EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate F - Health, Consumers and Reference Materials
Food & Feed Compliance

12TH WORKSHOP OF NATIONAL REFERENCE LABORATORIES UNDER REGULATION (EC) NO 882/2004 AND 26TH ENGL PLENARY MEETING

21-22 September 2016

Ispra, Italy

Meeting Report

SESSION: NATIONAL REFERENCE LABORATORIES AND OFFICIAL CONTROL

1. WELCOME AND APPROVAL OF THE AGENDA

The Chair welcomed the participants and the guest Dr. Kazumi Kitta, of the National Food Research Institute of Japan. The agenda (see Annex 1) was approved without modifications.

2. APPROVAL OF THE 11TH WORKSHOP REPORT

The report of the last meeting was adopted without modifications.

3. Tour de table: issues/opinions/training needs of NRLs

The participating NRLs designated by their Member States under Regulation (EC) No 882/2004 were invited to summarise their control activities in the past year. Most NRLs are analysing 200-300 official control samples of food/feed/seed per year; some smaller Member States analysed fewer samples, some larger ones more samples, up to 3700 samples. Most or all of the food and seed samples analysed were found to be negative for GMO presence, and most of the feed samples were only containing authorised GMOs. A few GM-positive papaya food samples were identified during last year, and some incidences of GM rice.

A major issue mentioned by many NRLs is the increasing workload due to the accumulating number of GM events to be tested. There is therefore a pertinent need for multiplexing approaches to cope with this workload, either in the form of more efficient screening assays, multiplex PCR or pre-spotted plates (PSP). Several NRLs are also active in other domains, e.g. allergen screening, developing a method for GM salmon, or detection of GMM.

With regard to training needs, another training in dPCR was mentioned by several NRLs, and further suggestions included NGS, implementation of multiplex PCR, DNA extraction, use (and production) of PSP, and the use of CRMs at 0.1 m/m % (for implementation of Regulation (EU) No 619/2011). The JRC concluded that a new training on dPCR will be organised in 2017, as well as a workshop/training on DNA extraction experiences (discussed under point 15.3).

4. UPDATE ON COMPARATIVE TESTING ACTIVITIES

W. Broothaerts (JRC) gave a summary of Comparative Testing (CT) round CT 01/16 and the observed reasons for obtaining an unsatisfactory performance by some of the laboratories. The next CT round

02/16 was announced. The NRLs were requested to reflect on the test items to be used in future CT rounds and the performance for which they would like to be tested. Most NRLs appreciated the balance between food and feed samples and between more complex and easier matrices.

The number of GM events to be tested should be limited and the type of crops and events should be in line with market prevalence. Events falling under Regulation (EU) No 619/2011 should not be included too often (and not too soon after validation of the method by the EURL GMFF) as these events are rarely found in control samples.

The EURL GMFF announced that the functional mailbox for CT was changed into jrc-comparative-testing@ec.europa.eu.

5. PRE-SPOTTED PLATES: STATUS OF ACTIVITIES AND WAY FORWARD

F. Gatto (JRC) gave a presentation on the status of the PSP project. There is a large interest among NRLs for implementation of such plates in the routine control activities, and DG SANTE continues to support the project in order to improve the harmonisation of GMO testing.

Some NRLs have started to develop their own PSP, and a dedicated training on this would be welcomed. Guidance may be needed for the incorporation of PSP under the accreditation.

6. AOB AND CONCLUSIONS

The chairman asked participants to communicate any issues with the revised guidance on GM rice screening. In line with the needs mentioned, the EURL GMFF will consider organising another training workshop on dPCR next year.

The detection method for GM salmon will be further monitored. Any need for guidance on the implementation of multiplexing in the laboratory, and proposals for a new research project under Horizon 2020, will be discussed during the next Steering Committee meeting.

Issues with CRM availability at a low level will need to be reflected to the EURL GMFF, e.g. through a discussion forum that will be set up on ENGLnet.

SESSION: NRL/882 WORKSHOP AND 26TH ENGL PLENARY MEETING

7. WELCOME AND APPROVAL OF THE AGENDA

The Chair welcomed the participants; the agenda was approved without modifications.

8. APPROVAL REPORT 25TH ENGL PLENARY

The report of the last meeting was approved without changes.

9. DYNAMIC ACTION LIST OF 25TH ENGL PLENARY

The Secretary reviewed the open points of the action list.

10. OUTCOME OF THE 31TH ENGL STEERING COMMITTEE MEETING (JUNE 2016)

The Secretary summarised the main points discussed by the Steering Committee (SC) during the last meeting. A proposal was made (by the Advisory Group on Selection of Methods for Validation) to validate a method for the detection of GM salmon and one for *B. subtilis*-overproducing vitamin B2.

With regard to setting up new working groups, the SC had concluded that it was too premature to start a WG on New Breeding Techniques, but to continue monitoring the evolution in this field.

On DNA extraction, a survey was proposed through ENGLnet to summarise the experiences from ENGL members on the application of extraction methods on different matrices.

Further, the upcoming JRC re-organisation was announced.

In the EURL GMFF validation reports a warning will be included in case deviations near the LOQ were observed during validation and further monitoring and signalling of any problems observed would be requested.

11. UPDATE FROM DG SANTE (I. CIABATTI)

An update was given on the US notification on GM wheat found in a farmer's field in July 2016 and the steps that were taken at EU level. C. Savini (JRC) and K. Kitta (Japan) clarified the experimental work that has been carried out so far.

The background on the implementation of the recently updated EURL GMFF guideline for the submission of DNA sequences by applicants was explained.

Regarding the EU notification of GM oilseed rape in the UK from 2015, no further findings were observed. The Commission requested the Member States concerned to provide their monitoring plans and the relative results.

An overview of the RASFF notifications submitted in 2016 was provided: a reduction in the number of notifications on GM rice was noted in 2016; Six new notifications of unauthorised GM papaya were also noted in 2016 up to now.

The discussions on synthetic biology continue under the CBD/Cartagena Protocol and at EU level. Main issues concern an operational definition of synthetic biology, potential positive and negative impacts, risk assessment methodologies and regulatory aspects.

12. JRC REORGANISATION (H. EMONS)

H. Emons (JRC) presented an update on the JRC reorganisation and the implications for the EURL GMFF and the ENGL. Due to the continued budget restrictions, there is a need for a more efficient use of resources to perform the tasks required. Possible networking in other fields such as allergens and species identification in general should be investigated outside the ENGL mandate.

13. PROGRESS REPORTS FROM ENGL WORKING GROUPS

13.1. Advisory group on the selection of methods

N. Roosens (ISP, BE) provided an update of the actions and discussions.

The report on the t-35S pCAMBIA ring trial has been drafted and the method will shortly be added to the GMOMethods database and published.

Validation of a pentaplex method for GM soybean, to be conducted by the JRC, is under planning.

A survey has been proposed on the use of taxon-specific methods, which should result in a table also incorporating the information on the copy number of the targets.

A similar table would be developed on the taxon-specific targets for animals.

It was further suggested to consider a dPCR method for validation through a ring-trial.

13.2. WG on the Update of Methods

U. Marchesi (IZSLT, Italy) presented the progress of this WG on behalf of R. Onori (ISS, Italy).

The goals of this WG are to establish general criteria based on which, for any issue identified with a validated method, a specified action is recommended, and to review on a regular basis the methods for which a renewal is foreseen and to provide advice to the EURL GMFF on their status (e.g. still fit-for-purpose, need for verification, need for re-validation etc.).

The issues identified in validated methods have been analysed and recommended actions proposed. It is suggested that the EURL GMFF would provide a template for collecting the existing validation data on method modifications and perform a meta-analysis on this. This would inform on the need for additional validation efforts.

A need was emphasised to revise the validation reports for those maize events using *adh1*-70 bp (MON863, GA21, NK603). The Chair agreed on the need for a stronger message on this issue.

13.3. WG Digital PCR

S. Pecoraro (LGL, DE) reviewed the draft report prepared by this WG on the applications of dPCR in various fields and providing recommendations for the application of dPCR. The comprehensive report is almost ready. The ENGL members are eagerly waiting to see the final version of this report.

13.4. WG on the Unit of Measurement

P. Corbisier (JRC) explained how comparable and metrologically traceable results can be established from qPCR or dPCR measurements, if the data are anchored to the certified value of a reference material. To do so, one option is to establish a conversion factor specific for each plant species, or to determine the copy number of all existing CRMs from both IRMM and AOCS. A small collaborative trial using dPCR is required to determine these factors. The report of this WG summarises the legal background on the unit of measurement, the available techniques for GM detection and quantification and the units used for method validation and CRM certification. The report will be finalised in early 2017.

13.5. WG on Method Verification (Mvrf2)

J. Zel (NIB, SI) presented the progress made on behalf of L. Houghs (FVST, DK). The existing guidance on the verification of methods has been updated and will be first reviewed by the SC, then presented to the ENGL Plenary.

13.6. WG on ENGL Procedures

M. Mazzara (JRC) stated that this WG has not really taken off yet, but may be launched now. The aim is to review the ENGL procedures and where needed, propose modifications.

14. SCIENTIFIC AND TECHNICAL SESSION 1

14.1. Update from the JRC Advisor for the Bioeconomy (J. Kreysa)

J. Kreysa (JRC) presented his views on the evolutions occurring in bioeconomy. The definition of this concept differs between the US and the EU, but it is clear that the key driver is the availability of biomass.

The big issue for the future is the foreseeable competition for biomass. This will require an increase in productivity, for which the developments in genomics will be key. Gene-editing, New Breeding Techniques, Synthetic Biology, animals as bioreactors are being developed and will enter the global markets (and the EU) in the future.

Whatever legal system of regulation is applied, methods for monitoring (detection, identification, quantification) will remain relevant and the ENGL should continue to play an authoritative role in this field.

14.2. Technical study to assess the need for harmonisation of sampling and analysis methods for GM material in food (I. Ciabatti)

I. Ciabatti (SANTE) presented the outcome of a study performed in 2015 by a consultant on behalf of DG SANTE to investigate the need to harmonise sampling and analysis methods for official controls of pending and expired GM material in food.

The findings collected from different stakeholders revealed divergent views, but in general it was concluded that there is no clear need for a policy action aimed at improving harmonisation in this context .

The final report of this study is now available on DG SANTE website.

Discussion took place on the possibility to improve harmonisation at technical level e.g. by promoting the use of JRC Pre-Spotted Plates.

15. SCIENTIFIC AND TECHNICAL SESSION 2

15.1. Enlargement and capacity building project

M. Querci (JRC) presented an overview of the JRC contribution to global harmonisation of GMO analysis over the past years. She mentioned that the capacity building activities covered 36 training courses on GMO analysis with participation of scientists from more than 300 laboratories. This activity will be continued through the JRC Unit F.7 - 'Knowledge management'. The support of ENGL is considered crucial and would need to be formally structured rather than be on demand. She proposed the development of an electronic platform where the JRC may act as a facilitator to transfer the regional request to ENGL experts.

15.2. The WAEMU Regional biosafety program and the Community regulation project

A delegation of West-African experts joined the technical and scientific session 2 as observers. S. Kina presented the projects on the management of risks related to plant biotechnology within the 8 countries of the West-African Economic and Monetary Union (WAEMU). The objective is to protect biodiversity and establish a common legal framework in line with the Cartagena Protocol to prevent biotechnological risks and to provide the tools for GMO analysis (guidelines, harmonized methods, provision of equipment, training, and networking). Different countries are at different levels of ratification of the Cartagena protocols; Burkina Faso is the only country with commercial GMO cultivation (Bt Cotton). The goal is that within 5 years each of the countries should be able to screen for and identify and quantify GMOs. The support from the JRC is highly appreciated.

15.3. Discussion on a training workshop on DNA extraction

M. Burns (LGC, UK) introduced the topic on DNA extraction from food and feed and pointed to the difficulties that laboratories regularly signal as a recurring issue during NRL/ENGL meetings. He presented an overview of a basic training designed by LGC on DNA extraction. The JRC proposes to organise a workshop on DNA extraction with presentations by experts, but also largely capitalising on the shared knowledge and collective expertise existing within the ENGL. The idea was supported by many ENGL members. Before the event, a summary table should be composed to collect best practices based on food/feed matrixes (to be initiated by ENGL Secretariat). The training will be organised at (the beginning of) 2017.

15.4. Available high-tech strategies for monitoring of unauthorised GMO on the EU market

M.A. Fraiture (ISP, BE) presented the results of the project in which three partners developed a new approach for unauthorised GMO detection utilising genome walking and NGS and then the analysis of the generated sequences against a database of EU authorised GMOs. In the first step they used primers specific for genetic elements identification combined with degenerate primers anchored on key targets. This approach allows a wide-scale and more comprehensive monitoring of market samples. See: Fraiture *et al.*, 2016, *Trends in Food Sci and Technol.* 52: 66-79.

16. SCIENTIFIC AND TECHNICAL SESSION 3

16.1. Multiplex dPCR method for GM soybean

A. B. Košir (NIB, SI), presented their work on the development of a 6-plex, 7-plex and 11-plex ddPCR method for the quantification of GM soybean events. The multiplex approach should reduce the workload of official control laboratories since half of the EU authorised soybean GMOs cannot be detected with methods targeting conventional screening elements. The performance of the multiplex methods was in line with the performance acceptance criteria established for multiplex methods within the Decathlon project and in general with the ENGL criteria. The cost of the ddPCR approach was comparable to real-time PCR when the screening step gave negative results, but it was cheaper in case the product was positive. ISO 17025 accreditation of the 6 and 7-plex methods is ongoing.

16.2. Validation of digital PCR methods

Ph. Corbisier (JRC) presented the results of the in-house validation of a ddPCR method for quantification of DNA copy numbers and the critical performance characteristics that have been analysed. The major sources of variability contributing to the measurement uncertainty were analysed and quantified. It was concluded that the extensiveness of the method validation depends mainly on the intended use of the method and on the acceptable level of measurement uncertainty.

16.3. On-site PCR for GMO detection

In order to speed up GMO testing on fresh samples from fields or supermarkets the performance and characteristics of a number of portable PCR instruments were evaluated. These instruments are

mainly suitable for positive/negative analysis due to the small number of reactions and the small sample sizes (risk of inhomogeneity).

17. BREAK-OUT GROUPS

Three topics were proposed for discussion in smaller groups.

18. REPORTS OF BREAK-OUT GROUPS

18.1. Future analytical challenges for the ENGL resulting from NBT and Synbio products

The main conclusion from the break-out discussion was that it is premature to initiate further actions until the legal situation has been clarified. The Chair remarked that ENGL could be of great value in addressing the issue.

18.2. Handling the increasing number of methods: how laboratories manage the challenge

This is a relevant issue for many laboratories. A number of solutions were proposed both at the level of analytical strategy and organisation. For the first level prioritisation of GMO testing was suggested based on a risk-based analysis, more screening, no unnecessary further testing for samples with high Cq and (adaptable) multiplex approaches. For the second level members proposed to increase specialisation of control laboratories for either screening or quantitative event-specific analysis (networking) or to enlarge the analytical capacity of a smaller number of laboratories. The ENGL may support these improvements by making the PSP available, keeping the GMO-matrix database updated, providing guidance on multiplexing, and collecting data from official control to fine-tune the risk analysis based approach.

18.3. Method dynamic ranges and units of conversion

Although different laboratories use different approaches when establishing the calibration range of a new method, the opinion of the seven participants to the discussion was that there is no real need for additional guidance on this issue. Most laboratories report their calculations in mass, however, some convert the certified value of the CRM to copies, and then back to mass using the same conversion factor. The EURL GMFF validation reports are mainly consulted for the zygosity factor reported. Members expressed the need of defining conversion factors for CRMs by dPCR techniques.

19. MEETING CONCLUSIONS

The Chair thanked the participants for the lively and useful discussions.

20. AOB AND DAL 26TH ENGL

The Secretary reviewed the updated dynamic action list (Annex 2).
He informed that for the following year the EURL GMFF will organize two workshops on DNA extraction and dPCR, respectively. He remarked that volunteers are needed for chairing the Advisory group on the selection of methods and on ENGL procedures.
The Secretariat informed also that the issue of an ENGL guidance on multiplexing will be included in the agenda of the next SC meeting. He finally proposed to strengthen the role of ENGL in research projects.

Annex 1: agenda

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12th WORKSHOP OF GMO NATIONAL REFERENCE LABORATORIES
REGULATION (EC) No 882/2004 and 26th ENGL PLENARY MEETING

21-22 September 2016, Ispra, Italy

Draft Agenda

Day 1: 21st September 2016

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 11th workshop report ▪ Tour de table: issues/opinions/training needs from NRLs (each NRL is invited to report orally) Follow-up discussion	Draft agenda Report Oral input from NRLs
	10:45	*Coffee Break*	
4 5 6	11.15	▪ Update on comparative testing activities (W. Broothaerts, EURL GMFF) ▪ Pre-spotted plates: status of activities and possible way forward (F. Gatto, EURL GMFF) ▪ AOB and conclusions	Presentation Presentation Presentation
	12:45	*End of session NRLs*	
		Buffet lunch	

Day 1: 21st September 2016

Session: NRL 882 WS and 26th ENGL Plenary meeting

AP	Time	Topic	Documents
7	14:00	▪ Welcome and approval of the Agenda	Draft agenda
8		▪ Approval Report 25th ENGL plenary	Report
9		▪ Dynamic Action List (DAL) of 25rd ENGL plenary	DAL ENGL25
10		▪ Outcome of the 31st ENGL SC meeting (June 2016)	Report SC31
11		▪ Update from SANTE (I. Ciabatti, SANTE)	
12		▪ JRC reorganisation (H. Emons, JRC)	Presentation
13		*Progress reports ENGL working groups:*	
13.1		▪ AG SMV (Advisory Group on Selection of Methods for Validation) - N. Roosens	
13.2		▪ WG Update of Methods – U. Marchesi	
13.3		▪ WG Digital PCR – S. Pecoraro	
	15:30	*Coffee Break*	
	16:00	*Progress reports ENGL working groups:*	
13.4		▪ WG Unit of Measurement – P. Corbisier	
13.5		▪ WG-Mvrf2 (Method Verification) – J. Zel	
13.6		▪ WG-Proc (ENGL Procedures) - M. Mazzara	
14		*Scientific and technical session 1*	
14.1		▪ Update from the JRC Advisor for Bioeconomy (J. Kreysa, JRC)	Presentation
14.2		▪ Technical study to assess the need for harmonisation of sampling and analysis methods for GM material in food (I. Ciabatti, SANTE	Presentation
	17:30	***End of day 1***	
	19:30	**Networking dinner at "La Playa" Cadrezzate**	

Day 2: 22nd September 2016

Session: NRL 882 WS and 26th ENGL Plenary meeting

AP	Time	Topic	Documents
15	09:15	*Scientific and technical session 2*	
15.1		▪ Enlargement and capacity building project (M. Querci, JRC)	Presentation
15.2		▪ The WAEMU Regional Biosafety Program and the Community Regulation Project (Saidou Kina)	Presentation
15.3		▪ Discussion on a training workshop on DNA extraction to be organised by the EURL GMFF	
15.4		▪ "Available high-tech strategies for monitoring of unauthorized GMO on EU market" (Marie-Alice Fraiture, ISP, BE)	Presentation
	11:00	*Coffee Break*	
16	11:30	*Scientific and technical session 3*	
16.1		▪ Multiplex dPCR method for GM soybean (Alexandra Bogožalec Košir, NIB, SI)	Presentation
16.2		▪ Validation of digital PCR methods (P. Corbisier, JRC)	Presentation
16.3		▪ On-site PCR for GMO detection (F. Debode, CRA-W – BE)	Presentation
	12:45	*Buffet lunch*	
17	14:15	*Break-out Groups* 1) Future analytical challenges for the ENGL resulting from NBT and Synbio products 2) Handling the increasing number of methods: how laboratories manage the challenge 3) Method dynamic ranges and units conversion	 Mandate Mandate Mandate
	15:30	*Coffee Break*	
18	16:00	*Reports of break-out groups*	
19	16:40	*Meeting conclusions*	
20	16:50	*AOB* and DAL ENGL 26th	
	17:00	***End of meeting***	

Supporting documents available at:
https://englnet.jrc.ec.europa.eu/26th%20ENGL%20Plenary%20and%2012th%20WSNRLs882/default.aspx?InstanceID=1

Annex 2: dynamic action list (DAL)

26th ENGL PLENARY ACTION LIST 22/09/2016				
ACTIONS	**Resp.**	**Timelines**	**Status**	**Comments**
ENGL MEETINGS				
Make available on ENGLNet report and presentations of 26th ENGL Plenary	SEC	Oct-16	Open	
ENGL WORKING GROUPS				
WG Detection Interpretation Reporting				
Publish final report	SEC	30/10/2016	Open	
WG UoM				
Final draft to ENGL members	SEC	31/10/2016	Open	
WG dPCR				
Final draft to the SC	SEC	31/12/2016	Open	
WG Mvrf2				
Send doc to SC for comments	SEC	30/09/2016	Open	
WG Procedures				
Prepare draft procedures	SEC	Oct-16	Open	
WG UpMeth				
Organise 4th meeting	SEC	30/09/2016	Open	Date to be decided in consultation with WG
Deadline to other WGs for comments	SEC	30/09/2016	Open	send email
Template for raw data submission to JRC	EURL	31/12/2016	Open	
Final draft to the SC	WG + SEC	31/12/2016	Open	
AG Method Selection for Validation				
Contact Decathlon to get info on new methods and on possible members	SEC	30/09/2016	Open	
Consider/seek the submission of a dPCR method	SEC + AG	30/09/2016	Open	soybean ddPCR developed by NIB?
Finalise the list of taxon-specific modules	AG	30/11/2016	Open	
Review existing methods for endogenous genes for animals	AG	31/12/2016	Open	To be started after finalisation of the task above
OTHERS				
Consider a letter from the EURL to ENGL members on the establishment of a network on "species identification"	SEC	31/12/2016	Open	proposed during the meeting
Provide table on DNA extraction methods	SEC	31/12/2016	Open	webform; wait till the workshop on DNA extraction
Organise training on dPCR for NRLs 882	EURL	30/3/2017	Open	Summer 2017?
Fish/salmon	EURL	31/12/2016	Open	put together info from SANTE and NRLs (DE, BE..)
ENGL Guidance on multiplexing	SEC	31/12/2016	Open	Put on the agend of the SC Feb 2017
Research project: ENGL role	SEC	31/12/2016	Open	Put on the agenda of the SC Feb 2017
Does the ENGL doc on UGM need revision?	SEC	30/11/2016	Open	On the agenda of the SC Feb 2017
Organise workshop on DNA extraction	EURL	31/10/2016	Open	Date to be decided
Position document identyfying issues with 619 and suggesting possible solutions, including approach for MU	EURL/ENGL	31/12/2016	Open	electronic forum. Moderator?