EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

23rd ENGL PLENARY MEETING

15-16 April 2015, Ispra, Italy

Meeting Report

1. Welcome

The Chair welcomed the participants and announced that a new schedule for the ENGL and Steering Committee (SC) meetings will be implemented to facilitate financial reporting. The ENGL plenary meetings will be organised in April and September/October, the SC meeting will take place in February and June.

2. Approval of the Agenda

The agenda (Annex 1) was approved without amendments.

3. Approval Report 22nd ENGL plenary

The report was endorsed without modifications.

4. Outcome of the 28th ENGL SC meeting (February 2015)

The Secretary summarised the main outcome of the ENGL SC meeting and reminded that the report is available in ENGLnet. It was highlighted that new WGs are established and that interested members can still join any of these working groups. A survey on methods and reference genes used by laboratories will be launched in June.

5. Dynamic Action List (DAL) of 22nd ENGL plenary

The Secretary reviewed the open points of the list. No expert on new breeding techniques and on alternative detection techniques was found to be available for the ENGL plenary meeting. New attempts will be put in place to identify experts on these topics for the next meetings.

6. Update from SANTE

SANTE provided an update on Directive (EU) 2015/412 amending Directive 2001/18/EC and explained the two options available to Member States for restricting GMO cultivation on their territory.

SANTE informed that a technical study was launched at the beginning of the year to assess the need for harmonisation of sampling and analysis of GM material in food. The study is focused on GM events whose authorisation procedure is pending or expired. The information will be collected from official control and competent authorities across the whole food supply chain and from relevant stakeholders. The study should be concluded in July. SANTE asked NRLs to contribute to the assessment by flagging up relevant issues.

SANTE further provided an update on the work of three Scientific Committees (SCENIHR, SCHER and SCCS) on operational definition, risk assessment methodologies and research priorities in the field of synthetic biology.

SANTE informed that an online forum on synthetic biology under the Convention on biological diversity will be set up in the coming weeks and invited members to contribute.

7. Progress reports ENGL working groups

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

A summary of the activities undertaken since December 2014 was presented. The candidate methods are collected through surveys or by submission using a web form available on the GMOMETHODS database web page.

The analytical gaps identified in the meeting of 3rd December 2014 were:
1) Methods for detecting wheat and potato reference genes
2) Assays for multiple detection of GM events containing rare or unique genetic elements not covered by current screening strategies and for which it would not be worth to develop element-specific methods
3) Methods for detecting GM animals such as GM salmon
4) Methods for GMM detection i.e. GM *B. subtilis*.

An event-specific pentaplex method developed by the ISP-BE was selected by the committee for further validation: this method contributes to covering the analytical gap 2.

The Chair invited participants to submit new method proposals.

7.2 WG on unit of measurement

The Secretary explained that conversion factors are needed to express the unit of measurement of the PCR analytical results in m/m as requested by EU legislation. He remarked the importance of using agreed values to assure consistency between laboratory measurements and described the mandate of the new WG. The Secretary announced that P. Corbisier will be the chair of the WG and that the first meeting will be organised in Ispra on the 30 June-1st of July 2015.

7.3 WG on digital PCR

The Secretary explained that digital PCR is a promising technology becoming more suitable for the ENGL purposes and explained the mandate of the new WG. The WG should produce a document addressing the various issues and summarising relevant existing experiences with dPCR, thus helping the laboratories in deciding on implementing the technology. Dr. Sven Pecoraro volunteered to chair the group. The first meeting will be take place on 1-2-July 2015.

7.4 WG on update of methods

The Secretary explained that the WG should review on a regular basis the methods of detection of GMOs, particularly those for which a renewal is foreseen, and provide advice on their validity to the EURL GMFF. Methods may become sub-optimal with time, thus needing re-assessment or re-validation. Given that only few ENGL members offered to participate, the Chair reminded that the entire control system is based on the use of validated methods that are considered fit for the purpose. The Secretary underlined the importance of the WG and invited more participation. Two new members were included in the group.

7.5 Secretariat report on workspaces breakout groups

The Secretary illustrated the workspaces defined on the ENGL net but noted their limited use. It was suggested that this may be due to the lack of a moderator stimulating the discussions. The Chair invited anyone who is willing to moderate a specific workspace.

8. Scientific and technical session

8.1 Decision system for DNA extraction from different types of samples (food, feed, seeds, plants etc.) (Dr. J. Zel, NIB, SI)

Efficient and reliable DNA extraction is a prerequisite for GMO analysis but the choice of the method to use is often based on the operator experience. A manageable decision system for choosing the most suitable method of extraction was proposed.

Samples were divided in different types (e.g. food, challenging and new matrices) and further classified in stable or variables according to the reproducibility of the results.

Different extraction strategies were tested and compared according to the samples classification. The nucleospin food DNA extraction kit resulted to be the most efficient.

8.2 Collaborative trials and statistical analyses of qualitative methods (Roy Macarthur, FERA, UK)

Different approaches for estimating the performance of a qualitative method in collaborative trials were presented. A simple assessment of a qualitative method (detected/ non detected) may be possible but is not using all information available. Quantitative data should also be used and provide useful information on reproducibility of qualitative methods.

8.3 Update on Pre-spotted Plates project (JRC)

The main findings of the pilot project were presented. In order to integrate the PSPs in the quality system of official testing laboratories the EURL GMFF further launched a proof-of-concept (PoC) project funded by the JRC Work Program "Intellectual Property and Technology Transfer".

The PSPs will be distributed to laboratories at the end of April, results will be analysed by July and reporting finalised by November. A survey is planned for estimating the potential market size/needs for PSPs. The speaker presented the PoC project layout covering screening and event-specific identification of maize and soy GMOs.

9. Scientific and technical session (2)

9.1 Multiplex GMO maize quantification with ddPCR (Dr. David Dobnik, NIB, SI,)

The speaker explained the underlining theory of ddPCR technology and presented the results of an *in house* validation of two multiplex methods (4-plex, and 10-plex) using that approach. A correlation between practicability and cost-effectiveness of ddPCR in comparison to existing qPCR approaches was presented. The cost of qPCR analysis resulted to be almost 3 times higher that digital droplet PCR. The multiplex methods will be first pre-validated (i.e. transferred to 3-4- labs) and then validated in an international collaborative trial within the Decathlon project.

9.2 Inter-laboratory characterisation of reference materials with dPCR (Dr. David Dobnik, NIB, SI

The project aimed at testing the applicability of dPCR to quantitative GMO analysis, compare methodologies (qPCR, dPCR and ddPCR) and assessing transferability of methods validated with the qPCR technology to digital PCR systems.

Both digital PCR systems provided values for relative GM quantification that were in line with ENGL acceptance criteria while a high variability was observed for the estimation of absolute copy numbers, especially for ddPCR platforms. Conversion to mass/mass unit

provided quite different values from the measured GM%/certified GM%. It was concluded that optimisation of the methods and new harmonised guidance may be needed.

10. Break-out Groups

The following break-out discussion groups were organised.

1) Practical implementation of Regulation (EC) No 619/2011
2) Experiences with multiplexing of PCR
3) Issues with national accreditation bodies
4) PCR on animals (GM, food authentication…)

11. Reports of break-out groups and discussion

1) Practical implementation of Regulation (EC) No 619/2011

The group had nine participants and concluded that only few cases of detection of GMOs falling under this Regulation were reported by member states. The absence of reference material for some GM events at the 0.1% GM level (mass/mass) and the diversity of approaches followed to circumvent this lack were remarked. It was suggested to prepare an ENGL position document to state that CRMs at different mass fraction (including the 0.1 % level) are needed.

2) Experiences with multiplexing of PCR

The group had 17 participants. The lack of specific performance criteria for multiplex methods was identified, although the new MPR documents provides for some criteria; it was suggested to prepare a guidance document for the development of new and the verification of existing multiplex methods. Validation of multiplex methods could be very complex and expensive but yet advantageous. The members proposed the establishment of a new WG or an extension of the mandate of the existing WG on verification. They further proposed to open a discussion forum in the ENGLnet with a moderator. The Chair asked for a volunteer reporting to the SC meeting in June and L. Hougs (DK) accepted the invitation.

3) Issues with national accreditation bodies

A significant number of laboratories reported difficulties with accreditation bodies. The following issues were identified:

- Difficulties in implementing flexible scope accreditation. In some MS, approval of the accreditation body was still required for each single method
- Coexistence of ISTA and ISO 17025 accreditation.
- Lack of technical auditors in accreditation bodies
- Non-harmonized interpretation of ISO 17025

The Chair reminded the existence of the guidance on flexible scope of accreditation endorsed by the European accreditation body and suggested informing the EURL GMFF and the European accreditation body on discrepancies in its implementation.

4) PCR on animals (GM, food authentication…)

An agreed starting point is meat identification and methods related, for which a web space for sharing information could be created. It was also proposed to provide a strategy document, with logical steps for implementing the analysis.

The Chair mentioned the review article on GM animals published by the EURL GMFF.

The Chair also reminded that one of the main outcomes of the 1st workshop on species identification was the suggestion to create a new network of species identification laboratories (ENSI). The mandate of the ENGL should not be extended to non-GMO, but

the considerable expertise existing within the network can be used for creating this new network.

12. AOB

None

13. DAL ENGL 23rd

The Secretary summarised the actions points following the discussion (Annex 2).

He communicated that the next ENGL plenary will be organised on the 22nd-23rd of September. The Chair asked members to share experiences, information, issues on the relative work spaces in ENGLnet and invited suggestions for expert's presentations for the next meetings.

The Chair thanked the participants and closed the meeting.

Annex 1 – Agenda

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Final Draft Agenda

Day 1: 15th April 2015

AP	Time	Topic	Documents
1	9:30	▪ Welcome	
2		▪ Approval of the Agenda	Draft Agenda
3		▪ Approval Report 22th ENGL plenary	Report 22nd ENGL
4		▪ Outcome of the 28th ENGL SC meeting (February 2015)	Report SC28
5		▪ Dynamic Action List (DAL) of 22th ENGL plenary	DAL-ENGL22
6		▪ Update from SANTE	
	10:45	*Coffee Break*	
7	11:15	*Progress reports ENGL working groups*	
7.1		▪ AG SMV (Advisory Group on Selection of Methods for Validation)	Presentation
7.2		▪ WG on unit of measurement	
7.3		▪ WG on digital PCR	
7.4		▪ WG on update of methods	
7.5		▪ Secretariat report on workspaces breakout groups	
	12:45	***Buffet lunch***	
8	14.00	*Scientific and technical session*	
8.1		▪ Decision system for DNA extraction from different types of samples (food, feed, seeds, plants etc) (Dr. J. Zel, NIB, SI)	Presentation
8.2		▪ Collaborative trials and statistical analyses of qualitative methods (Roy Macarthur, FERA, UK)	Presentation
8.3		▪ Update on Pre-spotted Plates project (JRC)	Presentation
	15.45	*Coffee Break*	
9	16:15	*Scientific and technical session (2)*	
9.1		▪ Multiplex GMO maize quantification with dPCR (Dr. David Dobnik, NIB, SI,)	Presentation
9.2		▪ Interlaboratory characterisation of reference materials with dPCR (Dr. David Dobnik, NIB, SI	Presentation
	17:30	***End of day 1***	
	19:30	**Social dinner at Hotel Europa - Ispra**	

Day 2: 16th April 2015

AP	Time	Topic	Documents
10	09:15	*Break-out Groups* 1) Practical implementation of Regulation (EC) No 619/2011 2) Experiences with multiplexing of PCR 3) Issues with national accreditation bodies 4) PCR on animals (GM, food authentication…)	 Mandate Mandate Mandate Mandate
	10:45	*Coffee Break*	
11	11:15	*Reports of break-out groups and discussion*	
12	12:00	*AOB*	
13	12:15	DAL ENGL 23rd	
	12:30	*End of meeting and sandwich lunch*	

Annex 2 – Action list

23rd ENGL PLENARY ACTION LIST 16/04/2015				
ACTIONS	**Resp.**	**Timelin**	**Status**	**Comments**
ENGL CONSORTIUM AGREEMENT				
Make available on ENGLNet report and presentations of 23th ENGL Plenary	SEC	30/4/2015	Open	
Send invitation for the 24th plenary ENGL	SEC	30/6/2015	Open	date: 22-23 September 2015
ENGL WORKING GROUPS				
WG Detection Interpretation Reporting (DIR)				
Publish final report	SEC	15/5/2015	Open	
Circulate the cutoff draft to SC	SEC	30/4/2015	Open	
WG UoM				
Organise kick--off meeting	SEC	30/4/2015	Open	
WG dPCR				
Organise kick-off meeting	SEC	30/04/2015	Open	
WG UpMeth				
Organise kick-off meeting	SEC	30/04/2015	Open	
AG Method Selection for Validation				
Survey on methods including ref genes	SEC	15/5/2015	Open	
OTHERS				
Invite NBT experts (detection) to the next ENGL plenary	SEC	15/01/2015	Open	to be discussed by the SC29
Invite experts on Alternative Detection Techniques to the next ENGL plenary	SEC	15/1/2015	Open	focus on isothermal (Litao + NIB) TBD by SC29
Postion document identyfying issues with 619 and suggesting possible solutions, including approach for MU	EURL	31/5/2015	Open	electronic forum
Open discussion forum on multiplexing in ENGLnet	SEC	30/4/2015	Open	Lotte moderator