events.
The group agrees that there is a need for definition of performance criteria for ddPCR and multiplex PCR and guidelines for their verification. In the respect it was reminded that within the Decathlon project some performance criteria for multiplex methods are under development, which could be later taken on discussed and possibly endorsed by ENGL. Another point of discussion was whether multiplexing previously validated singleplex methods would require a full re-validation or only a verification of some performance characteristics.

3) Interpretation of results at the limit of detection
The group underlined that there are different ways of implementing cut-off values in different laboratories. Cut-off values are method and sample specific, therefore it may not be advisable to provide guidance on precise values to be applied. The group suggested, starting from the existing draft prepared by the WG DIR, providing recommendation on how to implement the different cut-off strategies, underlying the factors to be considered when establishing such a decision support system. The new guidance document could also suggest performance tests or provide guidelines for validation of these strategies. It was suggested to generate data and evaluate the different approaches. Participants suggested adding a sample at the LOD level in the CT rounds where laboratories are asked to apply the strategies for their validation.

ENGL matters

Pre-spotted plates, update (EURL GMFF)
The main goal of the PSP proof-of concept project was the integration of the PSPs in the routine workflow of official control laboratories. A series of screening PSPs and PSP specific for maize and soy had been distributed to five laboratories. Some of the laboratories have already started the accreditation procedure for the PSP. Among the requirements for accreditation, specificity did not need to be re-investigated and only the LOD needed to be tested. Most of the screening targets had a LOD value close to five copies while the soy and maize PSPs had a partial result close to 20 copies.
The results of the PSP survey and market assessment were also presented. Most of the respondents reported a workload of more than 100 samples analysed per year, showing interested in using PSP and considering the estimated price acceptable given the number of analysis that could be performed.
The EURL GMFF finally presented the demonstration project aimed at testing needs and feasibility of a systematic PSP production/distribution done in-house by the JRC. The results of stability tests (based on accelerated ageing theory) performed on plates stored at different temperatures demonstrated that the plates were quite stable and can be distributed at room temperature.

Scientific and technical session 3

Practical demonstration on EURL GMFF bioinformatics tools (JRC)
JRC explained that the increased number of GMOs on the market and the relative wealth and disparity of sources of information demanded the development of bioinformatics decision supporting tools to help defining time and cost-effective routine testing strategies to GMO official laboratories.
The JRC GMO-Matrix tool provides in silico prediction of GMO detection by the EU reference methods included in the GMOMETHODS database. These predictions are not purely based on annotations from public available resources but are based on sequences supplied by the applicants for the GM event under

authorisation and stored in the internal CCSIS database. The *in silico* predictions resulted to be quite reliable and did not require heavy investment of human resources as for experimental evaluation.
A pipeline was developed for enriching the CCSIS database with GMO sequences from public resources and patents collections. The JRC carried out a practical demonstration of the JRC GMO-amplicon tool recently released which allows mining and retrieval of GMO sequences from public repositories. An article on the application will be published soon.

AOB
None

DAL ENGL 24th
The Secretary reviewed the DAL, available in Annex 2.

Annex 1: list of participants

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11th WORKSHOP OF GMO NATIONAL REFERENCE LABORATORIES REGULATION (EC) No 882/2004 and 24th ENGL PLENARY MEETING

22-23 September 2015, Ispra, Italy

Final Draft Agenda

Day 1: 22nd September 2015

Session: National Reference Laboratories and Official Control

AP	Time	Topic	Documents/comments
1 2 3	9:00	▪ Welcome and approval of the agenda ▪ Approval of the 10th workshop report ▪ Tour de table: issues/opinions from NRLs (each NRL is invited to report orally)	Draft agenda Report Oral input from NRLs
	10:45	*Coffee Break*	
4 5 6 7	11.15	▪ Preliminary results of EURL GMFF survey on reference methods used by NRLs (L. Bonfini, EURL GMFF) ▪ Update on comparative testing activities (W. Broothaerts, EURL GMFF) ▪ Discussion on NRLs training needs specific to their function as NRLs ▪ AOB and conclusions	Presentation Presentation Oral input from NRLs
	12:45	*End of session NRLs*	
		Buffet lunch	

Day 1: 22nd September 2015

Session: NRL 882 WS and 24th ENGL Plenary meeting

AP	Time	Topic	Documents
8	14:00	▪ Welcome by Directors IHCP and IRMM ▪ Approval of the Agenda	
9		▪ Approval Report 23rd ENGL plenary	Draft agenda
10		▪ Dynamic Action List (DAL) of 23rd ENGL	Report
11		plenary	DAL ENGL23
		▪ Outcome of the 29th ENGL SC meeting (June	
12		2015)	Report SC29
		▪ Update from SANTE	
13			
		Progress reports ENGL working groups:	
14		▪ AG SMV (Advisory Group on Selection of	
14.1		Methods for Validation)	
		▪ WG DIR (Detection Interpretation Reporting)	
14.2		▪ WG Seed Testing	
14.3		▪ WG Update of Methods	
14.4		▪ WG digital PCR	
14.5		▪ WG Unit of Measurement	
14.6			
	15:30	*Coffee Break*	
15	16:00	*Scientific and technical session 1*	
15.1		▪ The application of isothermal nucleic acid amplification techniques in GMO analysis. (L. Yang, PRC)	Presentation
15.2		▪ Multiplex quantification of individual GM events by droplet digital PCR (Dr. D. Dobnik, National Institute of Biology, Ljubljana)	Presentation
15.3		▪ Event-specific multiplex PCR - screening for six GM soybean lines (L. Grohmann, DE)	Presentation
	17:30	*End of day 1*	
	19:30	**Networking dinner at Hotel Europa, Ispra**	

Day 2: 23rd September 2015

Session: NRL 882 WS and 24th ENGL Plenary meeting

AP	Time	Topic	Documents
16	09:15	*Scientific and technical session 2*	
16.1		▪ Risk-based inspection of import flows (Martijn Slot, MSc, RIKILT Wageningen UR, NL)	Presentation
16.2		▪ Detection of GM fish (F. Debode, CRA-W)	Presentation
16.3		▪ Wheat reference genes: seeking a suitable taxon-specific module for GMO analysis (U. Marchesi, IZSLT, Italy)	Presentation
	11:00	*Coffee Break*	
17	11:30	*Break-out Groups* 1) DNA extraction (follow-up) 2) Multi-target methods 3) Interpretation of results at the limit of detection	 Mandate Mandate Mandate
	12:45	*Buffet lunch*	
18	14:30	*Reports of break-out groups and discussion*	
19	15:30	*ENGL matters*	
19.1		▪ Pre-spotted plates, update (S. Rosa & F. Gatto, EURL GMFF)	Presentation
	15:45	*Coffee Break*	
20	16:15	*Scientific and technical session 3*	
20.1		▪ Practical demonstration on EURL GMFF bioinformatic tools (A. Angers, JRC)	
21		*AOB*	
22	16:45	DAL ENGL 24th	
	17:00	***End of meeting***	

Annex 2: action list

24rd ENGL PLENARY ACTION LIST 23/09/2015				
ACTIONS	**Resp.**	**Timeline**	**Status**	**Comments**
ENGL CONSORTIUM AGREEMENT				
Make available on ENGLNet report and presentations of 24th ENGL Plenary	SEC	Oct-15	Open	
Set dates for the 25th plenary ENGL	SEC + SC	31/10/2015	Open	
ENGL WORKING GROUPS				
WG Detection Interpretation Reporting (DIR)				
Publish final report	SEC	30/10/2015	Open	
WG UoM				
Organise 2nd meeting	SEC	30/09/2015	Open	16-17 November 2015
WG dPCR				
Organise 2nd meeting	SEC	30/09/2015	Open	Nov or Dec 2015
WG UpMeth				
Organise 2nd meeting	SEC	30/09/2015	Open	End October 2015
AG Method Selection for Validation				
Organise next meeting	SEC	30/09/2015	Open	November/December 2015
OTHERS				
Report on EURL survey on reference methods	SEC	31/12/2015	Open	
Provide table on DNA extraction methods	SEC	15/10/2015	Open	Send table by email. Webform ? Deadline for replies end of November
Send SOP for PCR inhibition	SEC	30/09/2015	Open	Proposed by BOG on DNA extraction
Establishment of "cut-off"	EURL/ENGL	30/11/2015	Open	follow-up BOG group, reflect on how to produce data
Postion document identyfying issues with 619 and suggesting possible solutions, including approach for MU	EURL/ENGL	31/01/2016	Open	electronic forum? WG?
Reactivate WG Verification	SEC	30/09/2015	Open	BOG multitarget suggestion, criteria for multiplex methods