EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

19th ENGL PLENARY MEETING

19-20 June 2013, Ispra, Italy

MEETING REPORT

1.1 Welcome

The President welcomed the participants and introduced the 19th ENGL Plenary meeting.

1.2. Approval of the Agenda

The President asked the participants if there were additional points that needed to be added to the agenda (annex 1). The agenda (Annex 1) was approved with some modifications on the sequence.

1.3 Minutes of 17th ENGL plenary

The ENGL Secretary presented the minutes to the participants and underlined the main points. No further comment was made.

1.4 Dynamic Action List (DAL) of 18th ENGL plenary

The ENGL Secretary presented the DAL and asked for comments. The 18th ENGL plenary DAL was approved.

1.5 Outcome of the 23rd ENGL SC meeting (17th September 2012)

The ENGL Secretary presented the minutes and underlined the main points.

The President suggested organising the next ENGL meeting the last week of November or the first week of December. He informed that some specific points regarding the NRLs appointed by Regulation (EC) No 1981/2006 could be added to the plenary discussion and that a separate workshop for the NRLs appointed according to Regulation (EC) No 882/2004 could be organised immediately before or after the plenary.

The President invited all NRLs 1981/2006 and 882/2004 in participating to the comparative testing organised by the EU-RL and specified that if in a country the areas of testing for official control are covered by the different NRLs that specialise in some tests (e.g. only food or only feed samples), these NRLs would not be required to perform analysis outside their area of specialisation.

Questions were raised on the progress and update of the ENGL website; it was specified that on the EU-RL web page a sub-site is dedicated to the ENGL, with basic information. The EU-RL is not in the position of dedicating further human resources to increase the amount of information provided in the website; if requested and if more efforts are deemed necessary, the SC could appoint a task force for deciding the subjects and the information to be uploaded.

2. Progress reports ENGL-groups

2.1 WG MPR (Method Performance Requirements)

The hardest task for the WG was the enlargement of the scope to Reg. (EC) No 882/2004 and the inclusion of new criteria for qualitative and taxon-specific methods without impairing the document readability.

New definitions covering the enlargement of the scope, qualitative and quantitative methods and the concept of modularity were introduced to apply the new criteria to each module.

The concept of specificity was enlarged to taxon-specific methods and screening methods while a new approach for assessment of robustness is proposed.

A new criterion, the probability of detection (POD), was introduced in conformity with the approach published by several authors. The document is almost finished and requires only some fine-tuning.

The final draft will be submitted to the ENGL for comments, then to Europabio and finally approved by the ENGL Steering Committee.

Hopefully it could be published by the end of the year. Criteria for multiplex methods (duplex only) need to be agreed and added to the document.

2.2 WG SPP (Sample Preparation Procedure)

The WG chairman informed that at the last meeting the 9th version of the document has been revised; the gaps were identified and covered.

A flowchart still needs to be added. In general all the points were addressed and sufficiently covered.

It was added that the tenth version will be ready for the next SC meeting in September 2013 for final comments and approval.

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

It was reminded that the group performed a survey among ENGL laboratories and identified five methods covering specific genetic targets.

For some targets many available methods are undergoing validation in Germany and therefore the list should be maintained as a dynamic reference.

The advisory group decided to upgrade the GMO matrix with the GMOs that were newly authorised or those under the LLP regulation.

To the request of including non-authorised GMOs into the matrix, it was reminded that the purpose of the project is to fill the gaps for detection of authorised GMOs and that CRMs for non-authorised GMOs are not available but added that the option could be further discussed.

The group will have a conference call for discussing the list of methods and their ranking. This should be based on priorities, but should also take into consideration the validation efforts undergoing in some member states.

The group is planning to submit a report proposing five methods for the SC meeting in September. If the proposal will be accepted a budget request could be submitted by the EU-RL for validating the methods in 2014.

A realistic expectation would be to validate two PCR methods and a reference gene assay with a modular approach giving priority to the Cry1Ab/Ac method.

2.4 WG DIR (Detection Interpretation Reporting)

Given the large-scale complexity of the mandate the task was divided in sub-groups and that the scope was broadened to include taxon and GMOs covered by the LLP legislation.

Cost-effective benefits were taken into consideration. He commented that the interaction with other WG was very effective.

A brief listing of all subjects covered in the report was presented. The main issue is the definition of cut-offs values, their expression unit and their transferability to other laboratories.

A survey in the WG was launched on the implementation of the cut-off approach in the laboratories using the LOD approach.

The chairman added that the next meeting will be organised in November 2013 to complete the document revision and that the report will be probably presented to the 26th SC and published by December 2014.

2.5 Technical guidance on flexible scope

The final document is published. The document is currently based on event-specific methods but could be extended to the screening elements in the future.

3. Scientific and technical session 1

3.1 Unit of measurement and conversion between mass/mass and copy/copy numbers: pragmatic guidance

Current guidance notes suggest reporting in the same unit of reference material used and avoid conversion between different units of measurements since the nature of the sample cannot be fully known.

It was further underlined that using digital PCR, the conversion factor for maize could range between 0.4 and 0.6 depending on the paternal or maternal GMO contribution as well as on the percentage of endosperm and embryo present in the sample.

It was remarked that while for soybean or maize the conversion factor is easier to establish, for other species its determination could be very complex.

It was further commented that it should be specified if the sample is derived from monoploid or alloploid genomes and that another level of complexity is added by the zygosity and ploidy level of the organism.

Participants remarked that discrepancies of results were observed in the ploidy and zygosity values of the CRMs and asked guidance on reporting results with DNA reference material.

The participants further requested information on the genetic make-up of the CRMs. It was replied that even if the CRM is fully known, the laboratory samples ploidy level may still not be known.

It was suggested to compile a table reporting all available information on genetic characteristics of all CRMs.

The participants further requested to invite an expert breeder as speaker at the next plenary meeting.

3.2 Issue of a positive control for CaMV and Agrobacterium tumefaciens (with regards to Chinese GM Rice Decision 2011/884)

The CaMV virus can be divided in families with different sequence variations. French laboratories have developed two methods (not published yet) to cover the different variations of the virus sequences and deposited the related plasmid constructs.

It was requested whether the two TaqMan methods previously published (Cankar et al. (2005) and Chaouachi et al. (2008) were included in the list by the ENGL Advisory Group.

Conversely it was added that nothing is known on Agrobacteria tumefaciens. Since the article Weller *et al.* (2002) has indicated that Agrobacteria strains may be T-nos negative because of sequence variations, it was suggested to use as a target the nos gene coding region which has functional more constrains for random mutations and it has been used only in one case for the construction of a GMO.

The President asked to share the relevant articles on the web.

3.3 An innovative integrated approach based on DNA walking to identify unauthorized GMOs – Marie Alice Fraiture (BE)

Since the number and diversity of GMOs is increasing, a surge of unauthorised GMOs is expected in the food and feed chains and their detection should therefore be part of a molecular biology platform strategy.

An inventory of transgenic rice events was build through searches in literature (500 articles) and noticed that the major part has been developed in Asia (China). New traits and a high diversity of vectors were described, with a predominance of vector pCambia (30%).

GMOs were classified based on the knowledge of the inserted sequences where class 1 corresponded to fully characterised inserts and class 4 to GMOs transformed with novel genetic elements not previously characterised (the majority belonged to level 3).

When unauthorised GMOs were suspected in the sample, a DNA walking strategy was used to characterise their junctions.

In addition to 35S and T-nos elements, reported in pCambia vector but also in GMOs, the T-35S element was used as a marker for the vector, which is not usually incorporated into GMOs.

Double nested PCR was used to avoid background effects. Three primers specific for the T-35S pCambia element were designed and the specificity, sensitivity and repeatability of the method assessed.

The approach was found to be fast, easy, not expensive and able to detect down to 0.1% of GM rice.

3.4 Use of CRMs and calibration

IRMM announced that it is offering a training course on proper calibration. A report on comparative testing highlighted indeed that 50% of participants still have a problem in expressing measurements of uncertainty and the results in correct units (incomplete and inconsistent manner).

The training will take place on the 21st-22nd of November at IRMM Geel Belgium and it will cover calibration, uncertainty estimation, traceability and reference material.

Deadline for registration was set for the 10th of October and the participation limited to a max of 30 trainees.

It was suggested to disseminate the information through the ENGLnet. After the training all the documentation will be made available on the ENGL web site.

4. Scientific and technical session 2

4.1 Update on the pre-spotted plates project (EU-RL GMFF)

An overview on the historical background and scope of the project was presented to the participants.

A survey launched in December 2012 suggested improving flexibility in terms of support and type of analysis.

As a result the EU-RL investigated plasticware compatibility and considered two types of supports, one being an update of the event-specific pre-spotted plates the other the development of 2x8 strips with element-specific and taxon-specific methods.

It was remarked that for the first updated version 17 new methods needed to be added to the platform.

The verification will be very complex since it will require the re-validation of all methods and therefore will be not be feasible for the current year.

For the second approach a new bioinformatics tool has been developed by the JRC to predict results for the different targets and each GMO analysed.

The second step will be the experimental verification of the methods.

The chairman asked for volunteer laboratories for experimentally testing the plates and providing feedback on the usefulness, cost effectiveness of the platforms.

4.2 In-house preparation of 0.1% m/m samples

For verification of analytical methods when implementing inter-laboratories validated methods, control positive material is required for determination of trueness.

For that purpose it is often necessary to produce intermediate concentrations of positive material from available CRMs. The procedure to produce the intermediate concentrations was presented.

It was remarked by the participants that the impact of the pipetting error could be considered quite significant.

The point of the stability of the solutions used, given the time required for their preparation, was raised; no data is yet available on the issue.

The EU-RL GMFF proposed testing the stability of the solutions in its laboratory.

4.3 Update on BTSF project (including the international workshop of GMO analysis networking)

The President provided a summary of the workshop on enlargement, international collaboration and capacity building organised in Ispra in April 2013 by the JRC.

The workshop was part of the "better training for safer food" program which aims at promoting high level of competence and harmonized approaches in control laboratories, safeguarding fair trade and safer food for third country consumers.

The particular aim of the workshop was to share the ENGL experience and support the establishment of regional networks.

Regional challenges and needs, prioritisation of actions and training needs were discussed and regional contact persons were nominated.

It was emphasized how the ENGL has functioned as a model. Volunteers for acting as experts trainers were requested.

5. ENGL matters

The JRC presented the main points of the revision of Regulation (EC) No 882/2004 and the results of the impact analysis performed on the GMOs activities of the JRC.

It was explained that in the new legal framework no major impact is foreseen for the function of the EU-RL GMFF.

The President envisioned the potential widening of the scope of the unit activities and the possibility that the expertise on DNA analysis may be transferred to other fields such as rare genetic diseases.

5.1 Update on GM glyphosate resistant wheat

The EU-RL GMFF presented the case on wheat contamination in Oregon where farmers noted volunteer plants resistant to glyphosate in the fields and asked the University of Oregon to further investigate the findings.

The USDA later involved in the investigation identified the event as being MON-71800-3.
The EU-RL GMFF underlined that only common white wheat was involved, whose import in Europe is very limited and added that it was assessing the end-point PCR method provided by Monsanto.

The EU-RL GMFF is trying adapting the method to real-time PCR instruments and is organising an in-house validation to test its performance.

The EU-RL GMFF announced an interim solution for allowing the laboratories to perform the test.

The document was prepared in conjunction with DG-SANCO and the ENGL.

The interim proposal suggests performing 35S, T-nos and CTP-CP4 specific methods to detect the GM event.

Since wheat has a large genome it was advised using as much DNA as possible to reach sensitivity close to an acceptable LOD.

5.2 Revision of Decision 2011/884 on Chinese rice

Commission implementing decision 2013/287/EU will enter in force in the second week of July.

New elements have been included into the EU-RL GMFF guidance document to establish Ct and Tm peaks cut-off for the SYBR Green methods.

A TaqMan method, validated in an international collaborative trial organised by the German Federal Office of Consumer Protection and Food Safety, has been incorporated into the guidance as a parallel analytical strategy in order to establish the equivalence of the two approaches.

The chairmen encouraged the participant in collecting data for advising policy assessments in October.

The participants remarked that the two methods target different sequences and that to perform the comparison they would need more information on the samples analysed.

6. New activities

6.1 Detection of stacked events (SmartStax case)

Strategies were discussed for distinguishing contamination of single events from the presence of the corresponding stacked events in a sample.

One possibility could be isolating intact nuclei from maize flower using cytometry, extract DNA from the single nuclei and PCR amplify.

6.2 Sequencing at the EU-RL GMFF

Objectives and the initial results of a project aiming at sequencing GM events and the corresponding stacks using the Junior sequencer provided by Roche were presented.

The goal of the project is to verify possible sequence differences between the single events and the same events combined in a stacked GMO.

The first experiment was the sequencing of the 1507 event; the sequence obtained resulted identical except for one base to the reference sequence.

Furthermore, a case on papaya was illustrated where from literature review it was possible to assemble in-situ the genome sequences of the transgenic events commercialized in Hawaii and Asia.

The information retrieved could be used for defining sequence strategies and develop specific detection methods to distinguish the different transgenic papayas.

7. DAL ENGL 19th and AOB

The ENGL Secretary presented the dynamic action list. It was announced that all presentations will be made available on the ENGL net.

It was asked if the participants were interested in having a paper copy of the Compendium in addition to the digital version provided on the EU-RL GMFF web page. No interest was shown for an extra printed version.

The 19th ENGL plenary meeting was closed. The 20th ENGL Plenary will take place on 4-5 December 2013.

Annexes:

- 1. Agenda

Annex 1: agenda

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Draft Agenda

Day 1: 19th June 2013

AP	Time	Topic	Documents
1.1 1.2 1.3 1.4 1.5	09:30	▪ Welcome ▪ Approval of the Agenda ▪ Approval Report 18th ENGL plenary ▪ Dynamic Action List (DAL) of 18th ENGL plenary ▪ Outcome of the 24rd ENGL SC meeting (March 2013)	 Agenda; Meeting report; DAL-ENGL18; Report SC24
	10:30	*Coffee Break*	
2 2.1 2.2 2.3 2.4 2.5	11:00	Progress reports ENGL-groups: ▪ WG MPR (Method Performance Requirements) ▪ WG SPP (Sample Preparation Procedure) ▪ AG SMV (Advisory Group on Selection of Methods for Validation) ▪ WG DIR (Detection Interpretation Reporting) ▪ Technical guidance on flexible scope	 MPR update SPP update AG SMV update DIR update AP2.5 in ENGLnet
	12:45	*Buffet Lunch*	
3 3.1 3.2 3.3 3.4	14:00	Scientific and technical session 1: ▪ Unit of measurement and conversion: pragmatic guidance ▪ Issue of a positive control for CaMV and Agrobacterium (with regards to Chinese GM Rice Decision 2011/884) ▪ An innovative integrated approach based on DNA walking to identify unauthorized GMOs – Marie Alice Fraiture (BE) ▪ Use of CRMs and calibration (P. Corbisier, IRMM)	 AP3.1 in ENGLnet AP3.2 in ENGLnet Presentation Presentation
	15:45	*Coffe Break*	
4 4.1 4.2 4.3	16:00	Scientific and technical session 2: ▪ Update on the Pre-spotted plates project (M. Querci, EU-RL GMFF) ▪ In-house preparation of 0.1% m/m samples (L. Hougs, DK) ▪ Update on BTSF project (including the international workshop of GMO analysis networking) (M. Querci, EU-RL GMFF)	 Presentation Presentation Presentation
	17:30	***End of day 1***	
	19:30	**Social dinner at Hotel Europa - Ispra**	

Day 2: 20th June 2013

AP	Time	Topic	Documents
5 5.1 5.2	09:30	ENGL matters ▪ Update on GM glyphosate resistant wheat ▪ Revision of Decision 2011/884 on Chinese rice	
	10:45	*Coffee Break*	
6 6.1 6.2	11:15	New activities ▪ Detection of stacked events (SmartStax case) ▪ Sequencing at the EU-RL GMFF (A. Patak, M. Petrillo, EU-RL GMFF)	Presentation
7	12:30	DAL ENGL 19th and AOB	
	12:45	*End of meeting and buffet lunch*	