Genetically Modified Organisms in food and feed

Annual Report 2009 of the Dutch National Reference Laboratory

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Distribution list:

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- Dutch Ministry of EL&I; B. van den Assum, E.R. Deckers, T. Greutink, J.L. Thio
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Summary

This is the annual report of the Dutch National Reference Laboratory (NRL) for Genetically Modified Food and Feed (RIKILT). The report gives an overview of the NRL activities carried out in 2009.
In 2009 RIKILT participated in several GeMMA proficiency tests and ring trials organized by the European Reference Laboratory for Genetically Modified Food and Feed (EU-RL-GMFF). Also RIKILT participated in CRL/NRL workshops and the NRL/ European Network of GMO Laboratories (ENGL) Working Group on Method Verification.
Since 2009 RIKILT has become NRL for GM food and feed whereas before RIKILT was NRL for only GM feed and the Food and Consumer Product Safety Authority (VWA) was NRL for GM Food). Therefore the relevant tasks and responsibilities of the two Dutch Routine Field laboratories for GMO detection (RIKILT and Food and Consumer Product Safety Authority (VWA)) were discussed and agreed.

RIKILT has a flexible scope accreditation for real time PCR GMO analysis in raw materials, food and feed.

Contents

1 Introduction

The Dutch Ministry of Health, Welfare and Sports and the Ministry of Economic Affairs, Agriculture and Innovation are responsible for the maintenance of the following
EU regulations in the area of GMOs, i.e. 'European Regulation (EC) 1829/2003 and 'European Regulation (EC) 1830/2003. Both legislations 1829/2003 and 1830/2003 came into force in 2004. Regulation (EC) 1829/2003 'European Regulation (EC) on genetically modified (GM) food and feed' states that food or feed products containing GMOs must be labelled as such. There is a 0.9% labelling threshold for the unintentional presence of GMOs that are authorized in the EU in non GMO batches. In Regulation (EC) 1830/2003 further rules concerning traceability of GMOs are laid down. The producer of a GMO to be authorized in the EU must supply reference material and an event-specific quantitative detection method to the CRL-GMFF. These methods are evaluated by the CRL-GMFF and subsequently validated in inter laboratory ring trials organized by the CRL-GMFF in cooperation with the European Network of GMO Laboratories (ENGL). RIKILT is a member of the ENGL. Regulation 882/2004 stipulates which institutes within the EU member states are NRL for GMO analysis tasks. Since 2009 RIKILT has become NRL for GM Food and Feed (prior to 2009 RIKILT was only NRL for GM Feed).
This report describes all NRL tasks in the area of GM feed and food, as stipulated in national and EU GMO regulations and as far as they are not yet part of other national projects (e.g. in the WOT Theme 4 project on the development, validation and accreditation of methods for GMO analysis).

2 NRL tasks

The NRL tasks are laid down in Directive 882/2004. RIKILT is the National Reference Laboratory for detection of genetically modified food and feed. There are two enforcement laboratories (Routine Field Laboratories) in the Netherlands. For the analysis of food samples this is the Food and Consumer Product Safety Authority (VWA) and the analysis of feed samples is done by RIKILT.
The following NRL tasks are carried out:

- Assisting the CRL in ring trials for GMO detection methods and delivering of information through e.g. CRL questionnaires
- Participate in CRL/NRL meetings
- Participate in proficiency tests
- Perform confirmative analysis on samples of other enforcement laboratories
- Provide relevant information and advice to Routine Field Laboratories
- Check proficiency test results of Routine Field laboratories

3 NRL activities 2009

3.1 Annual CRL/NRL workshops 2009

In 2009 two NRL meetings were attended.
The first NRL Plenary Meeting organized by CRL-GMFF in the frame of Regulation (EC) 882/2004 was held at 28-29 may 2009. The following topics were discussed:

- CRL mandate
- Results from two CRL working groups
- Update of list of National Reference Laboratories by DG SANCO
- Overview of CRL GM food and feed activities
- Organisation of comparative testing schemes by the CRL
- Analytical method requirements under Regulation 882/2004. There is a need for screening methods for several elements.
- ENGL: mandate, functioning and management
- Participation of NRLs to ENGL. It was decided to integrate the NRL meeting into the ENGL meeting and organize separate additional meetings if necessary.
- Training activities

The minutes of this meeting are available in Annex 1.

The second NRL meeting was held on 15 and 16 December 2009 and was integrated in the 12th ENGL plenary meeting. In the second NRLs workshop the following topics were discussed:

- Administrative items, e.g. the NRLs nominated under 882/2004 were invited to check if they were on the official 882/2004 NRL list on the DG SANCO website
- Definition of reference method. Definition and classification of reference methods will be further discussed in the WG on Minimum Performance Requirements and in the WG on Method Verification.
- Sampling and micro-sampling issues - sample preparation. Results of the Questionnaire of Co-Extra on sampling were presented. Sampling will be addressed at the 18th ENGL Steering Committee.
- Update on training needs and training on sampling.
- Harmonization of ISO17025 accreditation. ENGL members were invited to express their interest and join the IRMM initiative for inviting the European Accreditation to form a task force/ experts group of Member State auditors to discuss the issue of accreditation harmonisation.
- Comparative testing schemes. In 2010 the CRL-GMFF will organize two proficiency tests in collaboration with IRMM. Participation will be mandatory for NRL laboratories.
- Collection of test materials from official controls

The minutes of this meeting are available in Annex 2.

3.2 CRL Working Groups

RIKILT participates in the NRL/ENGL 'Working Group on Method Verification'. In 2009 preparatory work was done by email exchanges and one meeting was attended on 14th December. The goals were presented at the 12th ENGL Plenary meeting. A mandate was given to the working group to prepare a guidance document for verification of inter laboratory validated GMO methods.

3.3 CRL Questionnaires

RIKILT, as a member of the ENGL, filled in a questionnaire on sample preparation which was prepared in the framework of Co-Extra (integrated European research project). The aim of this questionnaire was to get an overview of the level of harmonization with regard to the critical steps in the sampling that contribute to the measurement uncertainty (probably more than the PCR step) and may result in differences between labs with regard to the final analytical result.
Also in 2009 RIKILT was asked to fill in a questionnaire for the Co-Extra Decision Support System on the potential presence of GMOs in food/feed supply chains.

3.4 GeMMA proficiency tests

In 2009 RIKILT participated in the GeMMA proficiency tests for DNA, food and feed matrices listed in Table 3.4.1. Satisfactory qualitative results and Z-scores were obtained in all tests.

Table 3.4.1. RIKILT participation in GEMMA proficiency tests

Test	Event	Matrix
GeM MP04	RR soya	Animal feed
GeM SU18	RR soya	Mixed flours
GeM D14	H7-1 sugar beet	Sugar beet DNA
GeMMP05	RR soya	Baked product
GeMMP05	NK603 maize	Baked product
GeMSP03	RR soya	Processed matrix
GEMD10	A2704-12 soya	Soya DNA

As part of the NRL tasks the Food and Consumer Product Safety Authority was asked to report their proficiency test results to the RIKILT. The VWA participated in the GeMMA proficiency tests for DNA, food and feed matrices listed in Table 3.4.2. Satisfactory qualitative results and Z-scores were obtained in all tests.

Table 3.4.2 - VWA participation in GEMMA proficiency tests

Test	Event	Matrix
GeM SU19	NK603 maize	Maize flour
GeM D12	EH92-527-1 potato	Potato DNA
GeM P04	RR soya	Animal feed
GeM SU23	MIR 604 maize	Maize flour
GeM MP05	RR soya	Baked product
GeMSU31	GA21 maize	Maize meal
GeMSU32	MIR604 maize	Maize meal

3.5 Participation in CRL-GMFF organized ring trials

In 2009 RIKILT participated, as a member of the ENGL, in the SIMQUANT validation study organized by the CRL-GMFF. This was a validation study on single molecule quantification (SIMQUANT) applied to the detection of soybean 40-3-2.
RIKILT also participated in the CRL-GMFF ring trial Pentaplex.
Both methods were developed within the Co-Extra project.

3.6 Assistance to other laboratories

In 2009 the RIKILT performed confirmatory MON88017 maize tests on maize food samples for the Food and Consumer Product Safety Authority (VWA) and carried out GMO maize tests for the NAK (Nederlandse Algemene Keuringsdienst voor zaaizaad en pootgoed van landbouwgewassen) on maize seed samples that were possibly contaminated with GMO.

3.7 Other aspects

Before 2009 the VWA was NRL for GM Food and RIKILT for GM Feed. In 2009 it was decided that RIKILT was NRL for both GM Food and Feed. Therefore the following tasks and responsibilities have been agreed with the Dutch Food and Consumer Product Safety Authority with relation to (the quality aspects of) GMO analyses in the Netherlands:

- No Proficiency test will be set up in the Netherlands. Both Food and Consumer Product Safety Authority and RIKILT (under 1829/2003) will continue to participate in FAPAS GeMMA proficiency tests. These proficiency tests are the standard in Europe at the moment. From 2009 RIKILT participates in tests for food as well as feed.
- In 2010 the VWA will remain the Dutch representative in the ENGL Steering Committee and RIKILT will be the official back-up representative. RIKILT and VWA will both continue to attend the ENGL Plenary Meetings.

4 Conclusions

In 2009 RIKILT became NRL for GMO Food and Feed. Before the Food and Consumer Product Safety Authority was NRL for Food and RIKILT was NRL for Feed. It was agreed that:

- No Proficiency test will be set up in the Netherlands. Both Food and Consumer Product Safety Authority and RIKILT (under 1829/2003) will continue to participate in FAPAS GeMMA proficiency tests.
- In 2010 the VWA will remain the Dutch representative in the ENGL Steering Committee and RIKILT will be the official back-up representative. RIKILT and VWA will both continue to attend the ENGL Plenary Meetings.

Also RIKILT assisted the Food and Consumer Product Safety Authority (VWA) with confirmative GMO analysis for unauthorized GMOs in maize samples and the Dutch General Inspection Service for agricultural seeds and seed potatoes (NAK) with confirmative GMO analysis in maize seed samples. In 2009 various NRL tasks were carried out e.g. participation, with satisfactory results, in proficiency tests and collaborative studies, CRL surveys, meetings and working groups.

References

Inter laboratory validated GMO methods: http://gmo-crl.jrc.ec.europa.eu/statusofdoss.htm.
Definition of minimum performance requirements for analytical methods of GMO testing: http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm.

Annex I Minutes of the first NRL Plenary Meeting organized by CRL-GMFF in the frame of Regulation (EC) 882/2004, 28th and 29th May 2009.

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

DRAFT MINUTES
1st NRLs Workshop
28-29 May 2009

A tour de table was done in order to introduce all participants of the workshop. The list of participants is available on the ENGLNET[1].

1. Approval of the Agenda

The agenda was approved without comments.

2. Introduction to the workshop

2.1. Background

General presentation on the mission of the JRC, overview of the activities on GMOs at corporate level, the Institute for Health and Consumer Protection (IHCP) and the Molecular Biology and Genomics (MBG) Unit activities on GMOs since 1997 and its mandates.

Comments:

- One comment was made on broader meetings as some NRLs deal with several activities related to Regulation (EC) No 882/2004.
    - This workshop covers the mandate of the CRL-GMFF on Genetically Modified Organisms and shall not deal with other activities. Moreover each CRL has its own mechanism for coordinating NRLs networks. However it was mentioned that the CRL-GMFF is closely working with other CRLs to streamline the processes on common issues, e.g. comparative testing schemes.
- Some of the participants were interested in different activities of the IHCP, e.g. activities on wine testing and ECVAM.
    - The activities on Nanobiosciences in the IHCP were also mentioned.

2.2. CRL mandate

Presentation of the CRL-GMFF mandate under Regulation (EC) No 882/2004.

2.3. CRL working group on 'Implementation of the quality criteria for the assessment of the quality systems of ENGL validating laboratories under annex 1 of Regulation 1981/2006'

Presentation of the outcome of the working group (draft report sent before the workshop).

Comments:

- The term "audit" or "evaluation" should be avoided in this context. It is a term specifically used in relation to accreditation. Alternatively it was proposed to use terms like 'on-site visits'.
- There is a lack of harmonisation in the ISO 17025 accreditation scheme among different countries. Moreover there is a lack of technical experts in some countries for GMO detection.
- There is a need to work closely with accreditation bodies, in particular with the European co-operation for Accreditation (EA), to explain the mandate of GMO detection laboratories and harmonise the accreditation process.
- Several participants requested the CRL-GMFF together with the ENGL to play a significant role in EU harmonisation of accreditation by providing guidance specifically on GMO testing.

[1] https://englnet.jrc.it/sites/engl/NRL882/1st%20NRLs%20Workshop/default.aspx?InstanceID=1

Conclusions:

- It was agreed to set up a group of technical experts on GMOs (internal auditors) with the aim to i) have a pool of available technical experts trained on GMOs detection issues to carry out internal audits in different laboratories in the EU and to provide assistance to the laboratories visited before their official accreditation audit; ii) to work towards an European harmonisation of the ISO 17025 accreditation process in relation to GMO detection and organise a joint meeting with EU accreditation bodies.

2.4. CRL working group on 'Implementation of the CRL working plan according to Regulation 882/2004'

Presentation of the outcome of the working group (report sent before the workshop). One comment was done on separate meetings between the CRL-GMFF and NRLs. The comment was discussed in item 8 of the agenda.

3. National Reference laboratories
Update from DG SANCO

According to Article 32.2.4 of Regulation (EC) No 882/2004, Member States communicate the name of their NRLs to the European Commission, to the CRL and to other Member States. The official list of all NRLs nominated for GMO detection is in the process of consolidation by DG SANCO. The list is expected to be transmitted to the next Standing Committee for agreement and subsequently publication on the DG SANCO web site.

4. Overview of CRL activities

Presentation of the different activities of the CRL-GMFF.

5. Organisation of comparative testing schemes

Presentation of the 2009 work programme of the CRL-GMFF.
The CRL-GMFF is in contact with GeMMA (UK) and IRMM for this activity (meeting in June 2009). The first comparative test is expected to be launched in spring 2010.

Comments:

- It was again suggested to use the term "audit" or "evaluation" carefully.
    - "Audit" should be seen as assistance to improve the quality of the analyses.
- A question was raised on the diversity of matrices and openness of the list of events for comparative testing schemes.
    - Despite the fact that the final experimental design of future comparative testing schemes should be discussed by the advisory board, the list of possible event will be opened, with all possible combination of events. Raw materials will be preferred in the first instance but processed products will also be part of the schemes.
- A concern was raised about the limited number of event-specific methods under the scope of accreditation in some laboratories.
    - NRLs are supposed to be accredited and to detect what is present in a sample. The CRL-GMFF will use GM events for which the event-specific method has been validated and published on its web site. There is no reason to exclude any of the EU- approved events by default.
- A suggestion was made regarding the reports of comparative testing. Reports from the GeMMA PT scheme are considered to be too general. The CRL-GMFF was asked to develop a model report with sufficient detail so that NRLs may understand the reason for their underperformance.
- The unit of measurement was questioned (mass fraction vs. haploid genome copy number).
    - This issue will be taken into consideration and will be indicated in the report.
- The mathematical model to analyse and thus interpret the results of comparative tests must be carefully chosen in order not to lead to misleading conclusions on laboratories results. "Z-scores" are relative figures measuring the performance of laboratories relatively to each other, and not the absolute performance of a lab. "Z-scores" were reported as not appropriate values for the CRL-GMFF comparative testing schemes because they are based on the statistical average of the results

and therefore always 5% of the results need to be outside the calculated Z-score. The CRL-GMFF is working in collaboration with other CRLs to harmonise the interpretation of results. The advisory board for the comparative testing schemes will provide guidance on this issue.

- A question was raised about which laboratories could participate to the CRL-GMFF comparative testing schemes.
    - Comparative testing schemes could be opened to ENGL members. However this needs to be considered based on capacity and financial resources and the priority will be given to NRLs nominated under Regulation (EC) No 1981/2006 and (EC) No 882/2004.

Conclusions:

- The CRL-GMFF will set the organising committee for comparative testing schemes.
- In first instance, NRLs nominated under Regulation (EC) No 1981/2006 and (EC) No 882/2004 are required to participate in comparative testing schemes. Depending on the feasibility and capacity these schemes may be expanded to all ENGL members and other laboratories.
- The data resulting from comparative testing schemes will be analysed and interpreted carefully.
- The CRL-GMFF will ask for the disclosure of proficiency testing results from NRLs (e.g. to GeMMA).

6. Analytical method requirements under Regulation 882/2004

Technical discussion.

6.1. Definition of analytical methods, reference methods

Comments:

- The Procedural Manual (18th edition) from the Codex Alimentarius Commission proposes a definition of reference method[2] and recommends the use of reference methods in case of dispute and for calibration purposes. A Codex Alimentarius document sets the procedure for legal disputes and potential counter analyses (Appendix II of Report of the Thirtieth Session of the CODEX Committee on Methods of Analysis and Sampling, Balatonalmádi, Hungary, 9 - 13 March 2009). However the text is still in the final phase of adoption by the Codex Alimentarius Commission and therefore changes in the text are still possible.
- The analytical method concerns only the PCR module. In addition the ENGL document on the "Definition of minimum performance requirements for analytical methods of GMO testing" defines the criteria on DNA extraction.
- Laboratories are free to use any suitable validated method for official controls. However there is a need to define reference methods for legal disputes.

Conclusions:

- Reference methods are methods that have been validated.
- The methods published by the CRL-GMFF are the only event-specific detection methods available and fully validated through a collaborative trial and should be used for legal disputes as reference methods.
- The issue of "definitions" will be further considered and a document will be drafted by the CRL-GMFF and presented to the group. If no consensus is reached, there may be the need to call for a working group or meeting of experts on this issue.

6.2. Harmonisation and practicability of methods used for official controls

Comments:

- Harmonisation and practicability are important issues in relation to e.g. different methods for event-specific detection, different reference genes for the same species.
    - The CRL-GMFF works in collaboration with EuropaBio on harmonisation of methods to e.g. restrict the number of reference genes used in applications for authorisation and to carry out bridging studies on existing reference genes. Fuzzy-logic established in the MBG Unit can also be applied to define the method of choice when different methods exist for the same event-specific detection.

[2] ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_18e.pdf

6.3. Method validation (screening methods and target-taxon specific assays)

The discussion was focused on NRLs needs in terms of validated methods and the type of support the CRL-GMFF can provide.

Comments:

- Validated screening methods were considered as a priority (qualitative, quantitative, multiplex PCR).
- There is a need as well on screening methods for new events coming in the authorisation pipeline.

6.3.1. Availability of validated methods

Comments:

- There are different screening methods available from different sources. These methods have been validated or are in the process of being validated. In all cases, publications of these methods are available or will be available in the coming months:
    - 35S method validated by the AFNOR (French standardisation committee);
    - 35S/tNos/Bar/CTP2-CP4/Pat (validated and published in German – the English version may be available);
    - COCIPS (under validation study);
    - GMOtrack (on-going 2-year project, started on 01/06/09);
    - "Pre-spotted plates" (on-going project);
    - Methods from the CO-EXTRA European project.
- The CRL-GMFF can assess the data from the validation study of e.g. the p35S screening test from the AFNOR.
- The "pre-spotted ready-to-use plates" developed by the MBG Unit and distributed to the ENGL members were mentioned as a useful tool for NRLs. As a consequence, the state of the art was presented concerning the development of "pre-spotted ready-to-use plates" as a tool for the control of GMOs present on the agrofood and feed.
    - The development of this tool was appreciated and its availability in the future was requested.
- There is a need to know the specificity of screening methods, in particular which GM event can be detected including new GM event (in the authorisation process). The CRL-GMFF proposed to support NRLs in relation to screening of new GM events by offering the possibility to blast primers/probes of NRLs screening tests against the MBG DNA sequence database (Central Core DNA Sequences Information System – CCSIS) which contains the DNA sequences of all GM events in the authorisation pipeline.

Conclusions:

- All available publications related to the above mentioned methods and/or validation studies thereof will be published on the ENGLNET.
- The MBG Unit will further develop the "pre-spotted plates" concept and is exploring the possibility to produce them in-house. The Unit also explores the creation of a "spin-off" that could allow the distribution of the plates at production/research costs. Few plates of the first generation can still be made available to the network providing that NRLs send their request to the CRL-GMFF together with the description of the project for which they intend to use the plates.
- NRLs willing to test the specificity of their primers/probes used for screening tests are invited to send their request for blast searches against the CCSIS database. This database is not publicly accessible via Internet but requests can be sent to the CRL-GMFF through the ENGL secretariat[3].

6.3.2. Support of national networks

National networks may support the implementation of validation studies.

[3] engl-secretariat@jrc.it

6.4. List of reference materials / control samples

Comment:

- A proposal was made to collect and share samples from official control identified to be positive in GMO testing and to use them for comparative testing schemes.

Conclusion:

- This issue will be followed up (feasibility study) and can be presented to the ENGL Steering Committee.

6.5. The role of the CRL and the ENGL

This point was discussed in items 7 and 8 of the agenda.

7. ENGL: mandate, functioning and management

An overview about the history, mandate, functioning and management of the ENGL was presented. The ENGL, informally established in 2000, was inaugurated in 2002. Since 2009, a new Consortium Agreement provides a new operational structure to the ENGL, where all Member States are represented together with EEA and EFTA member states as well as Candidate Countries, in order to cover all EU GMO regulatory frameworks. The majority of NRLs nominated under Regulation (EC) No 882/2004 are already ENGL members. The benefits of integrating NRLs into the ENGL were discussed in item 8 of the agenda.

8. Participation of NRLs to ENGL

Discussion on the integration of NRLs scientific and harmonisation issues within the ENGL context to facilitate a smoother organisation and reduce the costs of meetings. The future structure of the ENGL meetings was outlined, i.e. the plenary meetings were agreed to foresee at least 3 parts (i.e. general scientific matters, Reg. 1829/2003 related issues and Reg. 882/2004 related issues) and guarantee that specific issues related to each Regulation would be dealt with, whenever it is needed, using either workshops or parallel working groups.

NRLs not yet members of the ENGL were encouraged to join the Network. The procedure for signing the new ENGL Consortium Agreement (CA) was explained:

- The ENGL secretariat must receive the name of the person legally entitled to sign the CA ("the signatory") [3];
- The CA is sent to this person;
- Where relevant, NRLs who want to become ENGL member are invited to contact the ENGL secretariat who will provide them with the instructions and legal documentation and the signature and associated laboratories.

Comments:

- All participants welcomed the initiative to join all the activities related to the legislation on GMOs. Some requests were received to deal in separate meetings with some specific issues, linked to e.g. the coordination of official controls in Member States and to share practical problems.

Conclusions:

- The NRLs topics will be integrated in the ENGL plenary meetings which will be divided into 3 parts (general matters, Reg. 1829/2003 related issues and Reg. 882/2004 related issues).
- Before each ENGL Steering Committee (SC) meetings, the NRLs will receive an invitation to send their proposals/topics/requirements to be included in the agenda of the ENGL plenary meeting.
- Specific meetings will be organised with the NRLs nominated under Regulation (EC) No 882/2004 whenever it will be necessary.

- All the participants to the Workshop will be granted access to the ENGLNET as well as specifically to a sub-site[4] dedicated to Regulation (EC) No 882/2004 (see on ENGLNet the link to "Site of the NRLs and CRL-GMO under Reg. 882/2004").

9. Trainings

General presentation on the "JRC activities on training and capacity building towards harmonization of GMO analysis", and presentation of the outcome of the survey on NRLs training needs (draft report sent before the meeting).

Comments:

- One of the identified priorities is linked to the implementation (in-house verification) of validated methods and availability of internationally recognised guidelines.
    - The ENGL SC already proposed to set up a technical working group on the implementation of method verification. In addition, an article has been published in *Food Anal. Methods* on "Method Validation and Quality Management in the Flexible Scope of Accreditation: An Example of Laboratories Testing for Genetically Modified Organisms".
- The capacity building project will require resources in terms of experts for regional seminars.

Conclusions:

- A working group led by the CRL-GMFF will draft a guidance document on the implementation of validated methods in accredited laboratories (method verification), based on available publications and including practical information.
- This document will be an "NRL document" and will be transmitted to the ENGL Steering Committee with the request to present it to the next ENGL plenary meeting. This document may be endorsed by the ENGL afterwards. This document may support the work of the group of technical experts (internal auditors).
- A call for assistance in regional seminars organised in the frame of the capacity building project will be launched to all ENGL members.

10. Miscellaneous

- The CRL-GMFF will include a specific budget for the group of technical expert (internal auditors) in the 2010 business plan to be proposed to and approved by DG SANCO in September 2009.
- Meetings schedule:
    - JRC working group (8/06/09, Ispra) on "unit of measurement in relation to seed labelling" (See ENGLNET: "Link to the JRC WG Measurement Unit");
    - JRC working group (9/06/09, Ispra) on "Improvement of the RASFF" (See ENGLNET: "Link to the JRC WG RAFFS");
    - Next ENGL SC: after the summer break;
    - Second Global Conference on GMO Analysis (20-24/06/2011).

[4] https://englnet.jrc.it/sites/engl/NRL882/default.aspx

11. Actions and decisions

1. Grant access to an ENGLNET sub-site dedicated to Regulation (EC) No 882/2004
CRL-GMFF: https://englnet.jrc.it/sites/engl/NRL882/default.aspx

2. Set up of a group of technical experts on GMOs (internal auditors).
- Launch a call for interest to all ENGL members;
- Define the terms of reference;
- Organise a first meeting with the technical experts;
- Organise a second meeting with the technical experts and the official accreditation bodies.

CRL-GMFF

3. Produce a one page document to clarify the definition of "reference methods".
Service Commun des Laboratoires du MINEFI - Laboratoire de Strasbourg (FR) / CRL-GMFF

4. Set up the organising committee for comparative testing schemes
CRL-GMFF

5. Ask NRLs for results in proficiency testing schemes
CRL-GMFF

6. Integration of the NRLs in the ENGL
- Send name of the person responsible for signing the CA and associated laboratories – *NRLs (those who did not so previously)*;
- Send the CA - *ENGL secretariat*;
- Send call for proposals to NRLs before the next SC - *ENGL secretariat*;

7. Feasibility study for collecting positive samples from official controls
Veterinary Public Health Institute for Lazio and Toscana Regions; National Reference Centre for GMO Analysis (IT)

8. Guidance document on the implementation of validated methods
National Institute of Biology (SI)
Danish Plant Directorate, Laboratory for Diagnostics in Plants, Seed, and Feed (DK)
Veterinary Public Health Institute for Lazio and Toscana Regions; National Reference Centre for GMO Analysis (IT)
Crop Research Institute - Reference Laboratory for GMO Detection and DNA fingerprinting (CZ)
CRL-GMFF

9. Launch a call for support to the JRC in the context of the capacity building project
CRL-GMFF

Annex II Minutes of the second NRL meeting, held on 15 and 16 December 2009, integrated in the 12th ENGL plenary meeting.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics

Ispra, 01 March 2010

12th ENGL PLENARY
(Ispra, 15-16 December 2009)

MEETING REPORT

1. Approval of the Agenda

The agenda was approved without comments.

2. Debriefing of the Chairman

2.1. Administrative items – debriefing from the Steering Committee

2.1.1. New consortium Agreement (membership, Annexes, Steering Committee)

The Chairman recalled previous decisions confirming that the Community Reference Laboratory for Genetically Modified Food and Feed (CRL-GMFF) will align the activities carried out under Regulation (EC) No 1829/2003 and under Regulation (EC) No 882/2004. As a consequence all National Reference Laboratories (NRLs) nominated under Regulation (EC) No 882/2004 have been invited to sign the ENGL Consortium. Accordingly, the 12th Plenary Meeting will be divided into three parts:

- ENGL common session, relevant to activities carried out under both Regulation (EC) 1829/2003 and Regulation (EC) No 882/2004
- Session 882/2004, relevant to activities carried out under Regulation (EC) No 882/2004 (2nd NRLs workshop)
- Session 1829/2003, relevant to activities carried out under Regulation (EC) 1829/2003

Malta did not sign the Consortium Agreement: this will affect the quorum of the ENGL Steering Committee (SC). The Chairman added that a copy of the ENGL Consortium Agreement, signed by the JRC, will be returned soon to all signatory laboratories. He also invited participants to indicate any possible mistakes still appearing in the ENGL or Steering Committee membership list. Any change of the annexes of the consortium Agreement will have to be approved by the Steering Committee.

2.1.2. Observers and non-signatories of CA (Confidentiality clause)

It was agreed that observers from official candidate countries could be accepted at both the SC and ENGL Plenary meetings. However any observer should sign the confidentiality rules of ENGL to get access to the whole plenary meetings. The previous observers, China, Morocco, ISTA and EFSA, were confirmed by the SC. Any new observers should be proposed and accepted by the SC.

The Jordan Institution for Standards & Metrology, the Food Control Authority of the United Arab Emirates and the Central Laboratory for certification of seed material from Moldova were granted the status of observers to the ENGL Plenary Meetings.

It was also agreed that a delegation of representatives from various Middle East countries would join part of the 12th ENGL plenary meeting on "common/general matters".

The Chairman also announced the dates of the following meetings:
- 18th ENGL Steering Committee on 25-26 February 2010
- 13th ENGL Plenary / 3rd NRLs Workshop, 19-20 May 2010

2.2. Capacity building project

In the frame of the project “Towards Global Harmonisation of GMO Analysis by Creating and Supporting Regional Networks of Excellence”, different events (i.e. training courses and workshops) were organised in 2009:

- International workshop on harmonisation of GMO detection and analysis (Turkey, April 2009);
- Regional Meeting on GMO Detection and training course for laboratory staff of ASEAN countries in GMO analysis (Malaysia, June 2009);
- Training course on "The analysis of food and feed samples for the presence of genetically modified organisms” for Latin American countries and the Caribbean (Cuba, October 2009);
- International Workshop on Harmonisation of GMO Detection and Analysis for Central and South America (Brazil, December 2009).

In 2010, further workshops and trainings are foreseen in Turkey, Asia and Africa. This important capacity-building programme will lead to the 2nd Global Conference on GMO analysis in June 2011.

2.3. Commercialisation of GMO detection kits

The Molecular Biology and Genomics (MBG) Unit is developing GMO detection kits (e.g. event-specific detection and screening). Available kits will be accessible for ENGL members and NRLs prior to their commercialisation. In addition to detection kits, the CRL-GMFF distributes control samples under the form of plasmid to NRLs and members of the ENGL, including associated laboratories, under a material transfer agreement.

2.4. Update on RASFF notifications (New Annex)

An expert working group meeting on RASFF notifications was held in June 2009. A “technical annex” to be attached to RASFF notifications, was sent to DG SANCO who presented it to the Member States for comments in summer 2009. DG SANCO now needs to look at the Member States comments before the Annex is finalized and in place. Several ENGL members commented that the harmonization brought by this “common” technical annex will be welcome.

Comments were made on the work on-going in standardization committees (CEN / ISO) to what concerns test reports. The possibility to launch the work on test reports from GMO detection, at the ENGL level will be submitted to the 18th ENGL Steering Committee (02/2010).

2.5. Update on 2001/18 Working Group on "new techniques"

A Member States experts working group is presently evaluating whether certain new techniques of plant biotechnology fall (or not) within the scope of the “GMO” Directive 2001/18/EC. The expert working group has identified 8 “new techniques”, which may (or not) be included in the scope of the “GMO” Directive 2001/18/EC. One topic under discussion is the “detectability” of products from these new techniques.

DG ENV has therefore asked JRC IHCP to produce a report (based on expert panels and/or literature surveys) on the detection issues presented by these new techniques. JRC IPTS will prepare a report on the “economic/commercial importance” of these new techniques (both inside and outside the EU). The final JRC reports should be available to DG ENV in August 2010. The ENGL expertise may be needed to produce the JRC IHCP report.

3. ENGL COMMON SESSION

3.1. ENGL Working Groups

3.1.1. Working group on Minimum Performance Requirements - WG-MPR

The “Working Group Validation” is replaced by a new “Method Performance Requirements (MPR)” working group. The mandate of the WG-MPR was presented and agreed by the ENGL. The output of the working group is expected in August 2011. A call for expression of interest for working group leader and participants will be launched by the MBG Unit.

3.1.2. Working Group on Method Verification - WG-MV

Following the 1st NRLs workshop, it was agreed that a NRLs working group would start working on a guidance document for the implementation of validated methods. During the 17th ENGL Steering Committee meeting (November 2009), it was agreed to have an ENGL working group on Method Verification – Implementation of Validated Methods for GMO detection (WG-MV). The consolidated mandate was presented and agreed by the ENGL. The output of the working group is expected in December 2011. A call for expression of interest for participants will be launched by the MBG Unit.

The Chairman added that in a 2001/18 Competent Authorities meeting last November, a proposal from Slovenia to work on "EU validation of GMO screening methods" was supported by the Member States and the Commission. The above two working groups should therefore address the topic of "EU validation of GMO screening methods".

3.1.3. Working Group on Unauthorised GMOs – UGM

In the absence of the Chairman of this working group, discussions on the topic were postponed to the 18th Steering Committee meeting and to the 13th ENGL Plenary meeting.

3.2. Linseed/flax sampling and testing

3.2.1. Guideline document on linseed/flax sampling and testing - perspective of the German §64 WG

A scientific presentation was done about the activities of the German §64 WG, who has developed in particular two sampling schemes, for authorized and unauthorized GMO testing. Screening is based on tNos. All details are available at www.bvl.bund.de.

Further to this presentation, the importance of using the same sampling protocols was highlighted, since different sampling protocols may lead to discrepancies in analytical results. In particular, the application of harmonized sampling schemes will be crucial for implementing future thresholds for adventitious presence of GM material in seeds.

3.2.2. Update from the JRC and results from member states

DG SANCO received the first alert in September 2009. Since that date about 100 notifications were submitted from many Member States. The origin of the unauthorised Flax event is from Canada (although there may also be some issues - to be confirmed - with linseed imports from China and Russia). DG SANCO had regular contacts with the Canadian authorities for the development of a sampling and testing protocol for the exports of linseeds to Europe. The agreed detection method for event FP967 (Genetic ID) was verified by the CRL-GMFF (in terms of limit of detection and specificity) and published on its website. The CRL-GMFF also made available control samples (DNA and plasmids) to be used for the qualitative detection of Flax event FP967. A testing protocol commonly agreed by the EU and Canada is therefore now in place and will start to be implemented by end of 2009.

3.3. Conversion factor for unit of measurement in seeds

An expert working group (incl. ENGL members and various Commission services) met in June 2009. Two IHCP communications on “unit of measurements in seeds” were sent to DG ENV (October and November 2009), including a paper from R. Mac Arthur (the latest version of this paper was distributed as supporting document to the participants).

The key points of the conversion factors for unit of measurement in seeds are:

- The analytical uncertainty is approximately a factor of 2 (independently of the unit of measurement used);
- The uncertainty associated with biological factors remains relatively small compared to total uncertainty;
- Consequently, a conversion between different units of measurement can be achieved using a simple conversion factor without the need for detailed knowledge of biological factors;
- For instance in the case of maize (heterozygous seeds): %DNA • %seed x 0.5 x stacking level (number of inserts per seed);
- For instance in the case of soya (homozygous seeds): %DNA • %seed x stacking level (number of inserts per seed).

The chairman invited ENGL members to provide further feed-back on this topic.

3.4. Botanical impurities

3.4.1. Update on DG SANCO document

The Chairman explained that discussions started with the Member States about a DG SANCO document on botanical impurities. Although this is only a first draft document at this stage, it contains some inconsistencies which need to be corrected.

3.4.2. Presentation on the WP4 deliverable from the Co-Extra project

It was recalled that one deliverable under the CoExtra project (WP4) was addressing the topic of botanical impurities. A scientific presentation about this work was made, which included some practical examples on how to address "botanical impurities".

3.5. CODEX and ISO/CEN standardisation

3.5.1 Codex

CODEX is referenced by the Word Trade Organisation (WTO) and applies to any commercial exchanges worldwide. CODEX has a step-wise procedure (8 steps) for document adoption (i.e. official CODEX standard).

A new document “Guidelines on criteria for methods for detection, identification and quantification of specific DNA sequences and specific proteins, in particular in foods derived from modern biotechnology” (CX/MAS10/31/3) will be on the agenda of the next CODEX Committee on Methods of Analysis and Sampling (CCMAS). US delegates proposed to broaden the scope of the document, to open it to allergens, pathogens… The consequence of this proposal is that all references to genetically modified organisms (GMOs) have been eliminated. During the last meeting, the outcome of an electronic working group was reported but no consensus was reach on the document.

The next CCMAS meeting will be in March 2010. All EU Member States should support the reference to “food derived from biotechnology” in the title and scope of the document. The ENGL members were asked to make comments on the document and forward their comments to their national CODEX contact point so that the EU delegations in CODEX could support the "European" perspective on the original scope of the document. If a consensus is reached during the next CCMAS meeting, the document can go to step 5 of the CODEX procedure.

3.5.2 ISO/CEN

The importance of discussions at ISO/CEN level was highlighted. For instance ISO standards become automatically CEN standards and CEN standards are referred to in Regulation (EC) No 882/2004. So ISO standards can definitely have an impact in Europe. ENGL members were therefore invited to attend future ISO/CEN WG meetings (for instance in December 2009 in Germany for CEN, in February 2010 in Japan for ISO)

3.6. Prospects for FP7 projects

Several projects related to GMO analysis were funded under FP5 and FP6. Some evaluation of the outcome of these projects may be needed before further projects are funded. Nevertheless, the ENGL has an opportunity to propose some topics to DG RTD to be considered for future FP7 funding.

4. SESSION 882/2004

4.1. Administrative items

NRLs nominated under Regulation (EC) No 882/2004 were invited to check their presence on the official 882/2004 NRL list published on DG SANCO website http://ec.europa.eu/food/food/biotechnology/gmo_reference_lab_en.htm), as well as their access to the ENGL Intranet: (https://englnet.jrc.it/default.aspx).

The few NRLs who have not yet signed the ENGL consortium agreement, for administrative issues will be invited to do it shortly.

4.2. Definition of "reference method"

During the 1st NRLs Workshop, the need to clarify the definition of reference method has been identified. A set of different definitions from ISO and CODEX was presented. It seems that the definitions and classification at CODEX level would be the most appropriate to follow for GMO detection methods. The four categories of methods given in the XVIIIth edition of the CODEX Procedural Manual were discussed. A reference method is for instance a type II method as described by the CODEX Procedural Manual. The CODEX nomenclature is however addressing food analytical methods in general and therefore may be too academic and general to apply to GMO detection methods, e.g. method validated by the CRL-GMFF. Method acceptance criteria and minimum performance requirements are the key elements of the process in that case. It was therefore agreed that definition and classification of methods should be further discussed in the two established ENGL working groups (see 3.1).

4.3. Sampling and micro-sampling issues - sample preparation

4.3.1. "Sample preparation : results of the Questionnaire of Co-Extra"

A Scientific presentation on a survey carried out under the CoExtra project was made. The need for further harmonization in the field of sampling seems clear (for instance a wide variety of practices was noted regarding the "test portion size"). Comments were made about existing documents and work on sampling being done for mycotoxins testing (incl. documents available on DG SANCO website), which may be a good reference and starting point to use.

It was agreed that the whole sampling issue needs to be addressed by the ENGL. Sampling will therefore be addressed at the 18th ENGL Steering Committee.

4.4. Update on training needs and announcement of Training on sampling, 4-5/02/2010 (Representative sampling of heterogeneous systems)

In 2008-2009, the CRL-GMFF carried out a survey on the evaluation of potential training needs of National Reference Laboratories in the context of Regulation (EC) No 882/2004. "Sampling and experimental design" gathered 48% of NRLs interest with emphasis on practical implementation of sampling procedures for different materials and representativeness of subsequent sub-samples from bulk samples to analytical samples.

A 2-day workshop on sampling issues, organised in Ispra on 4-5/02/2010, was announced during the meeting.

4.5. Harmonisation of ISO 17025 accreditation

A new process for harmonisation of accreditation bodies in Europe is under development and may result in a very long process. As a consequence, the IRMM proposed to lead a parallel initiative looking at a more rapid process. The initiative consists at inviting the European Accreditation to form a task force / Experts group of Member State auditors to discuss the issue of accreditation harmonisation.

ENGL members were invited to express their interest and to join this IRMM initiative.

4.6. Comparative testing schemes

In 2009, the CRL-GMFF has set up the activity foreseen in Regulation (EC) No 882/2004 on comparative testing schemes.

In 2010, the CRL-GMFF will launch the first comparative testing schemes in collaboration with the JRC IRMM (production of test materials and software). Two rounds of comparative testing are planned in the first and third quarter of 2010, respectively. In first instance, NRLs nominated under Regulation (EC) No 1981/2006 and (EC) No 882/2004 will be required to participate in comparative testing schemes. Depending on the feasibility and capacity, these schemes may be expanded to all ENGL members and other laboratories. The advisory board has been selected and will decide on future test materials to be prepared, and will be involved in the assessment of results.

Following discussions with DG SANCO, the JRC will organise a Scientific Forum for Community Reference Laboratories in February 2010 (Geel, BE) where comparative testing schemes will be discussed.

4.7. Collection of test materials from official controls

Following the proposal made during the 1st NRLs Workshop to collect and share samples from official control in GMO testing, the idea of collection both positive and negative samples was welcomed by the meeting participants as such samples collection could be useful for e.g. validation purposes, comparative testing schemes, etc…

Answering a question on availability of reference material for the latest GM events, IRMM confirmed that applicants for GMO authorisation are free to choose their producer of reference material; it can be IRMM but also for instance AOACS.

4.8. Miscellaneous

4.8.1. Information from EFSA

- New revised guidance document for GM plants

An EFSA guidance document for the submission of applications for authorisation of GM plants will be incorporated in EU legislation and will therefore be legally binding, which will be new. The document has been sent to DG SANCO for Member States' comments and may be finalised in the first-half of 2010.

- New working group on "comparator"

A new working group has been set to work on the definition of good comparator (control/conventional counterpart) for risk assessment of GM plants. It was commented that the requirements for comparator for risk assessment and for GMO detection may not be the same (for instance a non-GM variety of the same crop may be sufficient for GMO detection).

4.8.2. FAO delegation

Some representatives from Jordan, Yemen, Lebanon, Syria and Sudan were present following a FAO training hosted by the JRC and were invited to attend part of the meeting and to introduce themselves.

5. SESSION 1829/2003

5.1. Low Level Presence in seeds and in Food and Feed

5.1.1. Presentation/discussion on technical solutions

A scientific presentation was given to illustrate the enforcement of a 0.1% threshold which has been decided in Germany for the presence of authorised GMOs in conventional seeds. The test plan is based on the qualitative detection of 1 GM seed in 3000 seeds and statistical analysis based on Seedcalc: If no GMO is detected in 3000 seeds the GMO-content is below 0.1% with a confidence level of 95%. The LAG working group for detection methods (UAM) proposes a two step test plan with qualitative analysis of subsamples (first step: analysis of 3x1000 seeds and depending on the outcome of the analysis of these sub-samples, additional 3x1000 samples can be analysed).

The importance of sampling plans adapted to the level of thresholds to be tested (e.g. 0.1% or 0.9%) was stressed and should be taken into account in any future regulation.

5.2. Fuzzy logic and expert weight

A scientific presentation of the fuzzy logic was given to the audience. Fuzzy logic could be applied to all validated methods in order to compare them. To this aim, validation criteria need to be assigned different weights/contribution to the final results (expert weights).

An expression of interest for the participation in a 2-day workshop of experts for the definition of fuzzy logic expert weights will be sent to ENGL members.

5.3. Harmonisation of screening methods in GMO testing

In November, in a 2001/18 Competent Authorities meeting, Slovenia raised the point of “harmonisation of screening methods” and proposed that CRL-GMFF gets involved in the validation of “GMO screening methods” (in addition to on-going validation of event-specific meetings). The proposal from Slovenia was supported by various Member States and by the Commission services (ENV, SANCO, JRC).

There is therefore consensus for CRL-GMFF to start working on the validation of screening methods. The issue will be dealt with by the ENGL WGs (see before): for instance to define first the needs and group the available information.

5.4. Digital PCR

A scientific presentation was given on applications of digital PCR. After a brief introduction to the technology, some preliminary results in relation to GMO analysis were presented and then the need for further validation was discussed.

5.5. Miscellaneous

The possible funding of research projects was discussed. The deadline for DG RTD process for project proposals is 15/01/2010. Different proposals should be collected before the deadline and sent to the Molecular Biology and Genomics Unit who will forward them to DG RTD.

6. Summary Action list

1. Contact NRLs that did not sign the ENGL Consortium agreement II;
2. Send the signed copy of the ENGL consortium agreement to all signatories;
3. Make the list of the BCCM plasmids available to the ENGL (16/02/2010);
4. To the ENGL Steering Committee: proposal to revise/enlarge test reports (in relation to the new RASFF annex and on-going work in CEN/WG11), at the ENGL level;
5. Invite ENGL members to participate to the work to be carried out in relation to the DG ENV expert group for new techniques;
6. Consolidate expression of interest of ENGL members for participation to the WG-MPR (+ WG leader) and WG-MV;
7. Follow up Member State support (via ENGL members) in preparing the next CCMAS meeting (March 2010) regarding the insertion of “food derived from biotechnology” in the CX/MAS10/31/3 document.
8. Launch a group of experts for the definition of expert weights in fuzzy logic;
9. To the ENGL Steering Committee: sampling issues.
10. Send the invitation and training programme for the workshop on sampling (Ispra, Feb 2010);
11. IRMM to gather expressions of interest for a group of experts working on harmonisation of accreditation;
12. CRL to evaluate the feasibility of a collection of tests materials with the Italian Network;
13. Send invitation for a 2-day workshop of experts for the definition of fuzzy logic expert weights.
14. Collect proposals for future research projects (15/01/2010) and forward them to DG RTD.