



REPORT ON THE 27th ENGL PLENARY MEETING 6-7 APRIL 2017

1. Welcome

The Chairman welcomed the participants (Annex 1) and presented the agenda of the meeting

2. Approval of the Agenda

The agenda (Annex 2) was approved.

3. Approval Report 26th ENGL plenary

The report previously circulated for comments was endorsed without modifications.

4. Outcome of the 32nd ENGL SC meeting (February 2017)

The Secretary summarised the main outcome of the ENGL SC meeting.

5. Dynamic Action List (DAL) of 26th ENGL plenary

The Secretary reviewed the open point of the list. He reminded that a moderator is missing for the forum on "issues with Regulation (EU) No 619/2011".

6. Update from SANTE (I. Ciabatti)

I. Ciabatti gave an update on the Commission's request to the Scientific Advice Mechanism (SAM) on new techniques in agricultural biotechnology. An explanatory note will be provided by the experts in the coming weeks¹.

She further informed that a request was addressed by the Court of a Member State to the European Court of Justice for defining the legal status of organisms produced by new mutagenesis techniques. A final opinion may be available by the first half of 2018.

SANTE announced that the Commission is also organising a high level conference on "Modern biotechnologies in agriculture: paving the way for responsible innovation" on the 28th of September meant to promote an open and informed debate on the topic with all relevant stakeholders.

She informed that the 13th meeting of the Conference of the Party (COP) - Convention on Biological Diversity (CBD) was held in Mexico in December 2016. One of the items that triggered more discussions was synthetic biology. The proposal of a quite broad definition of synthetic biology which is in line with the one provided by the scientific committees of the Commission did not obtain a full endorsement. The COP-CBD took note that living organisms developed through existing applications of synthetic biology could be considered in line with the current definition of LMOs under the Cartagena Protocol but recognized the need for

¹ At the time of issuing this report, the SAM explanatory note has been published and is available at https://ec.europa.eu/research/sam/pdf/topics/explanatory_note_new_techniques_agricultural_biotechnology.pdf

discussing future applications. It was decided to extend the mandate of the Ad Hoc Technical Expert Group and to establish an open-ended online Forum. The COP asked the Parties to submit information on synthetic biology products and to nominate experts for the online forum. This should feed the discussions of the Ad Hoc Technical Expert Group which would provide recommendations for the COP future meeting in Egypt in 2018.

I. Ciabatti reported also that a new draft guidance document on the characterisation of microorganisms used as feed additives or as production organisms prepared by EFSA FEEDAP Panel is being reviewed by other panels of EFSA and will go through public consultation. In parallel an ongoing revision of Regulation (EC) No 429/2008 concerning the application for feed additives is considering more detailed requirements for the applicants.

Participants asked if the methods for detecting Genetically Modified Microorganisms (GMM) could be made publicly available, it was explained that more detailed requirements are being considered in the amendment of the Regulation so that the applicant will have to use analytical methods sensitive enough to verify the compliance of the product. The Commission, however, does not have a legal basis for requesting the provision of the specific method of detection.

It was remarked that the ENGL could play a role in the discussion on detectability of new organisms generated with new techniques. SANTE explained that the ENGL is a very important network, but that the Commission decided to have a more open debate on this issue looking into the future from a broader perspective.

7. Collection of issues from ENGL members

Orange petunias were observed on the market for sale during summer 2016 in Finland. In 1987 a mutant petunia plant with pink flowers had been transformed with a maize gene and to display an orange colour. At the moment petunia flowers with orange colours are being grown by producers. Suggestions for determining if the flowers are genetically modified were requested. Members proposed identifying by PCR the presence of border sequences, genetic elements or constructs not naturally occurring and evaluating their ratio with species-specific genes copies. It was asked whether information on a petunia reference gene could be provided.

The Chairman remarked that other two topics raised by participants, namely adaptation of GMO methods to ddPCR and species identification, will be addressed in the scientific topic discussions of the current ENGL meeting and at the ENGL plenary of September, respectively. The latter topic will be covered by a presentation of a JRC colleague from the Food Fraud Unit.

An ENGL laboratory received the request of verifying the presence of GM cotton in T-shirts made with organic cotton. The laboratory tested different samples. It was able to extract 50 copies of DNA and performed PCR analyses. It was asked whether other laboratories were interested in collaborating on the DNA extraction optimisation.

M. Burns (UK) summarized the feedback received from a survey launched among UK control laboratories. The replies indicated that the laboratories would appreciate assistance and support on GMO screening and receiving information on bioinformatics decision support tools, dPCR, NGS sequencing and DNA extraction from difficult matrices.

The Chairman commented that all these points are going to be discussed at this ENGL Plenary. The Secretariat commented that a presentation and a JRC Technical Report on using the EURL GMFF online bioinformatics resources were already available on the related website at <http://gmo-crl.jrc.ec.europa.eu/jrcgmmatrix/>. Concerning the screening approaches he indicated

that the EURL GMFF is committed on making the PSP available and is working in setting up the necessary arrangements.

8. Progress reports ENGL working groups

8.1 AG SMV (Advisory Group on Selection of Methods for Validation) (N. Papazova, BE)

The Secretary informed that Nina Papazova volunteered to replace Nancy Roosens as chair of the working group and that Nancy will remain as a member. New members have also joined the group. He informed that the 35SpCambia method has been validated and that the multiplex method selected by the WG is under validation. The Secretary reported that in 2016 no method had been submitted while in 2017 two new methods, a qPCR for detecting potato and a multiplex digital PCR for detecting soybean GM events, have been proposed for validation. A meeting will be organized to discuss the two methods submitted.

8.2 WG on unit of measurement (P. Corbisier, JRC)

The Chair announced that the document on the unit of measurement has been published (see http://bookshop.europa.eu/is-bin/INTERSHOP.enfinity/WFS/EU-Bookshop-Site/en_GB/-/EUR/ViewPublication-Start?PublicationKey=KJNA28536).

8.3 WG on digital PCR (S. Pecoraro, DE)

The Chair informed on the progress of the work and commented that in his opinion would be interesting to develop an Annex on dPCR platforms and method verification, but cautioned on the additional time required. These subjects are already covered in the text, but could be treated in the Annex as more in depth experimental recommendations for laboratories. Participants supported the proposal and asked whether performance requirements were also discussed in the document. The Chair explained that the criteria are treated only in those cases where they are different from those of the qPCR methods. He informed that the main document was almost ready for submission to the SC and that a revised Annex will be provided to the group for input.

8.4 WG on Method Verification (L. Hougs, DK)

F. Gatto announced that the group finalized a first draft and further addressed most of the comments. It is foreseen having another meeting for finalizing the document before summer. It was suggested leaving the old version of the document on the EURL GMFF website as a reference for laboratories and publishing the new version with a date so that it could be clearly distinguished.

8.5 WG on update of methods (R. Onori, IT) (presentation on way forward and discussion)

It was explained that the document covers also modifications performed at single laboratory level which could be used for studies of robustness or practicability issues. It was proposed to include in the document new definitions of PCR measurement method, PCR measurement procedure and modified/updated PCR procedure. The Chairman suggested referring to ISO standards or international definitions. The Chairman proposed creating an online forum for this purpose. The group needs only one additional meeting to consolidate the document and finalize the draft.

8.6 WG on ENGL Procedures (M. Mazzara, JRC)

The document should be ready and approved by the end of the year.

9. Scientific and technical session

9.1 Food allergens: Current and future criteria for measurement methods (G. O'Connor, JRC)

The speaker highlighted the importance of harmonisation of food allergen measurements for providing consistent labelling of allergenic ingredients in food products, especially in cases of unintentional presence. Statistics indicate that 17 million Europeans suffer from food allergies. Reliable and harmonised labelling approaches in the EU could help preventing fatal allergic reactions and decrease unnecessary food product recalls. EU labeling legislation requires that any allergen used as ingredient is declared on the label while for an allergen unintentionally present the producer can provide the information on a voluntary basis. Since the publication of an EFSA document, threshold levels are now provided for milk and egg allergens in comparison to a reference value based on the amount used. The choice of the peptide for measuring the allergen is critical, (i.e. for milk and eggs alone 40 different targets could be identified), while there is a broad performance variability for the detection kits available on the market. The speaker informed that a workshop for establishing an infrastructure for the harmonization of food allergen measurements in the EU will be organized by the JRC on 13 & 14 June 2017 in Geel, Belgium.

9.2 Experience with accreditation of dPCR methods (D. Dobnik, NIB)

The speaker informed that following some consultations with the auditors the Slovenian accreditation body agreed to consider the dPCR method as a 'small deviation' from the original qPCR assay. Consequently, the laboratory did not perform a full validation but a verification of the method already validated as qPCR using a different platform. The dPCR method (a multiplex for detecting soybean GM events) was found to be compliant with the ENGL requirements. The method was audited and added to the list under the flexible scope accreditation in autumn 2016. There were no discussions on the buffer changes or requirements for testing the full specificity of the single methods. The laboratory just verified the absence of cross reactions or interferences between the measurement targets.

9.3 Recommendations on flexible scope accreditation of (d)PCR methods (H. Emons, JRC)

The Chairman reminded that a European Technical Guidance document for the flexible scope accreditation of laboratories quantifying GMOs which received approval from European Accreditation (EA) has been published in 2014. The document covers only quantitative real-time PCR (qPCR) and not screening or digital PCR methods. During method validation/verification for dPCR methods some parameters, such as absence of extracted compounds partially (temporarily) inhibiting the PCR reactions and amplification efficiency, are not significant for digital PCR assays, while other parameters such as the dilutions of the initial extracted solution and mixed presence of single/double stranded DNA fragments may contribute considerably to the final measurement uncertainty. The Chairman requested opinions on a potential revision of the European Technical Guidance to include dPCR. Seven members were in favour, others did not express an opinion. Members suggested introducing only a brief clarification in the document. The Chairman remarked that the document should not only cover dPCR alternatives to qPCR assays but also dPCR methods that have been newly developed.

Scientific and technical session (continued)

9.4 Evaluation of PTs/CTs considering different scoring parameters (W. Brothaerts, JRC)

The speaker remarked that up to now the assigned value in the CT rounds was calculated as the consensus value of the participant's (NRLs only) results, and a performance evaluation by the z score was applied, where a $z > 2$ was considered as unsatisfactory. Since the role of NRLs in verifying compliance with food and feed law will be further emphasized in the new

Regulation on official controls, new EURL GMFF procedures will be implemented in 2017 and 2018. For the performance evaluation more emphasis will be placed on the evaluation of the measurement uncertainty using zeta scores in addition to z scores. A presentation on how to perform estimations of measurement uncertainty will be offered in the following ENGL plenary meeting. The procedures for evaluating the outcome of the CT will be also modified in line with ISO 13528, with z scores <2 being satisfactory, 2<z<3 considered as questionable, and z>3 as unsatisfactory. A compliance statement of the tested sample to Regulation (EC) 1829/2003 and Regulation (EU) 619/2011 will be requested as from 2018. For the determination of the assigned value the consensus values will be replaced by a consensus value from "expert laboratories" or by the result from one lab, i.e. the EURL GMFF. This approach will be tested in 2017, but evaluation results using consensus values will be reported as well.

The standard deviation for PT evaluation will be reduced to 0.15 for all samples. The matrix and the GM events to be tested in the upcoming CTs and their dates will be displayed on the EURL GMFF website, to enable the ordering of the relevant primers, probes and CRMs.

The Chairman further clarified that the assessment of the lab performance will still be based on the z score, but that as an additional service the assessment will alert when an unrealistic measurement uncertainty is reported.

9.5 Results of the DECATHLON project (E. Kok, NL)

The speaker illustrated the objectives and results of the EU research project DECATHLON which brought together a broad range of experts for developing cost-efficient advanced DNA-based methods for specific traceability issues and high level on-site applications. The project included 19 partners coming from 11 different EU countries and China with members also from the ENGL network. The project covered the main areas of 1) food pathogens, 2) traceability of GMOs and 3) custom issues. Specific working groups addressed the development of methods in those three areas, the definition of minimum performance parameters taking into consideration cost efficiency and performed validation trials on the methods developed.

Four inter-laboratory studies were organized to validate the methods developed, 14 papers are being drafted or in the pipeline, and more than 25 abstracts have been published. -- See more at: <http://www.decathlon-project.eu/article/objectives#sthash.K18l80D6.dpuf>

10. Break-out Groups

The Chairman indicated the moderators and note takers for the following break-out groups:

- 1) *Identifying issues with ISO 17025 accreditation*
- 2) *Identify potential research topics*

He requested the participants to indicate their interests for one of the two topics.

11. Report of break-out groups and discussion

1) Identifying research topics

The rapporteur summarised the research topics covered in the discussions.

Members suggested starting a project on NGS for detection of non-authorized GMOs analyzing costs, establishing a reference database of sequences, standardization and development of bioinformatics pipelines. They suggested a WG to cover that area.

Other topics were SNP detection and quantification for gene editing. Members discussed but not reached a conclusion on the usefulness of addressing analytical solutions for detecting products derived from synthetic biology or new breeding techniques. They further suggested continuing the discussion on the ENGLnet. Members suggested

organizing a workshop on genome screening.

It was commented that in Belgium a project is initiated for detecting products derived from GMM since a strategy is needed for assessing their quality and purity. It was remarked that it could be performed as a collaborative research project under the flag of the ENGL. The Chairman asked for a volunteer moderator for the forum on research topics on the ENGLnet. J. Ovesna offered to be a moderator for the forum.

2) Issues with ISO 17025 accreditation for PCR methods

Members found the requests and interpretation of 'flexible scope' by accreditation bodies to vary greatly in the Member States. The following problems were encountered:

- 1) Definition of the matrix; in some cases it was described to be food/feed, in others it corresponded to specific food matrices, food vegetables, dairy products, etc.
- 2) National bodies are aware of the European accreditation guidance on 'flexible scope' but require an official communication (by whom?).
- 3) Equipment qualification is provided by the company for real-time PCR, not yet for ddPCR.

Many laboratories considered it useful to design the CT rounds according to food/feed matrices and solicited more information on the types of modifications requiring a more extensive verification. They suggested providing a link in the EU technical guidance on flexible scope to the verification and ddPCR documents. They further proposed adding a definition of matrix in the guidance and inviting a speaker from European Accreditation for a presentation to the ENGL plenary.

The Chairman asked the participants to express their level of satisfaction with their accreditation bodies. The majority did not vote, while half of the remainders declared to be partially satisfied.

The Chairman suggested inviting a representative of EA to the next plenary and preparing a collection of concrete issues. Members supported the proposal.

12. AOB

Participants did not propose subjects for this agenda item.

13. Conclusions & DAL 27th ENGL Plenary

The Secretary reminded that a moderator was still needed for the discussion forum on issues with Regulation (EU) 619/2011. W. Broothaerts reminded that a workshop on DNA extraction will be organized in June 2017 and invited replying to the EU survey launched on that topic. The action list was presented (Annex 3).

Closure of meeting

The Chairman reminded that as agreed by the ENGL SC for the following years only one ENGL meeting will be organized lasting 2.5 days and including the 882 NRL workshop, a scientific seminar and participation from regional networks. He encouraged using more virtual meeting tools. Given the positive feedback received, presentations of relevant projects will be included also in future meetings. The Chairman encouraged keeping the Secretariat informed on the topics considered relevant and on sharing information in advance before the meeting. The Chairman clarified that the reduction in the number of meetings offered by the JRC has been driven by the limited resources and the possibility of having other advanced means for discussions. He thanked the members for the active participation and closed the meeting.

Annex 1 – List of participants



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JOINT RESEARCH CENTRE

ENGL 27TH PLENARY MEETING
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EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

ENGL 27TH PLENARY MEETING
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ENGL 27TH PLENARY MEETING
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ENGL 27TH PLENARY MEETING
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Annex 2 – Agenda



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



27th ENGL PLENARY MEETING

6-7 April 2017, Ispra, Italy
ROOM 11 Auditorium – Building 58/C

Draft Agenda Day 1: 6th April 2017

| AP | Time | Topic | Documents |
|-----|-------|---|---|
| 1 | 9:30 | <ul style="list-style-type: none"> ▪ Welcome | Draft Agenda Report 26 th ENGL Report SC32 DAL ENGL26 To be sent to ENGL Secretariat by 2 April 2017 |
| 2 | | <ul style="list-style-type: none"> ▪ Approval of the Agenda | |
| 3 | | <ul style="list-style-type: none"> ▪ Approval Report 26th ENGL plenary | |
| 4 | | <ul style="list-style-type: none"> ▪ Outcome of the 32th ENGL SC meeting (February 2017) | |
| 5 | | <ul style="list-style-type: none"> ▪ Dynamic Action List (DAL) of 26th ENGL plenary | |
| 6 | | <ul style="list-style-type: none"> ▪ Update from SANTE (I. Ciabatti) | |
| 7 | | <ul style="list-style-type: none"> ▪ Collection of issues from ENGL members | |
| | 10:45 | <i>Coffee Break</i> | |
| 8 | 11:15 | <i>Progress reports ENGL working groups</i> | Presentation Presentation Presentation Presentation Presentation Presentation |
| 8.1 | | <ul style="list-style-type: none"> ▪ AG SMV (Advisory Group on Selection of Methods for Validation) (N. Papazova, BE) | |
| 8.2 | | <ul style="list-style-type: none"> ▪ WG on unit of measurement (P. Corbisier, JRC) | |
| 8.3 | | <ul style="list-style-type: none"> ▪ WG on digital PCR (S. Pecoraro, DE) | |
| 8.4 | | <ul style="list-style-type: none"> ▪ WG on Method Verification (L. Hougs, DK) | |
| 8.5 | | <ul style="list-style-type: none"> ▪ WG on update of method (R. Onori, IT) (presentation on way forward and discussion) | |
| 8.6 | | <ul style="list-style-type: none"> ▪ WG on ENGL Procedures (M. Mazzara, JRC) | |
| | 12:30 | <i>Buffet lunch</i> | |
| 9 | 13:30 | <i>Scientific and technical session</i> | Presentation Presentation Presentation |
| 9.1 | | <ul style="list-style-type: none"> ▪ Food allergens: Current and future criteria for measurement methods (G. O'Connor, JRC) | |
| 9.2 | | <ul style="list-style-type: none"> ▪ Experience with accreditation of dPCR methods (D. Dobnik, NIB) | |
| 9.3 | | <ul style="list-style-type: none"> ▪ Recommendations on flexible scope accreditation of (d)PCR methods (H. Emons, JRC) | |
| | 15:45 | <i>Coffee Break</i> | |
| 9.4 | 16:15 | <i>Scientific and technical session (continued)</i> | Presentation Presentation |
| 9.5 | | <ul style="list-style-type: none"> ▪ Evaluation of PTs/CTs considering different scoring parameters (W. Broothaerts, JRC) ▪ Results of the DECATHLON project (E. Kok, NL) | |
| | 17:30 | <i>End of day 1</i> | |
| | 19:30 | <i>Social dinner at Corner 08 – Travedona Monate</i> | |

Day 2: 7th April 2017

| AP | Time | Topic | Documents |
|--------------|-------|--|--------------------|
| 10 | 9:15 | <i>Break-out Groups</i> | |
| 10.1 10.2 | | 1) Identifying research topics 2) Issues with ISO 17025 accreditation for PCR methods | Mandate Mandate |
| | 10:30 | <i>Coffee Break</i> | |
| 11 | 11:00 | <i>Report of break-out groups and discussion</i> | |
| 12 | 12:00 | <i>AOB</i> | |
| 13 | 12:30 | Conclusions & DAL 27 th ENGL Plenary | |
| | 12:45 | <i>End of meeting and sandwich lunch</i> | |

Meeting documents available at:

<https://englnet.jrc.ec.europa.eu/27th%20ENGL%20Plenary/default.aspx?InstanceID=1>

Annex 3 – Action list

| 27 th ENGL PLENARY ACTION LIST 04/07/2017 | | | | |
|--|-----------|------------|--------|---|
| ACTIONS | Resp. | Timelines | Status | Comments |
| ENGL MEETINGS | | | | |
| Make available on ENGLNet report and presentations of 27th ENGL Plenary | SEC | 21/04/2017 | Open | include link to the report of the 2016 allegens meeting |
| ENGL WORKING GROUPS | | | | |
| WG dPCR | | | | |
| Work to be done on Annex (condensed part) | WG | 30/06/2017 | Open | Meeting needed ? |
| WG Mvrf2 | | | | |
| Send doc to SC for comments | SEC | 15/05/2017 | Open | |
| WG Procedures | | | | |
| Prepare next draft | SEC | 31/05/2017 | Open | |
| WG UpMeth | | | | |
| Discussion forum on definitions | SEC | 30/04/2017 | Open | |
| Template data submission | EURL | 30/6/2017 | Open | |
| Organise meeting | SEC | 30/06/2017 | Open | |
| AG Method Selection for Validation | | | | |
| Organise next meeting | SEC | 30/04/2017 | Open | Main agenda point 2 methods submitted |
| Finalise the list of taxon-specific modules | WG + EURL | 30/06/2017 | Open | |
| Review existing methods for endogenous genes for animals | WG | 31/12/2017 | Open | To be started after finalisation of the task above |
| OTHERS | | | | |
| Contact EA for participation to ENGL meeting | SEC | May-17 | Open | |
| Collection of issues with accreditation | SEC | May-17 | Open | |
| Update the Guidance on flexible scope of accreditation | SEC | 30/09/2017 | Open | Minor amendments, contact task force |
| Planning update of the MU doc | SEC | 30/06/2017 | Open | Include dPCR. Add to the agenda of the SC June |
| Forum on research topics | SEC | 30/04/2017 | Open | J. Ovesna moderator |
| Position document identifying issues with 619 and suggesting possible solutions, including approach for MU | SEC | 30/04/2017 | Open | Discussion forum. Moderator? |