EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

22nd ENGL PLENARY MEETING

1-2 December 2014, Ispra, Italy

Meeting Report

1. Welcome

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

2. Approval of the Agenda

The agenda (Annex 1) was approved without modifications.

3. Approval Report 21st ENGL plenary

The report was adopted without modifications.

4. Dynamic Action List (DAL) of 21st ENGL plenary

The Secretariat reviewed the open points of the list. A workshop on species/allergens detection will be organised in 2015.

5. Outcome of the 27th ENGL SC meeting (September 2014)

The Secretariat informed that the report of the last SC meeting was published on the ENGL web site and highlighted its main points. No amendments were requested.

6. Progress reports ENGL working groups:

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

The WG leader reviewed the criteria for method selection and illustrated the relative workflow. A pCambia T35S detection method (Breitler *et al*. 2004) was selected at the last WG meeting especially to target unauthorised GM events.
It was reminded that a form is available on the GMOMETHODS home page for submitting method proposals to the Secretariat and invited contributions from the laboratories. It was reported that the WG has taken on-board the task of identifying for each crop the best reference gene method.

6.2 WG DIR (Detection Interpretation Reporting)

The WG leader presented the structure of the document. As decided at the last SC meeting, the section on "cut-off" will be addressed in a separate document possibly by a different WG with an ad-hoc mandate. The final commenting phase of the document is completed and the final version will be submitted to the ENGL in January 2015.

6.3 WG Seed Testing

The WG leader reviewed the structure of the report and announced that it will be circulated to the ENGL in December/January. Comments should be provided by the end of January. The report will be then finalised and delivered to SANCO.

7. Recent emergencies (Vit B2, Chlorine Chloride, papaya, wheat) status, lessons learned (EU-RL GMFF, SANCO, ENGL members)

Dr. L. Grohmann (DE) provided background information on the vit. B2/GM *Bacillus subtilis* contaminations reported in two recent RASFF notifications and proposed a PCR method for detecting transgenic vector sequences also present in the GM *B. subtilis* strain. The forward and reverse primers of the method target respectively a construct of the *lac* operon sequence of *E. coli* and a fragment derived from the vector pBR322. With a master mix free of bacterial DNA contamination the laboratory obtained a LOD of 16 copies.

The EU-RL GMFF provided a literature review and a summary of the NGS (next generation sequencing) analysis performed on the GM *B. subtilis* sample provided by a German laboratory. Based on these analyses, it appears that the Chinese producers are probably using multiple strains/subspecies of *B. subtilis* developed in Russia and containing more than one plasmid.

No remarks or new concerns were raised on the previously notified GM chlorine chloride, papaya and wheat contamination cases.

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

The document CEN/TS 16707:2014, providing technical specification for screening strategies in GMO detection, is published.

The WG 11 is waiting for the results from collaborative trials conducted by the GMOval project to evaluate the POD statistical approach proposed in ISO/NP 16393 for validation of qualitative methods.

Regarding the activities of ISO/TC 34/SC 16 (horizontal methods for molecular biomarker analysis), new standards were proposed for identification of meat species, chicken, pig, sheep, horse, cow and buffalo meat in meat products. A new working group will be formed with the mandate to define performance criteria for methods of detection of animal species.

9. Scientific and technical session 1

9.1 Presentation on synthetic biology (Dr. I. Hafner, Laboratory of Biotechnology Institute of Chemistry, Ljubljana)

The speaker presented a general overview on the new emerging field of synthetic biology and its approaches: bottom-up (build life from scratch starting with a protocell) or top-down (eliminating the problem of natural complexity from an existing cell).

It was remarked that a high-level expert group of the European Commission has defined synthetic biology as the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms.

The speaker presented some case studies of synthetic biology applications for heterologous production of artemisinin (antimalarial drug), engineering of DNA-guided assembly of biosynthetic pathways to improve catalytic efficiency and facilitate the production of useful compounds, design of activators or repressors of DNA expression for construction of logic circuits in mammalian cells and modelling of a single-chain polypeptide tetrahedron.

9.2 Update on Decathlon project (Dr. E. Kok, RIKILT Wageningen UR)

The objective of the project is the development of cost efficient and advanced DNA-based methods for specific traceability issues and high level on-site applications. An overview of the project and a description of the activities of the eight work packages were provided.
A ddPCR (droplet digital PCR) method has been selected for validation in 2015 and methods for identification of tobacco are being tested (PCR, LAMP). Expert webinars to discuss the activities were organised. Many ENGL members are involved in the project, including the MBG unit of JRC.

10. Scientific and technical session 2

10.1 Digital PCR: report from discussion day and NRL training (W. Broothaerts, EU-RL GMFF)

The EU-RL GMFF reported on the training on digital PCR organised in Ispra on.

The Secretary presented a summary of a discussion day organised on the 26th of November by the JRC. Advantages/disadvantages of the dPCR technology, evaluation of practical experiences and comparison of different platforms were among the topics discussed.

The Secretary informed that on the ENGL-net a discussion group on dPCR is open, and invited members to share articles, data and keeping a live discussion on the subject.

10.2 Digital PCR: technical and legal requirements for GMO quantification (P. Corbisier, JRC-IRMM)

The features of real-time and digital (droplet) PCRs were presented. The legal requirements for GMO quantification and expression of measurement units were reviewed. It was reminded that a conversion factor (Cf) provided by the EU-RL GMFF needs to be used for reporting in terms of mass fraction the copy number percentages obtained in real-time PCR analysis. No laboratory is accredited yet for performing d(d)PCR analysis and methods validated with real-time PCR must be tested in digital PCR.

The Secretary asked to reflect on the conversion factors issue during break-up sections. It was discussed that it would be useful providing standardised conversion factors to achieve harmonisation in official control.

10.3 Statistical relevance of NGS data for GMO detection in food and feed (Dr. S. Willems, WIV-ISP, BE)

The speaker presented three NGS approaches for GMO detection, their developed statistical framework and feasibility. Three approaches were presented:
1) Detection approach (determine if the generated fragments align with the insert) 2) Proof approach (determine the integration of the insert in the host genome, in particular if one part of the generated fragments aligns to the host genome and the other overlaps the transgenic sequence 3) Identification approach (precision sequence identification).

After a brief overview of the NGS technology, the formulas to calculate the number of reads necessary for obtaining a 95% probability of successfully detect a target read in a sample for the three approaches were presented. The author presented the results of experimental work where NGS analysis of Bt rice samples at different concentration level (100% and 10%) and complexity (noodle samples) were compared with the developed theoretical statistical framework.

GM rice was detected for all approaches and samples. In all cases the experimental data was found to fit the theoretical framework. The minimum number of reads (in million) necessary for

GM detection was increasingly higher for the first, second and third approach and is depending on the genome size, % of GM contamination and complexity of the sample.
It was concluded that NGS could be used in a general framework for detecting samples containing pure GMOs or a high GM % and could be easily expanded to new GMOs. However the technology is still unfeasible and too expensive for samples at low GM level and with complex matrices.

11. Break-out Groups

The following groups with the relative moderators and note takers where organised:

1) Analytical challenges of New Breeding Techniques

2) Alternative detection technologies (e.g. NGS, isothermal amplification….)

3) Detection and identification of GMM

4) EU-RL GMFF requirements for renewals of GMO applications

12. Reports of break-out groups and discussion

1) *Analytical challenges of New Breeding Techniques*
The group concluded that it is premature to establish an ENGL working group on the topic since the defining of the legal aspects is still underway. It was suggested organising a workshop for discussing detection techniques and strategies.
The ENGL agreed with the conclusion but suggested inviting an expert for a seminar at the next ENGL plenary meeting.

2) *Alternative detection technologies*
The group mainly focused on digital PCR, NGS, high resolution melting and loop-mediated technologies. Members commented that real-time PCR is still the technique mostly used for GMO analysis, while the cost of NGS technology is excessive for routine applications but it could be used for unauthorised GMO detection and considered dPCR the method of choice for future development of high throughput applications. Members felt there was not enough practical experience for establishing a WG on digital PCR and that a platform/forum to share information and experimental data, preferably via an ENGL website would be preferable for involving a larger number of laboratories.
The Secretary remarked that spontaneous participation has never been successful and proposed a forum moderator. He further remarked that information on platforms used or planned to be acquired by laboratories could be collected from CT rounds. It was suggested organising an ad-hoc workshop and a brainstorming section with experts from other fields to produce a report for ENGL.

3) *Detection and identification of GMM*
Twenty-six members attended the discussion of which nineteen laboratories already working in microbiology and two laboratories involved on the Vitamin B2 testing. The group agreed that an ENGL working group should be established. It was also suggested opening a GMM space on the ENGLnet for sharing experiences.
Most of the participants agreed on the break-up group proposal and commented that guidance was needed for laboratories. A draft mandate will be circulated and discussed by the SC while clarifications with SANCO will be requested.

4) EU-RL GMFF requirements for renewals of GMO applications
Several applications for renewal of authorisations are expected in the coming months. Requirements for detection methods in case of renewal are not specifically laid down in the Regulation, although it is the responsibility of the owner of the authorisation to ensure that the detection method remains fit for purpose at any time during the authorisation.

Members suggested surveying ENGL laboratories on the modifications implemented in their analytical procedures. They proposed creating an advisory group for discussing renewals on a case by case manner. Participants supported the idea of an inventory of methods currently implemented in the laboratories and suggested including availability and quality of CRMs. It was finally agreed to perform a survey on methods currently implemented by ENGL laboratories and to clarify the legal framework with SANCO.

13. ENGL matters

13.1 Pre-spotted plates, update (Dr. M. Querci, EU-RL GMFF)

The pilot project on PSP showed concordance of results (over 90%) between screening and identification steps. The cost of the screening step resulted lower than the control procedures currently employed by the laboratories, while the identification step was more expensive. The PSP workflow allowed overall a major reduction (30.9%) of staff time. The SPS were evaluated by the participants with a score ranging between 6 and 8.3.
A demonstration project will be launched in January 2015 aiming at integrating the PSP in official testing laboratories. Soon there will be a call for participating to the study. PSP would be distributed free of charge to be used in real-life situations.

13.2 Possible future ENGL working groups

Proposals:

- WG on renewals
- WG on "unit of measurement"
- WG on DNA extraction (It was suggested identifying a moderator for establishing a forum discussion. The Chair asked for volunteers. Since no candidatures were proposed the Chair concluded that no active support from the network really existed on the issue).
- WG on dPCR
- WG GMM

Participants also proposed addressing on-site methods and providing a platform for exchanging ideas, experiences or solutions. It was suggested organising an ad-hoc workshop with experts from other fields and producing a guidance document for laboratories interested in applying this new technology.

14. Scientific and technical session 3

14.1 Presentation (via Skype) on Genetically Modified Microorganisms (Dr. L. Herman, ILVO, BE)

A specific EFSA committee performs safety assessment of enzymes, amino acids and vitamins produced by GMM following the GM guidance document (EFSA Journal 2011; 9 (6): 2193). For confidentiality reasons GMM assessments are only partially publicly available. The committee evaluate the safety of the parental and recipient strains according to their capability of leaving undesirable metabolites in the final product. The products are divided in four categories according to their purity. Category 1 and 2 are not supposed to contain recombinant DNA or genetically modified organisms and the applicant has to demonstrate their absence in the final product. The methods of assessment should be described and the levels of detection documented. Absence of recombinant DNA is confirmed by PCR-based methods. Environmental risk assessment is not considered necessary if recombinant DNA or GMMs are not detected in the final product. The assessment does not follow a standard protocol (no amplicon length, number of PCR cycles, LOD defined for detection of recombinant DNA), is performed on a case by case basis and is dependent on the genetic modification.

15. AOB

None

16. DAL ENGL 22nd

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

The Secretary communicated that for budgetary reasons the dates of the SC and ENGL meetings will anticipated respectively to February and April so that the second meetings of the year could be respectively organised in June and October.

The Chair thanked the participants and closed the meeting.

Annex 1: final agenda

EUROPEAN COMMISSION
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10th WORKSHOP OF GMO NATIONAL REFERENCE LABORATORIES
REGULATION (EC) No 882/2004 and 22nd ENGL PLENARY MEETING

1-2 December 2014, Ispra, Italy

Final Draft Agenda

Day 1: 1st Dec 2014

Session: National Reference Laboratories and Official Control

AP	Time	Topic	Documents/comments
1 2 3	9:30	▪ Welcome and approval of the agenda ▪ Approval of the 9th workshop report ▪ Tour de table: issues/opinions from NRLs	Draft agenda Report Oral input from NRLs
	10:45	*Coffee Break*	
4 5 6 7 8	11.15	▪ Status of revision of Regulation (EC) No 882/2004 (including new requirements) (I. Ciabatti, SANCO) ▪ Update on comparative testing activities and role of NRLs in this context (W. Broothaerts, EU-RL GMFF) ▪ Discussion on NRLs training needs specific to their function as NRL ▪ Update on requirements of the EC work programme on food and feed controls (EU-RL GMFF and SANCO) ▪ AOB and conclusions	Presentation Oral input from NRLs
	12:45	*End of session for NRL*	
		Buffet lunch	

Day 1: 1st Dec 2014

Session: NRL 882 WS and start of 22nd ENGL Plenary meeting

AP	Time	Topic	Documents
9	14:00	▪ Welcome	
10		▪ Approval of the Agenda	Draft agenda
11		▪ Approval Report 21th ENGL plenary	Report
12		▪ Dynamic Action List (DAL) of 21th ENGL plenary	DAL ENGL21
13		▪ Outcome of the 27th ENGL SC meeting (September 2014)	Report SC27 + presentation
14		*Progress reports ENGL working groups:*	
14.1		▪ AG SMV (Advisory Group on Selection of Methods for Validation)	AG SMV update
14.2		▪ WG DIR (Detection Interpretation Reporting)	DIR update
14.3		▪ WG Seed Testing	Final draft for approval
15		Recent emergencies (Vit B2, Chlorine Chloride, papaya, wheat) status, lessons learned (EU-RL GMFF, SANCO, ENGL members)	Oral inpout from NRLs involved in emergency cases
16		Update on CEN/ISO activities (Dr. L. Grohmann, DE)	Presentation
	15:45	*Coffee Break*	
17	16:15	*Scientific and technical session 1*	
17.1		▪ Presentation on synthetic biology (Dr. I. Hafner, Laboratory of Biotechnology Institute of Chemistry, Ljubljana)	Presentation
17.2		▪ Update on Decathlon project (Dr. E. Kok, RIKILT Wageningen UR)	Presentation
	17:30	***End of day 1***	
	19:30	**Social dinner at Ristorante Montelago - Ternate**	

Day 2: 2nd Dec 2014

Session: NRL 882 WS and 22nd ENGL Plenary meeting (continued)

AP	Time	Topic	Documents
18	09:15	*Scientific and technical session 2*	
18.1		▪ Digital PCR: report from discussion day (Dr. S. Pecoraro, LGL, DE) and NRL training (W. Broothaerts, EU-RL GMFF)	Presentations
18.2		▪ Digital PCR: technical and legal requirements for GMO quantification (P. Corbisier, JRC-IRMM)	Presentation
18.3		▪ Statistical relevance of NGS data for GMO detection in food and feed (Dr. S. Willems, SIPH, BE)	Presentation
	10:45	*Coffee Break*	
19	11:15	*Break-out Groups* 1) Analytical challenges of New Breeding Techniques 2) Alternative detection technologies (e.g. NGS, isothermal amplification….) 3) Detection and identification of GMM 4) EU-RL GMFF requirements for renewals of GMO applications	Mandate Mandate Mandate Mandate
	12:45	*Buffet lunch*	
20	14:30	*Reports of break-out groups and discussion*	
21	15:30	*ENGL matters*	
21.1		▪ Pre-spotted plates, update (Dr. M. Querci, EU-RL GMFF)	Presentation
21.2		▪ Possible future ENGL working groups	
	15:45	*Coffee Break*	
22	16:15	*Scientific and technical session 3*	
22.1		▪ Presentation on Genetically Modified Microorganisms (Dr. L. Herman, ILVO, BE)	Presentation (via skype)
23		*AOB*	
24	16:45	DAL ENGL 22nd	
	17:00	***Dates of 2015 meetings and end of meeting***	

Annex 2: action list

22th ENGL PLENARY ACTION LIST 12/02/2014				
ACTIONS	**Resp.**	**Timeline**	**Status**	**Comments**
ENGL CONSORTIUM AGREEMENT				
Make available on ENGLNet report and presentations of 22th ENGL Plenary	SEC	15/01/2015	Open	
Send invitation for the 23th plenary ENGL	SEC	31/1/2015	Open	23rd plenary April 2015
ENGL WORKING GROUPS				
WG Detection Interpretation Reporting (DIR)				
Final draft for adoption by SC28	SEC	15/01/2015	Open	
Circulate the cutoff draft to SC	SEC	20/12/2015	Open	
WG Seed Testing (ST)				
Circulate the final draft to ENGL for comments and approval	SEC	31/12/2015	Open	
AG Method Selection for Validation				
Organise ring trial pCanbia method	chair + EURL	31/3/2015	Open	
OTHERS				
Define a mandate for a possible working group on GMM detection (with selected experts coming from the breakout group)	SEC	15/01/2015	Open	propose a mandate to the SC28
Setup a standing advisory group for renewals (electronically)	SEC	15/1/2015	Open	
Survey for the fisrt methods subject of renewals	SEC	15/01/2015	Open	
Setup WG on unit of measurement (copy/copy, mass/mass)	SEC	15/01/2015	Open	propose a mandate to the SC28
Setup WG on dPCR	SEC	15/012/015	Open	propose a mandate to the SC28
Invite NBT experts (detection) to the next ENGL plenary	SEC	28/2/2015	Open	ask ENGL for names
Invite experts on Alternative Detection Techniques to the next ENGL plenary	SEC	28/2/2014	Open	ask ENGL for names; include on-site methods
Open GMM space in ENGLnet to all members	SEC	20/12/2014	Open	
Organise workshop on species detection/allergens	SEC	Nov-14	Open	point left from previous DAL
Consider a Workspoace for each of the BOG	SEC	31/01/2015	Open	and report at the next plenary