EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

28th ENGL STEERING COMMITTEE MEETING

10-11 February 2015, Ispra, Italy

Meeting Report

1. Introductory activities

1.1 Welcome, apologies

The Chair welcomed the participants and informed that representatives from Denmark, Finland, Greece, Hungary, Ireland, Malta, Norway, Romania and Slovenia were excused.

1.2 Approval of the agenda

The Secretary asked including in the last part of the meeting a discussion on the ENGL survey. The agenda (Annex 1) was approved with this modification.

1.3 Approval of the report of the 27th SC meeting

The report of the last meeting was endorsed without changes.

1.4 Review of Dynamic Action List (DAL SC27)

The Secretary reviewed the DAL of the previous meeting. He informed that a working space on the SC web section including documents in a draft format has been made accessible to all ENGL members.

1.5 Update from SANTE

DG SANTE reported that for import of microorganism and enzymes Turkish authorities asked documents certifying that their production did not comprise GMOs. The Commission defined the request as a trade barrier. A bilateral WG will be established between Turkish authorities and the Commission for debating the issue.

DG SANTE reported that the Commission is discussing implementation of a harmonised approach for the analysis of botanical impurities.

DG SANTE informed that the Commission has launched a technical study on the assessment of the need for harmonisation of methods of sampling and analysis for GM material in food. The study is focusing on GM events whose authorisation is pending or expired. The results of the study will be finalised in June and will be used for deciding whether a policy action is needed.

DG SANTE informed that three Scientific Committees (SCCS, SCHER and SCENIHR) have been requested by the Commission to provide opinions on an operational definition for Synthetic Biology, on risk assessment methodologies and safety aspects and on research priorities in this field. The first opinion on an operational definition is already publicly available at

http://ec.europa.eu/health/scientific_committees/consultations/public_consultations/scenihr_consultation_21_en.htm;

the second one on risk assessment methodologies and safety aspects has been recently submitted to public consultation:

Joint Research Centre I-21027 Ispra (VA), Italy
Tel: direct line +39 0332 78 6735 · Fax: +39 0332 78 6159
E-mail: Joachim.kreysa@ec.europa.eu
http://ihcp.jrc.ec.europa.eu

http://ec.europa.eu/health/scientific_committees/consultations/public_consultations/scenihr_consultation_26_en.htm);
the third one on research priorities is under preparation. The work should be completed by summer 2015.

2. Progress reports ENGL working groups

2.1 AG SMV (Advisory Group on Selection of Methods for Validation): update

The WG leader informed that the pCambia T35S method selected in 2014 will be validated in a ring trial early in 2015 and that a form for submitting methods proposals has been published on the web page of the GMOMETHODS database. She reported that the advisory group had identified the current following gaps in GMO analysis:

1) Alternative 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 (i.e. GM salmon)
4) Methods for GMM detection (i.e. GM *B. subtilis* overproducing Vitamin B2)

In the last meeting the advisory group agreed to recommend for validation a qualitative multiplex event-specific PCR method for analysis of compound feed products covering five GM events not detected by commonly used screening approaches that had been proposed by the Belgian WIV-ISP laboratory.
To define the best available reference gene methods and identify corresponding analytical gaps, the WG suggested surveying ENGL laboratories on the reference genes targeted in their analysis and on the modifications implemented in the protocols of the relative methods.

2.2 WG DIR (Detection Interpretation Reporting): final exchange

The speaker reported that the comments provided by ENGL members were being incorporated into the document.

2.3 WG-ST (seed testing): final exchange

The WG leader informed that the final document had been endorsed by ENGL members. It will be transmitted to DG SANTE, who will discuss the report with SCoPAFF prior to publication.
The speaker remarked that new points not covered in the document may emerge and suggested keeping the WG dormant for future updates. The Chair agreed with the proposal and recommended using the same strategy for the other WGs.

3. New activities

3.1 Draft mandate for a WG on GMM detection

Since the relevant legislation is under revision SANTE proposed postponing the discussion to the next SC meeting when the mandate could be more clearly defined. The suggestion was agreed.

3.2 Draft mandate for a WG on unit of measurement

The Secretary explained that GM quantification by PCR is often reported using copy numbers of target sequences while recent Regulation (EC) No. 619/2011 requires the expression of the measurements in mass/mass values and that a harmonized approach for conversion factors is needed. He presented a draft mandate for the WG (see Annex 2). The mandate was discussed and will be finalised accordingly by the Secretariat. The Chair requested focusing on the technical aspects. Participants agreed to set-up the WG, which is expected to produce a final draft for the SC of February 2016. Philippe Corbusier accepted to be the chair of the WG ((to be confirmed by WG members at the kick-off meeting).

3.3 Draft mandate for a WG on digital PCR

The Secretary explained that laboratories are interested in the new technology and in receiving guidance on the relative decision-making and implementation phases. He presented the draft mandate for the WG (see Annex 3). Participants agreed to establish the WG with the proposed mandate. The WG is expected to submit a first draft to the ENGL SC in June 2015 and a consolidated version for the following ENGL plenary in October. Sven Pecoraro accepted to be the chair of the WG (to be confirmed by WG members at the kick-off meeting).

3.4 Draft mandate for a standing WG on renewals

The Secretary reported that many applications for renewals are expected in the near future and that it needs to be verified if the originally validated methods still maintain their functionality. He proposed establishing a WG to assist the ENGL in this task and presented a draft mandate (Annex 4). Participants agreed adding the task to the mandate of the AG SMV. The Secretary also mentioned that according to the new MPR document the methods using the delta-Ct approach will not be any longer valid and that the method verification guidance document should be regarded as a baseline for the evaluation. The AG SMV is expected to report on the implementation of the task at the next SC in June 2015.

4. ENGL topics

4.1 Preparation of the 23rd ENGL plenary (15-16 April 2015)

The Chair requested suggestions on scientific topics for the ENGL plenary. The participants provided and agreed on the following proposals:

- Presentation on detection of products of new breeding techniques (speaker to be invited) providing a general overview of the new technologies.
- Alternative detection techniques (speaker to be invited)
- Decision system for DNA extraction from different types of samples
- Multiplex GMO maize quantification
- Interlaboratory characterisation of reference materials with dPCR (GMOVal project)
- Collaborative trials for qualitative methods (GMOval Project)

The Secretary proposed including in the plenary a break-up group section. Participants suggested and agreed on the following topics:

1) Practical implementation of Regulation (EC) No. 619/2011
2) Experiences with multiplex PCR
3) Issues with national accreditation bodies
4) PCR on animals (GM and species authentication…)

5. Scientific / technical topics

5.1 Update on CT (EURL GMFF)

The speaker presented an overview on the results of the CT rounds and on the changes implemented. He remarked that in the future the unit of expression of results will be restricted to m/m % as this is required in Regulation (EC) No 619/2011 and that laboratories will need to report the calibrant and, if applicable, the conversion factor used.

The speaker announced that two CT rounds will be launched respectively in March and May 2015.

He anticipated the possibility of outsourcing the tasks for the preparation and characterisation of the tests items used in the CT rounds, which was deemed acceptable by the SC. The Chair requested suggestions for suitable service providers, possibly within the EU.

6. Scientific / technical topics

6.1 Pre-spotted plates (PSP), pilot and demonstration project (JRC)

The speaker presented the final results of the PSP pilot project and the follow-up demonstration project.
The practical work of the pilot project was finished in 2014 and the final analysis confirmed the finding that PSP for screening are seen to be very useful while event-specific PSP require more fine tuning.

In 2015 a demonstration project will be carried out, 800 screening PSP will be produced and made available to control laboratories for routine analysis. The aim is to demonstrate that pre-spotted screening plates can be introduced into routine analysis. In order to establish an estimate for the needed numbers of PSP, the EU-RL GMFF will survey the control laboratories in the EU to learn how many samples they analyse annually.

A discussion followed on the harmonization of the decision support systems and interpretation of ambiguous results during routine analysis (analysis performed in duplicate). The following e-mail address was provided for sending comments and suggestions: JRC-EURL-GMFF-PSPP@ec.europa.eu.

6.2 Proof of Concept project on pre-spotted plates (JRC)

A project of the MBG unit was awarded an internal grant as a proof-of-concept (POC) project. Its aim is to proof the concept of using PSP for official regulatory control.
DNA samples will be distributed to five laboratories that will perform *in-house* validation of the PSP with the aim to generate all data required for obtaining accreditation. The POC project will result in successfully including PSP in the flexible scope accreditation of the participating laboratories, thus proofing that it is possible.

7. Information on upcoming workshop on species/allergens detection (including proposal for experts) (JRC)

The speaker explained that the MBG unit was asked exploring new fields to which it could apply its DNA analysis expertise. For that purpose the unit launched a survey for mapping the non-GMO activities performed by ENGL laboratories and for collecting suggestions. She informed that many ENGL members are apparently occasionally or regularly involved in species identification. Participants of the breakout group at the last plenary meeting in December 2014 suggested including into the GMOMETHODS database species identification methods, establishing an EU network of accredited DNA-analysis laboratories (not only GMOs) and organizing a dedicated workshop to recommend concrete steps forward. She presented a draft outline for such a workshop (Annex 5). SC members suggested splitting the workshop in two parts, dedicated respectively to allergens and species identification, while maintaining a focus on methods and sequence information. The Chair invited the SC members to provide suggestions of experts/speakers for the workshop that will take place, on 14th of April, the day before the ENGL plenary meeting.

8. Information on 2nd international workshop on GMO analysis networking (JRC)

The speaker announced that the workshop, supported by SANTE, will be organised on the 14th-16th of July 2015. Participants will be invited from existing regional networks, including Europe, to favour networking and capacity building. She informed that up to 100 contributors could be welcomed to the workshop and that therefore only a limited number of ENGL members could participate. The speaker asked to propose interesting technical approaches to the Secretariat.

9. AOB (including date SC29)

- Participants agreed organising the following SC in June, in conjunction with the AB-CT meeting. The secretariat will confirm the dates.
- The Secretary recalled once more that a survey of methods employed by ENGL members for routine analysis will be carried out in the near future. The aim is also to learn about modifications introduced by the laboratories in comparison to the reference methods as included in the GMOmethods database. He presented the forms used in 2011 for a similar scope. Participants suggested
    - including in the forms a check-list/thesaurus for modifications to facilitate their description; and a space for providing comments.
    - visualising in the questionnaire the information regarding the methods PCR reaction and amplifications conditions.
    - including validation status and accreditation of the methods.
    - incorporating the country code in the questionnaire to possibly ascertain national scenarios.

  It was requested that NRLs should obtain the survey results on methods employed by the labs in their country in order to comply with their national obligations. The Secretary thanked the SC members for their input and remarked that the survey had to be completed by summer to report the results to SANTE.

10. Dynamic Action List (DAL) of SC28 and End of Meeting

- The Secretariat presented the DAL, summarising the agreed follow-up actions (Annex 6), and the SC agreed on this list.
- The participants also agreed on organising the 23rd ENGL plenary on the 15th-16th of April 2015. It was suggested that at that occasion it should be decided if the practical implementation of Regulation (EC) No 619/2011 will need further support.
- Referring to an earlier side discussion, the Secretariat asked if a WG on detection, interpretation, and reporting at the LOD level was needed. No decision was taken but the participants proposed circulating to the SC members the commented draft of the DIR document, covering the LOD subject.

The Secretary thanked the participants and closed the meeting.

Annex 1: agenda

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

28th ENGL STEERING COMMITTEE MEETING

10-11 February 2015, building 58C, room12 a/b, Ispra, Italy

Draft Agenda

AP	Time	Topic	Documents in ENGLnet
	Day 1		
1.1	09:30	▪ Welcome, apologies	
1.2		▪ Approval of the agenda	Agenda
1.3		▪ Approval of the report of the SC27 meeting	Report SC27
1.4		▪ Review of Dynamic Action List (DAL SC27)	DAL SC27
1.5		▪ Update from SANTE	Presentation
	10:30	*Coffee Break*	
2	11:00	*Progress reports ENGL working groups*	
2.1		▪ AG SMV (Advisory Group on Selection of Methods for Validation): update	Presentation
2.2		▪ WG DIR (Detection Interpretation Reporting): final exchange	Final draft
2.3		▪ WG-ST (seed testing): final exchange	Final draft
	12:30	*Buffet lunch*	
3	14:00	*New activities*	
3.1		▪ Draft mandate for a WG on GMM detection	Draft mandate
3.2		▪ Draft mandate for a WG on unit of measurement	Draft mandate
3.3		▪ Draft mandate for a WG on digital PCR	Draft mandate
3.4		▪ Draft mandate for a standing WG on renewals	Draft mandate
	15:15	*Coffe Break*	
4	15:45	*ENGL topics*	
4.1		▪ Preparation of the 23rd ENGL plenary (15-16 April 2015)	Draft agenda
5		*Scientific / technical topics*	
5.1		▪ Update on CT (EU-RL GMFF)	Presentation
	17:15	*End of day 1*	
	19:30	*Social dinner at Hotel Belvedere, Ranco*	
	Day 2		
6	09:30	*Scientific / technical topics*	
6.1		▪ Pre-spotted plates, pilot and demonstration project (JRC)	Presentation
6.2		▪ Proof of Concept project on pre-spotted plates (JRC)	Presentation
	10:45	*Coffee Break*	
7	11:15	▪ Information on upcoming workshop on species/allergens detection (including proposal for experts) (M. Querci, JRC)	Presentation
8		▪ Information on 2nd international workshop on GMO analysis networking (M. Querci, JRC)	Presentation
9	12:00	*AOB (including date SC29)*	
10	12:15	*DAL SC28 and End of Meeting (12:30)*	DAL SC28
	12:30	*Sandwich lunch*	

Annex 2: mandate of ENGL Working Group on unit of measurement (WG-UoM)

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

Mandate for an ENGL Working Group on Unit of Measurement (WG-UoM)

Approved by the ENGL Steering Committee on 11th February 2015

Background

Quantification of genetically modified ingredients in food and feed is typically achieved by polymerase chain reaction (PCR). qPCR provides quantification (relative or absolute) of target sequences that are the molecules template for specific amplification.

The GM content is expressed as ratio between a given GM ingredient over the total of that ingredient in a sample [e.g. (GM maize/total maize)*100]. The quantification is typically done by calibrating the PCR assay with certified reference materials (CRM) characterised for a given GM %. As most of the CRM are certified for mass fraction, the results are to be expressed in mass fraction.

Over years a certain degree of confusion was generated and still remains on the unit of expression of the GM percentage when using qPCR, i.e. if this unit should be haploid genome copy number (as provided by Recommendation 787/2004) or mass. Hence the relation between the two units is relevant, particularly because new technologies, like digital PCR, allow the direct quantification of DNA copies without the link to a CRM.

This relation is crop specific and influenced by biological factors, e.g. sexual reproduction, grain morphology, ploidy, etc. While for some crops, e.g. maize and soybean, these factors have been sufficiently identified and described, for other main GM crops this is not the case.

Tasks

The WG shall prepare an overview of the state of discussion about the relationship between copy numbers and mass for the main GM crop species and prepare a reference list of conversion factors that should be used by test laboratories in order to ensure comparability of results.

The WG may decide to take on board additional tasks if agreed. It may also consider inviting non-ENGL experts to join the discussion.

Timeline

The working group is expected to meet before June 2015. At that first meeting it shall establish a detailed work plan to be presented to the ENGL SC on 17/18 June 2015. A final draft report shall be discussed at ENGL SC in February 2016 and the final report should be approved by the ENGL plenary in April 2016.

Annex 3: mandate of ENGL Working Group on digital PCR (WG-dPCR)

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

Mandate for an ENGL Working Group on use of digital PCR for GMO and other DNA analysis (WG-dPCR)

Approved by the ENGL Steering Committee on 11th February 2015

Background

Digital PCR (dPCR), in its different formats (chamber dPCR, droplet dPCR) is rapidly evolving in the area of DNA analysis. Digital PCR brings various advantages over the traditional real-time PCR, among which the large number of parallel repetitions (from few hundreds to thousands per sample), the possibility to conduct an absolute quantification without standard curves, and the reduced sensitivity to PCR inhibitors affecting DNA analysis.

During 2014 the ENGL has discussed the current application of dPCR to GMO analysis and identified that the technology has the potential to advance regulatory DNA analysis. Pros and cons were identified during an ENGL discussion day where experts also identified some issues to be solved to facilitate routine application of dPCR for DNA analysis.

Tasks

The WG should review the following issues, identify future needs and propose approaches to address them:

- Transferability of existing qPCR methods into a digital PCR format
- Accreditation (including in-house validation)
- Applicability to difficult matrices
- Applicability to analytical areas other than GM food/feed
- Definition and assessment of relevant method performance criteria
- Multiplexing

Other issues may be added to this initial list.

As a result, a document should be produced, addressing the various issues discussed and summarising relevant existing experience with dPCR, thus helping laboratories to decide if dPCR would meet their specific needs.

Timeline

The working group is expected to meet before the next ENGL plenary (15/16 April 2015) and to produce an initial draft for discussion by the ENGL Steering Committee of 17/18 June 2015.

A consolidated version should be presented in autumn 2015 at the ENGL plenary meeting.

Annex 4: mandate of ENGL Standing Working Group on Update of Methods (SWG-UpMeth)

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

Mandate for an ENGL Standing Working Group on Update of Methods (SWG-UpMeth)

Approved by the ENGL Steering Committee on 11th February 2015

Background

According to Regulation (EC) No 1829/2003, the authorisation of GMO for food and feed use is granted for ten years. The applicant has the possibility to apply for a renewal of such authorisation for another ten years.

The legal text does not clearly specify the requirements for the methods of detection in case of renewals, thus leaving room for interpretation.

The first requests for renewal will be submitted soon; potentially, about 10-15 applications for renewal will be submitted in 2015.

Technological advances may allow the identification of issues with the original validated methods which, at the time of first validation, could not be identified, e.g. reference gene copy number, sub-optimal reference gene assays, commercial unavailability of some reagents, real-time PCR machines not anymore on the market, delta-Ct methods not anymore implementable due to the lack of CRMs, etc.

Regardless the specific case of a renewal, the authorisation owner holds the responsibility of ensuring that at any time the detection method remains fit for purpose; thus the identification of flaws in the method should trigger a corrective action not only at the point of renewal but at any time during the authorisation.

Tasks

The standing WG should:

- Establish general criteria based on which for any issue identified with a validated method a specified action is recommended (e.g. in-house verification of some method parameters by the EURL GMFF, by a volunteering control laboratory)
- Review on a regular basis the methods of detection of GMOs for which a renewal is foreseen and provide advice to the EURL GMFF on their status (e.g. still fit-for-purpose, need for verification, need for re-validation etc.)

Timeline

The group is expected to meet at least once a year. The first meeting should take place within the first half of 2015. At this meeting a work plan for the coming 12 month will be established.

Annex 5: draft agenda of Workshop on Species/allergens Detection

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

1st Workshop on Species Identification

14 April 2015 (9:30 – 17:00)

DRAFT AGENDA

9:30 – 13:00 (coffee break 11:00 – 11:20)

Participants' registration

Opening

- Welcome and Opening Remarks (Joachim Kreysa)
- Framing the workshop (the context, why we are organising it, summary from ENGL break-our groups and Needs Assessment Report, fields of application)

Session 1: The use of molecular DNA-based species identification methods for regulatory purposes: fields of application, current status and challenges (chair: Joachim Kreysa)

- Species identification: introductory overview (available methods, qualitative vs. quantitative approach, performance requirements, standardization, case studies etc.) (Dietrich Mäde)
- Molecular methods in allergens detection: current status, challenges and needs (Marc de Loose)
- Molecular methods in food fraud (e.g. fish species substitution): current status, challenges and needs (Alain Maquet)
- Molecular methods for custom controls (e.g. endangered or hazardous species): current status, challenges and needs (Martijn Staats)
- Molecular methods for species identification in feed: current status, challenges and needs (Gilbert Berben)

13:00 – 14:30 Lunch

14:30 – 17:00 (coffee break 16:00 – 16:20)

Session 1: (cont.)

- Molecular methods for microbes identification: current status, available methods and opportunity of novel approaches, challenges and needs (Gabriele Casadei)

Session 2: The way ahead (chair: Joachim Kreysa)

- Panel discussion (with moderator illustrating key messages from the different presentations)

- Conclusions / Recommendations for future actions

Annex 6: Dynamic Action List (DAL) SC28

28th ENGL STEERING COMMITTEE ACTION LIST 11/02/2015				
ACTIONS	**Resp.**	**Timelines**	**Status**	**Comments**
ENGL GENERAL				
Circulate report of 28th ENGL SC	SEC	Mar-15	Open	
Organise 23rd ENGL Plenary in Ispra	SEC	Feb-15	Open	15-16 April 2015: send draft agenda ASAP
Organise 29th ENGL SC	SEC	Apr-15	Open	June 2015 17-18; send draft agenda by 30-4-2015
WORKING GROUPS				
Advisory Group on "selection of methods for validation"				
Advance with pentaplex validation	EURL	30/6/2015	Open	
organise a virtual meeting (VC) and a physical meeting	SEC	Apr-15	Open	June 2015 (VC) and November 2015
VARIOUS				
Organise meeting of the WG on ENGL procedures	SEC	28/02/2015	Open	Gimara, Lotte, Esther, by VC
Prepare questionnaire on methods used by NRLs 882	SEC	30/3/2015	Open	Answers ready by end of June 2015
Call for WG-dPCR members	SEC	15/02/2015	Open	
Call for WG-UoM members	SEC	15/02/2015	Open	
Call for WG-renewals	SEC	15/02/2015	Open	
Establish WG on DELOD	SEC	15/02/2015	Open	circulate the draft cutoff - commented draft
WG GMM	SEC			wait for the clarification on legal frame

ENGL: European Network of GMO Laboratories
SEC: ENGL Secretariat
EURL: European Union Reference Laboratory for GM food