



Genetically Modified Organisms in Food and Feed

Annual Report 2012 of the Dutch National Reference Laboratory

I.M.J. Scholtens-Toma, B. Molenaar, S. Zaaijer, T.W. Prins and E.J. Kok

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RIKILT report 2013.006

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Summary

This is the annual report of the Dutch National Reference Laboratory (NRL) for Genetically Modified Food and Feed (RIKILT Wageningen UR). The report gives an overview of the NRL activities carried out in 2012.

In 2012 the two Dutch Official Laboratories participated in several proficiency tests with good results.

Furthermore RIKILT participated in two EURL/NRL meetings and the Working Group on Detection, Interpretation and Reporting.

RIKILT advised the other Official Laboratory on the application of a 'SYBRGreen' detection method for unauthorized GM rice in food from China. Also changes in the method were discussed.

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

1 Introduction

The Dutch Ministry of Health, Welfare and Sports and the Ministry of Economic Affairs are responsible for the maintenance of EU regulations in the area of GMOs, i.e. 'European Regulation (EC) 1829/2003' and 'European Regulation (EC) 1830/2003'.

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. The producer of a GMO to be authorized in the EU must supply reference material and an event-specific quantitative detection method to the EURL-GMFF. These methods are evaluated by the EURL-GMFF and subsequently validated in interlaboratory ring trials organized by the EURL-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. In the Netherlands RIKILT is NRL for GM Food and Feed. In the Netherlands there are two Official laboratories (OL) that carry out GMO analysis for the Dutch government. This report describes all NRL tasks and activities 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 'Validation and accreditation of GMO detection methods', WOT-02-004-005, funded by the Dutch Ministry of Economic Affairs).

2 NRL tasks

The official NRL tasks are laid down in Directive 882/2004. The following NRL tasks have been carried out in the 'NRL GM feed/food' project, WOT-02-004-003), funded by the Dutch Ministry of Economic Affairs):

- Assist the EURL in interlaboratory validation studies for GMO detection methods and exchange of information on detection methods
- Participate in EURL/NRL meetings and workshops
- Participate in proficiency tests
- Perform confirmative analysis on samples of other enforcement laboratories, if requested
- Provide relevant information and advice to Official Laboratories
- Check proficiency test results of Official Laboratories

It is also an NRL task to be able to perform all EURL interlaboratory validated GMO detection methods under accreditation. The in-house validations of these methods are performed in the 'Validation and accreditation of GMO detection methods', WOT-02-004-005, funded by the Dutch Ministry of Economic Affairs.

3 NRL activities 2012

3.1 Annual NRL workshops 2012

In 2012 two NRL meetings were attended. On June 7 there was a joined session with SCFCAH 'Standing Committee on Food Chain and Animal Health'. At this workshop RIKILT presented the Dutch GM feed control. Because almost all animal feed imported into the Netherlands is labelled as GM the emphasis was on the screening for unauthorized GMOs. For this reason the feed samples were screened using an informative screening plate that contained 24 different taxon specific, GM element and GM event specific PCR tests. Also RIKILT cooperates with BVL (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, Berlin) in a GMO database project (EUGenius).

From 3-5 December 2012 the 8th workshop of GMO national reference laboratories under Regulation (EC) No 882/2004, the 18th ENGL Plenary Meeting and the 20th workshop of national reference laboratories under Regulation (EC) No 1981/2006 were held in Ispra, Italy. The Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL), Germany) and the RIKILT Wageningen UR presented the new EUGenius database on GMOs during this meeting. This database is a joined effort of BVL and RIKILT. The database contains information on GMO biosafety, genetic structure, detection methods and has an analysis tool for screening results. The database is expected to become publically available in 2013.

The following topics were discussed at the NRL meetings: detection methods, interpretation and reporting of results, sampling, EURL proficiency tests, progress reports of several EURL/NRL working groups, international harmonization, genetically modified pollen in honey, experiences with 'Low Level Presence' Regulation (EU) No 619/2011, experiences with testing of GM rice from China according to Regulation (EU) Nr. 884/2011. More information can be found in the final EURL reports of the above meetings (Annex 1-5).

3.2 EURL working groups

RIKILT participated in the NRL/ENGL working group on 'Detection, Interpretation and Reporting'. This working group started in 2012. The mandate of this working group is to review the 2011 ENGL document 'Overview on the detection, interpretation and reporting on the presence of unauthorised genetically modified material'. The goal is to broaden the scope of the document to also include authorised GMOs and uncovered plant species. The document should provide practical guidance for harmonized detection, interpretation and reporting of GMOs in Europe. Advantages of targeted, knowledge based and non-targeted, unbiased approaches for screening will be discussed within the document. Gaps in necessary technology will be identified and proposals for closing these gaps will be made. The working group is divided into three subgroups dealing with different topics. RIKILT will primarily be involved in harmonization of the reporting of results and harmonization of screening matrices as used in several countries.

3.3 Surveys

In 2012 RIKILT gave feedback on a draft Technical guidance on flexible scope (IRMM) that was distributed to the ENGL/NRL network for comments. This document will provide guidance for a harmonised flexible scope accreditation in Europe.

In 2012 there were no EURL surveys.

3.4 Proficiency tests (GeMMA and ILC-EURL-GMFF-CT)

In a proficiency test unknown samples are analysed with a method of choice. The results are compared with other laboratories. For quantitative analyses Z-scores are calculated. A Z-score between +2 and -2 means that the result was satisfactory in comparison with other laboratories. In 2012 RIKILT participated in the GeMMA and ILC-EURL-GMFF-CT proficiency tests for DNA, food and feed matrices listed in Table 3.4.1. For NRLs it is mandatory to participate in the ILC-EURL-GMFF-CT proficiency tests. RIKILT obtained satisfactory qualitative results and Z-scores in the GeMMA 2012 tests.

Table 3.4.1
Results RIKILT proficiency tests 2012.

Test	GMO event	Matrix	Z-score	Date
GeMD34	Ms8, RT73, Rf3 canola	DNA	Not applicable	Cancelled by FAPAS Feb. 2012
ILC-EURL-GMFF-CT-01/12	RT73 canola	DNA	0.16	July 2012
ILC-EURL-GMFF-CT-01/12	DAS59122-7 maize	DNA	0.02	July 2012
ILC-EURL-GMFF-CT-01/12	RT73 canola	DNA	-0.12	July 2012
ILC-EURL-GMFF-CT-01/12	DAS59122-7 maize	DNA	-0.08	July 2012
GeMD36	LL62 rice	DNA	No z-scores assigned, because there were not enough participants. RIKILT value agreed with mixed percentage.	Aug. 2012
GeMMP12	35S promoter, NOS terminator, Roundup Ready soy, Bt176, Bt11, MON810, GA21, NK603, TC1507, MON863, MIR604, MON88017 maize Quantitative: Roundup Ready soy	meal	0.7	Oct. 2012

As part of the NRL tasks the proficiency test results of the Official Laboratory were monitored. In 2012 the Official Laboratory participated in one EURL proficiency test and four GeMMA tests. All reported qualitative and quantitative results were satisfactory. Not all planned PT tests were carried out by the Official laboratory mainly because the rice analyses were of higher priority (see also 3.5).

3.5 Assistance to other laboratories

In 2012 RIKILT has given priority to the validation of a mandatory screening method for Chinese rice (Directive 2011/884/EU). The method detects three GMO elements: P-35S, T-nos and cry1Ab/Ac. The in-house validation of this method was done in a separate project ('Validation and accreditation of GMO detection methods', WOT-02-004-005, funded by the Dutch Ministry of Economic Affairs). The validation revealed some drawbacks resulting in a risk of false positives. The method does not have a background cut-off. Very low signals, especially for the cry test have to be scored as positive. Also it is possible that positive signals are caused by contamination with Cauliflower Mosaic virus (CaMV) that also contains P-35S, or other non-rice GMOs like Roundup Ready soy that also contain P-35S and/or T-nos.

RIKILT and the other Official laboratory have frequently discussed the sample results of both laboratories obtained with this method. Together with the other Official laboratory proposals for improvement of the method were communicated within the ENGL and to European policy makers.

4 Discussion en conclusions

In 2012 RIKILT and the second Official Laboratory participated in several proficiency tests with good results.

RIKILT attended two NRL Workshops in Belgium and Italy and participated in the ENGL Working Group Detection, Interpretation and Reporting.

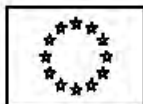
References

Commission Regulation (EU) No 619/2011 of 24 June 2011 laying down the methods of sampling and analysis for the official control of feed as regards presence of genetically modified material for which an authorisation procedure is pending or the authorisation of which has expired.

Directive 2011/884/EU: Commission Implementing Decision of 22 December 2011 on emergency measures regarding unauthorized genetically modified rice products originating from China and repealing Decision 2008/289/EC (2001/884/EU).

EU-RL GMFF Guidance on the Application of P-35S, T-NOS and Cry1Ab/Ac Methods for the Detection of Genetically Modified Rice Originating from China Using Real-Time PCR, 21-12-2011.

Annex 1 17th ENGL-SCFCAH Meeting Report



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



17th ENGL PLENARY

JOINT ENGL SESSION WITH THE

STANDING COMMITTEE ON FOOD CHAIN AND ANIMAL HEALTH

Thursday 7 June 10.00 am – 1.00 pm
(Borschette Conference Center, Room 2A)

MEETING REPORT

In introduction, Ms D. Andre from DG SANCO highlighted the useful work of EURL and ENGL, which is often referred to in the SCFCAH discussions.

1. Introductory presentation on the JRC, the EURL and the ENGL: J. Kreysa (JRC)

J. Kreysa briefly introduced the JRC, the EURL mandates under regulations (EC) No 1829/2003 and 882/2004 and the ENGL.

2. Presentation on ENGL achievements: G. Van den Eede (JRC)

G. Van den Eede recalled the various achievements from ENGL over its 10 years of existence (2002-2012) and the successful cooperation between the Commission and the Member States on GMO analysis over that time. He also highlighted international cooperation and global networking as "the next steps" beyond ENGL.

3. Presentation on EURL activities (Reg.1829/2003 and 882/2004): M. Mazzara (JRC)

M. Mazzara presented the various activities undertaken daily at the EURL to support harmonised GMO detection as a critical part of the implementation of EU GMO-legislation.

At this opportunity, France recalled a pending question to DG SANCO about the participation of NRLs, listed in annex to Reg. 1981/2006, to Proficiency Testing rounds, requested by Reg. 882/2004. France has three separate laboratories that are specialised according to the matrices from which DNA needs to be extracted and France asked if participation to Proficiency Testing could be adapted according to the matrices daily used by NRLs and the matrices used in the PT rounds. DG SANCO responded that they would coordinate with JRC to answer this and suggested the matter to be discussed within the ENGL.

4. Presentation on activities of a national GMO control lab: example from Netherlands

Ms Esther Kok from the RIKILT Institute made a detailed presentation about GMO controls on feed in the NL. She highlighted the challenges related to detection of "unapproved GMOs", for which the NL use as a first step an "element screening" approach (based on a standard plate with 27 different screening elements). In this frame RIKILT is cooperating with BVL in Germany to develop a "GMO Reference database", Euginius. She added that a pragmatic approach could be to focus detection efforts to unapproved GMOs that have not been assessed for safety in the EU. In the future sequencing could offer new options for GMO detection.

5. Update on RASFF notifications incl. GM rice

DG SANCO first recalled that input from the Member States on their experience with implementation of the Chinese rice Decision was welcome in order to prepare the revision of the December 2011 Decision. So far Member States have mainly expressed concerns about the number of replicates to be analysed (which increases the analytical costs) and the occurrence of false positives. DG SANCO distributed an early draft of a future revised decision.

Annex 2 17th ENGL Plenary Meeting Report



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



17th ENGL PLENARY
Borschette Conference Center, Room 3A
7 – 8 June 2012

MEETING REPORT

Day 1: Thursday, 7 June: 14.30 - 17.30

Note: the 17th ENGL plenary meeting had been preceded by a half day joint meeting with the SCFCAH (Standing Committee for the Food Chain and Animal Health).

ENGL Common Session

1. Approval of the 17th ENGL Plenary Agenda

The co-chairs J. Kreysa (new Head of the JRC Unit Molecular Biology and Genomics) and G. Van den Eede (newly appointed Advisor for Bio-Economy at the JRC Headquarters) opened the meeting. The agenda was adopted without modification.

DE commented that the sessions for the general ENGL, the NRLs under Reg. 1981/2006, and the NRLs under Reg. 882/2004 were not clearly separated. It was agreed to improve this in future ENGL agendas in order to better address the specific needs from the different NRL-mandates.

2. Debriefing by the ENGL Chairman

2.1. ENGL organisation

Since April 2012 G. Van den Eede and D. Plan have moved to new positions in the JRC headquarters in Brussels. Accordingly responsibilities for the ENGL chairmanship and secretariat will need to be re-allocated within JRC. This should be done smoothly by end 2012, for the time being the J. Kreysa and G. Van den Eede co-chaired the meeting. Both underlined that these changes will have no impact on the mandate and functioning of ENGL.

2.2. Action List from 16th ENGL Plenary (Nov 2011)

2.3. Outcome of the 22nd ENGL SC meeting (March 2012)

D. Plan presented an updated version of the ENGL DAL (dynamic action-list based on both the ENGL Plenary of Nov. 2011 and the ENGL SC of March 2012).

The main open topic is the ENGL website which is not yet upgraded. J. Kreysa confirmed that the new ENGL website will be available on-line by October 2012 (i.e. before the Global GMO Network Forum, planned for 16/17 of October 2012).

The following comments were made:

- Regarding the Accreditation Task Force, S. Trapmann confirmed that a final document will be available in time for discussion at the next ENGL SC meeting in September 2012.

- Regarding Proficiency Testing, D. Charels confirmed that the reports for the 3rd and 4th round will be published shortly.

France, Germany and Czech Republic raised the issue of obligatory participation of NRLs to Comparative Testing (CT), which may raise problems for some NRLs that are specialised on some matrices (eg seeds)

Annex 3 18th ENGL Plenary Meeting Report

Text from the official meeting report written by EUROPEAN COMMISSION, JOINT RESEARCH CENTRE, Institute for Health and Consumer Protection, Molecular Biology and Genomics Unit.

ENGL PLENARY MEETING

4-5 December 2012, Ispra, Italy

MEETING REPORT

1.1 Welcome

The President welcomed the participants (annex 1) and introduced the 18th ENGL Plenary meeting.

1.2 Approval of the Agenda

The President asked the participants if there were additional points that needed to be added to the agenda. Since there were no requests, the agenda (Annex 2) was approved.

1.3 Minutes of 17th ENGL plenary

The ENGL scientific Secretary presented the minutes to the participants and underlined the main points. The minutes had been published on the ENGLnet in June 2012. No further comment was made.

1.4 Dynamic Action List (DAL) of 17th ENGL plenary

The ENGL scientific Secretary presented the DAL and asked for comments. The 17th ENGL plenary DAL was approved.

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

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

The President announced that more clear criteria on the participation of observers to the ENGL plenary meetings will be decided. It was added that guidance will be produced on the use of CRMs (Certified Reference Materials) at 0.1% GM level. Concern was raised by the participants on the quality of CRMs produced by AOCS (The American Oil Chemists' Society). It was proposed to draft a new document defining minimum requirements for the quality of CRMs.

A question was raised on the update of the ENGL public web site considering that a more comprehensive explanation of the ENGL activities carried out may be important for the member states' authorities, to motivate their financial support. The President replied that at the last SC meeting this activity was not considered to be worth the effort. It was underlined that a dedicated web site should be dynamic, updated with new material and transparent. The President suggested adding this point again in the agenda of the 24th SC for further discussion.

2. Progress reports ENGL-groups

The President commented that members that are interested could still propose their participation to the different WGs.

2.1 WG MPR (Method Performance Requirements)

M. Mazzara reported on the progress of the draft document 'Definition of minimum performance requirements for analytical methods of GMO testing'. The mandate of the WG-MPR is to broaden the scope of the current document to qualitative, taxon-specific, DNA extraction and multiplex methods. The modularity approach has been embedded into the document and new criteria for

false positives, false negatives and performance assessment of qualitative detection have been established. A protocol for testing robustness has been included and general criteria for DNA extraction methods have been agreed. Criteria for multiplex methods still need to be developed. The next meeting has been scheduled for 24th and 25th January 2013, with the intention to progress towards the finalisation of the document. It is foreseen that a final draft will be presented to the SC in March 2013.

2.2 WG SPP (Sample Preparation Procedure)

G. Berben provided an update on the preparation of the guidance document on Sample Preparation Procedure. The draft document is at an advanced stage. Performance tests need to be added for the different steps of the sample preparation. It was suggested to agree on good working standards to harmonize the procedures and to include them in the flexible scope for accreditation. The chairman of the WG stated that an additional meeting would be necessary to finalise the document.

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

A. Holst-Jensen provided an update of the activity of the Advisory Group. Existing gaps have been identified on taxon, element and construct methods. A template to provide information on methods was submitted to the ENGL members, including a template for DNA extraction modules. A number of proposals were received based on which a priority list for methods to be validated was defined. The chairman of the AG SMV announced that for time constraints he can no longer act as leader. The President asked the members of the WG to propose a new candidate for the position. After the meeting, N. Roosens (IPH, BE) offered to take the chairmanship of the advisory group.

2.4 WG DIR (Detection Interpretation Reporting)

I. Ciabatti reported on the activities of the WG. She informed the participants that the title of the WG has been amended to include the unauthorised GMOs. GM animals will not be covered by the future guidance document. To embrace the broad scope of the document, activities were divided in three sub-groups, each covering two topics. A final draft should be available by the end of January 2014. A survey on matrix approaches currently in use in control laboratories was launched and the results are under evaluation by the WG. The first draft texts have been already produced by the sub-groups. The main challenge seems to remain the transferability of cut-off values determination among different laboratories. The second meeting of the WG is scheduled on 22-23 January 2013.

2.5 Technical guidance on flexible scope

S. Trapmann (IRMM) updated the participants on the progress of the guidance document for a harmonised flexible scope accreditation in Europe. She explained that the task force is reporting not only to the ENGL but also to the European Accreditation body and that the procedure for finalising the document is therefore more complex. A first document on the needs for flexible scope had been already presented to the European Accreditation (EA) body. A second document providing guidance on quantitative PCR methods has been reviewed by ENGL members. The final version will be submitted in spring 2013 to EA for comments and approval. A third document providing guidance on qualitative PCR methods will be prepared at a later stage.

3. Update on revision of Decision 2011/884/EU

G. Van den Eede, JRC Adviser for Bio-Economy, update the ENGL on the status of revision of Decision 2011/884/EU. The proposal of the EC will probably be presented to the Member States in January, for comments before voting. The main modification regarding the analytical strategy outlined in the Decision concerns the reduction of the number of sub-samples to be analysed for processed rice products, from four to one.

L. Grohmann (DE) reiterated the request of organising soon an ENGL ad-hoc meeting to discuss issues related to the implementation of the Decision on GM rice originating from China.

4. Scientific and technical session 1

4.1 Euginius (P. Heinze, DE, T. Prins, NL)

P. Heinze presented a new database on GMOs called Euginius, developed through a collaborative effort between the RIKILT (NL) and BVL (DE) institutes. The database provides information on GMOs, their genetic structure, biosafety and validated methods. Approximately 200-250 GMOs have been included in the database. The data are currently undergoing internal validation, for this reason Euginius is not publicly available yet.

4.2 Droplet digital PCR for GMO quantification (D. Morisset, SI)

D. Morisset illustrated the drawbacks and difficulties in quantifying DNA with the traditional real-time PCR approach and explained how these could be solved by using droplet digital PCR technology. Advantages and drawbacks of the droplets and array dPCR devices were presented. Dr. Morisset presented the results of a study where the performance of methods already validated was evaluated with the ddPCR approach. The linearity range covered 5 logs of DNA concentration and the trueness resulted to be lower than 10% for samples containing down to ten genome copies. Specificity was tested on milk samples and the rate of false positives resulted to be very low. No inhibition effect was observed. The technology required less hand-on time, but had about the same cost than the traditional qPCR approach.

4.3 Droplet digital PCR, comparison of instruments (G. Berben, BE)

G. Berben presented a comparative study between different ddPCR devices for measuring low amounts of targets (8-10 copies/uL). Bio-Rad-QX100 drop digital PCR, Fluidigm –Biomark and Life science open array devices were compared. They expected to obtain an equal spreading of the values with the different devices. Significant differences in the absolute copy number values were observed, with the values grouped according to the device used. These preliminary results may suggest an effect of the instrument on the quantification of DNA molecules by ddPCR.

4.4 Study on maize reference genes in baby-corn (B. Spilsberg, NO)

B. Spilsberg explained that during the analysis of baby corn samples dramatic difference in genome copy number when were obtained when using as target the reference genes *adh1* or *hmg*. The samples were analysed by capillary electrophoresis and a very small average size of the DNA fragments was observed, thus suggesting extensive DNA degradation. Following this observation, a comparative study with new maize methods amplifying smaller amplicons from different reference genes was conducted: the maize reference targets used were *hmg A*, *ivr1*, *zein*, *adh1* and *zSSI1b*. The authors observed a dramatic difference in amplification efficiency between amplicons that were 155 to 79 bp long. It was concluded that the size of the amplicon for the reference gene target is a crucial factor when analysing maize canned products.

4.5 Study on DNA extraction from maize gluten (B. Spilsberg, NO)

B. Spilsberg presented a new method for extracting DNA from gluten. For the protein purification step a large volume to a small column was applied, then washed thoroughly to eliminate inhibitors and used filtration with an ionic exchange column to concentrate the DNA solution. An in-house validation was performed on samples deriving from China and USA. Values of LOD and LOQ correspondently equal to 0.05% and 0.4% were obtained. No PCR inhibition on dilution regression analysis was observed on the samples analysed. The protocol takes about 7.5 hours.

5. Celebration of the 10th anniversary of the ENGL

Keynote speeches:

- E. Anklam, Director of IHCP, described the activities on GMOs since 1997 and presented the significant steps that brought to the official signing in 2002 of the first ENGL agreement.
- Guy van den Eede, previous head of the Molecular Biology and Genomics Unit, highlighted the developments over time on GMO legislation, commercialisation and research activities. He emphasized the contribution of the ENGL network on the implementation of GMO legislation and harmonisation of official controls not only at European level but also in the world.
- I. Ciabatti, as a representative of the ENGL, described the current activities and scientific contributions of the network.

– J. Kreysa, current Head of the Molecular Biology and Genomics Unit at the JRC and President of the ENGL, provided his personal foresights on the future challenges and developments in the GMO field.

6. Scientific and technical session 2:

6.1 Next Generation Sequencing (D. Wahler, DE)

D. Wahler presented the use of next generation sequencing approach for GMO characterisation, even when the genetic structure of the event is not known. LLrice 62 was used as model system for testing the technology; more than 300 million reads of the corresponding genomic DNA were sequenced.

As a bioinformatics strategy for assembling the transgenic genome, the LLrice 62 read sequences were aligned to the reference rice genome. With this strategy 93% of the host genome was covered and 65 x coverage of the transgenic component was obtained. The insertion sites could be identified as breakpoints gaps in the reference genome. The authors used paired-end sequencing for mapping the read sequences to the breakpoints. The results were verified by comparing the assembled sequences with the known genomic data of the transgenic event.

6.2 Chinese-Norwegian-Slovenian Next Generation Sequencing study on GM-rice. (A. Holst-Jensen, NO, D. Morisset, SI)

A. Holst-Jensen presented a collaborative project for applying the next generation sequencing approach to GMO characterisation. Different approaches, varying according to the information available on the transgenic event, were discussed. For GMOs of unknown structure, the genome fragments were sequenced and the reads sequences were categorised into A, B, C, D classes according to their similarity to the reference genome, plasmids or patent sequence collections.

6.3 Next Generation Sequencing study of composite/processed food/feed samples for GM detection (A. Holst-Jensen, NO)

A. Holst-Jensen presented the results of a project applying the next generation sequencing approach for GMO detection in complex food samples. A large sequence dataset from mix of CRM samples was generated; the sequences were then compared to pre-assembled collection of patent, species and genetic element sequences. The objective was to test the sensitivity and bias of the 'de-novo assembling matrix approach'. Three samples, containing 7% GM soybean, 0.7% GM maize and 0.07% GM cotton were tested. Single genetic elements at a concentration lower than 1% could be reliably detected. The quality of the DNA solution was determined to be the critical factor for the success of the strategy.

The presentations raised genuine interest and were followed by intense discussion.

7. Scientific and technical session 3

7.1 Interlaboratory testing of CoSYPS in GMO analysis (M. Van den Bulke, BE)

M. Van den Bulcke presented an inter-laboratory trial conducted to test CoSYPS. The study involved thirteen laboratories randomly selected from a pool of 23 ENGL volunteers. 10 samples containing varying combination of species and GMs materials (from 0.1% to 2.5% GM) were provided to the participants. Deviation was observed in the results possibly due to GM contamination of some samples. Significant differences in Ct values among different laboratories were observed, which could be explained by an inadequate application of the protocols. It was concluded that the system needs prior training of the laboratories involved. Overall, values of specificity and sensitivity higher than 95% were obtained, with more than 98% correct decisions for all methods in all samples.

7.2 Pre-spotted plates (M. Querci, EU-RL GMFF)

M. Querci summarised the previous development and progress of the pre-spotted plates project. She reminded that the LLP legislation requires GMO detection at the 0.1% level and proposes to adapt the plates to the new needs of the laboratories. M. Querci invited ENGL members to inform the EU-RL GMFF on their needs/wishes/constraints and preferred designs with respect to the future pre-spotted plates. It is the intention of the EU-RL to produce one or two pilot models of the plates. The Unit will evaluate the possibility of outsourcing the plate production. It was asked to

provide information on the analysis cost-saving connected to the use of the plates. The plates will be distributed after summer 2013.

7.3 Update on CEN/ISO activities (L. Grohmann, DE)

L. Grohmann provided an overview of the standardization activities organized on GMOs at national, regional (CEN) and international (ISO) level. He reported that the scope of the ISO/TC 34/SC16 committee has been broadened from GMOs to food products and announced that the 4th plenary meeting of the Sc16 will take place in April in London. It was noted that the participation of European countries to ISO meetings is low and encouraged the ENGL members to promote a more vigorous involvement of their standardization organization to the ISO activities. He announced that in February 2013 in Berlin there will be also a CEN/TC 275/WG 11 (Working group genetically modified foodstuffs) meeting, to prepare for the ISO SC16 meeting. It was suggested to post information on CEN and ISO activities and the corresponding agendas on the ENGL web site.

8. DAL ENGL 18th and AOB

M. Mazzara presented the outcome of a satisfaction survey conducted in 2011 and informed the ENGL members of the actions undertaken in response of the comments received. He informed the participants that another survey will be performed in January 2013.

The ENGL Secretary reviewed the newly updated action list and described the open points. He announced that on the 4-8th of March 2013 an international GMO meeting will be organised in Ispra.

The chairman asked for volunteers for the AG SMV WG leader position. He encouraged uploading on the ENGLnet web site projects and information on GMOs that could be important for the network.

The 18th ENGL plenary meeting was closed. The 19th ENGL Plenary will take place in May-June 2013 in Ispra.

Annex 4 8th Workshop NRLs 882 Report

Text from the official meeting report written by EUROPEAN COMMISSION, JOINT RESEARCH CENTRE, Institute for Health and Consumer Protection, Molecular Biology and Genomics Unit.

8th WORKSHOP OF GMO NATIONAL REFERENCE LABORATORIES

REGULATION (EC) No 882/2004

3 December 2012, Ispra, Italy

MEETING REPORT

1. Welcome and approval of the agenda

The chairman asked the participants (see Annex 2) if there was any point to be added to the agenda (see Annex 1). No suggestions were formulated.

2. Minutes 7th workshop

The chairman informed the participants that he received a request from SANCO to organise a workshop on GMOs to favour dialogue among Member States. He asked for suggestions on topics and for contributions.

3. Tour de table: points/opinions from NRLs

The chairman asked the participants to highlight issues or needs that could be important for the National Reference Laboratories (NRL) activities. The participants introduced their main activities. Some NRLs are responsible within the Member State (MS) only e.g. for feed, food or seed analysis, while others are the only NRL for GMO testing in the Member State, thus conducting analyses on all types of samples.

Attention was raised to the following points:

- Cost effectiveness of GMO analysis should be increased
- Harmonisation of methods of GMO analysis, at least for screening methods and use of matrix methods
- Availability of methods of analysis for highly processed food
- Implementation of flexible scope for accreditation
- Availability of certified reference materials (CRMs), especially at the 0.1% GM level
- Guidance on conversion of analytical results from mass/mass fraction to DNA copy number ratio
- Availability of test materials for proficiency tests

The participants shared the challenge of keeping the NRLs updated, taking into consideration the ever increasing number of GMOs approved. NRLs asked the EU-RL GMFF to intervene on the issue of flexible scope accreditation in all Member States and highlighted the importance of sharing information and experiences on the topic.

4. Update on comparative testing activities (EU-RL GMFF)

The EU-RL GMFF informed the participants on the ISO 17043 surveillance audit conducted by DakkS (the German accreditation body) on November 20th 2012. The formal assessment of the EU-RL GMFF activities resulted positive. The results of the 2011-2012 comparative rounds and the upcoming rounds for 2013 were presented; the EU-RL GMFF organises two comparative tests per year with about 100 participants per round. The EU-RL GMFF informed that the second round for the year 2012 will be launched early in 2013, due to the taking over of the management of the MILC database for on-line registration and submission of results. In the previous rounds the majority of the participants had a good performance, but some laboratories showed underperformance when results were expressed in copy number. This is mainly due to the wrong

interpretation of the CRMs certified for the mass fraction. It was suggested to use mass/mass % to prepare the calibration curves and perform the quantifications and then to convert the values in copy/copy % taking account of the zygosity statement and the DNA extractability. The colleagues from IRMM will be asked to draft a guidance document on the correct use of CRMs and the conversion of results expressed in mass/mass % to copy/copy %.

5. Comparative Testing (CT): discussion on practical needs of NRLs and other GMO control laboratories regarding the type of test item and GM events to be tested
The EU-RL GMFF and the Advisory Board for Comparative Testing agreed to provide samples for the comparative tests that would be more similar to the samples analysed during the NRLs' daily activities. Some NRLs questioned the mandatory participation in CT since they consider it difficult to perform analyses on samples that are outside the range of their specialisation and outside their scope of accreditation. The chairman commented that, given their coordinating role, the NRLs should be able to perform all type of analyses and noted that their competences should be defined by the Competent Authority of each Member State. It was stressed that the EU-RL GMFF does not have the authority to address the issue and it was promised to flag the reported legal aspects to SANCO. It was suggested to perform a survey among laboratories to compile a list of samples/matrixes analysed.

It was also pointed out that there is lack of DNA extraction reference methods, especially for complex materials and it was suggested to consider adding a section on validated DNA extraction methods in the JRC Compendium of Reference Methods (http://ec.europa.eu/dgs/jrc/downloads/jrc_reference_report_2010_11_gmo_analysis_compendium.pdf).

The chairman noted that the EU-RL GMFF would not have the capacity to test all DNA extraction methods, but that it could collect the protocols from the NRLs to share successful experimental approaches. It was also commented that this task could be taken on board by the ENGL Advisory Group on selection of methods for validation.

6. Comparative Testing: provision of official control samples by NRLs to the EU-RL GMFF for use in future CT rounds
The EU-RL GMFF asked about the possibility to provide real samples (leftover of samples analysed by the NRLs in their role of official control) for creating a bank of control samples to be used in CT. It was specified that the EU-RL GMFF would need at least 6 Kg of material for each type of sample. The participants noted that the NRLs would not have the required quantity and could provide, at a maximum, 2.5 kg of material and in most cases samples of only 500-600 grams. It was also proposed pooling different feed samples to provide a sufficient amount of official control samples to the EU-RL GMFF.

7. Proposal for minimum criteria for NRLs under Reg. (EC) No 882/2004 to implement the requirements of the labelling rules and emergency decisions
The request stems from a request made by the Food and Veterinary Office (FVO) conducting inspections in NRLs and Official Control laboratories of different MS. Following this request, DG SANCO asked the EU-RL GMFF to draft a guidance document specifying minimum requirements for NRLs nominated under Reg. (EC) No 882/2004. The comments on this document will be discussed at the next SC meeting taking place in March 2013. Once there is agreement, a kind of checklist could be made available to help FVO experts during their inspections.

The participants raised the issue on distinguishing between official control laboratories and NRLs. According to the legislation, NRLs should be able to perform all tests and not only the analyses related to their specialisation. This issue was previously discussed and needs to be addressed by DG SANCO. It was asked if there was a legal reference specifying that the NRL should have a screening methodology for identifying unauthorised GMOs. The chairman replied that when there is a Commission Decision for unauthorised GMOs, NRLs should be able to enforce it.

8. Discussion on NRLs training needs specific to their function as NRL (e.g. unit of measurement and conversion between measurement units, preparation of 0.1 % matrix samples, estimation of measurement uncertainty, quality management)

The chairman asked if the participants agreed with the indicative list of likely needs or if there

were other elements to be added. The participant answered that a guidance document (under preparation) could be enough to solve the issue on measurement units and that they would evaluate further training needs once this guidance would be made available.

For the in-house preparation of reference material at 0.1 % GM level it was reminded that there is a procedure described on the EU-RL GMFF web page (<http://gmo-crl.jrc.ec.europa.eu/doc/ENGL%20MV%20WG%20Report%20July%202011.pdf>).

Quite a few NRLs encountered problems in using the procedure, especially when American Oil Chemists' Society (AOCS) reference materials. It was pointed out that the legislation demands the provision of reference materials but does not specify its quality requirements and that this should be corrected in the legal text. Moreover, for certain GMOs, AOCS remains the only organisation providing 100 % CRMs. It was asked to assure continuity in the supply of these materials.

The chairman asked to have a list of the defective reference materials. The issue of quality of AOCS CRMs will be flagged to DG SANCO by the EU-RL GMFF.

It was also specified that in the flexible accreditation scope the uncertainty needs to be measured on all methods used by the laboratory and that a guideline for its determination is available (http://irmm.jrc.ec.europa.eu/reference_materials_catalogue/user_support/Documents/eur22756_en.pdf). From the comparative testing data it appeared that laboratories use quite different approaches to estimate the measurement uncertainty and present highly variable results. The chairman underlined that the measurements of uncertainty should be harmonised for all NRLs and that it would be useful to have more clear guidelines.

NRLs generally agreed that they needed training on the flexible scope.

9. Control samples

The EU-RL GMFF prepares and distributes positive and negative control samples to all ENGL members in the form of plasmids containing single GM targets for authorised GM events. The EU-RL GMFF asked whether the plasmid control samples are useful and effectively used. The participants appreciated the service provided and stated that ideally it would be useful to receive reference plasmids that can be used for calibration (i.e. with a certified number of molecules). The plasmids were considered especially valuable when CRMs are still not available. It was highlighted that there are no validated methods available for detecting viral contamination and it was proposed to modify the plasmid sequences to distinguish between a positive control sample and a carry-over contamination. The Advisory Group on selection of methods for validation should recommend which targets should be suitable for this scope.

Annex 5 1st Workshop NRLs 1981 Report

Text from the official meeting report written by EUROPEAN COMMISSION, JOINT RESEARCH CENTRE, Institute for Health and Consumer Protection, Molecular Biology and Genomics Unit.

1st WORKSHOP OF NATIONAL REFERENCE LABORATORIES

REGULATION (EC) No 1981/2006

December 2012, Ispra, Italy

MEETING REPORT

1. Welcome and approval of the agenda

No point was risen on the agenda (Annex 1)

2. Tour de table

Representatives of the NRLs present (Annex 2) at the meeting were asked to introduce themselves and give a short description of their tasks, report on how often they undertake validation ring trials, and their experiences with them.

Reported below are the main comments made:

- In general NRLs try to participate to as many validations as possible, nothing relevant to point out on the validation itself.
- NIB (SI) observed that the best solution is to ship the samples on Tuesday due to occasional problems with postal service; if shipments are done on Wednesdays, the risk that packs are received only the next Monday increases.
- The UK NRL asked if it would be possible for interested laboratories to participate in a validation studies for which they were not randomly selected, without receive the financial contribution. The EU-RL GMFF replied that it could in theory be possible, but due to the fact that samples are prepared and available in limited amounts, it would be possible only for a limited number of laboratories and it would have to be decided on a case by case basis.
- NRL from Poland asked whether all NRLs have to participate to comparative testing rounds organised by the EU-RL GMFF. The EU-RL GMFF replied that this point is on the agenda and will be discussed later on.

3. Revision of Regulation (EC) No 1981/2006

The EU-RL GMFF summarised the main modifications foreseen for the ongoing revision of Regulation (EC) NO 1981/2006

Changes will concern:

- the financial contribution due by applicants to the EU-RL GMFF for method validation;
- the list of NRLs assisting the EU-RL GMFF in validation studies;
- requirements for NRLs.

The financial contribution requested to the applicants has the purpose to support the costs of the EU-RL GMFF for validation of the submitted methods. It is only a partial contribution and currently the rules are:

- the flat rate contribution due at method submission, that supports the costs for data analysis and in-house testing (steps 1, 2 and 3), of 30000 €;
- for single lines, an additional contribution of 60000 € when an interlaboratory trial is needed;
- for stacked GMOs with one or more methods that need to be validated with an inter-laboratory, a flat of 30000 € is due plus 60000 € for each new method that needs to undergo an interlaboratory trial.

The current proposal of the EU-RL GMFF to update the fees for method validation takes into account the annual inflation rate, a higher staff costs, balanced by lower costs of means and a higher operational efficiency. Consequently, the proposal of the EU-RL GMFF is to update the fees according to the following:

For single events, an increase of the flat rate contribution of 30000 €, due to higher complexity of dossier assessment, including bioinformatics analyses, and higher costs for testing due to the introduction of the regulation on low level presence (LLP). An increase of the 60000 € additional contribution, due to higher costs of data analysis and increased costs for testing following the introduction of the regulation on LLP.

For stacked GMOs, for which the methods for all events have already been previously validated, a decrease flat rate contribution and additional fees whose value would increase with the number of events composing the stacked GMO.

For stacked GMOs for which one or more methods need to undergo a full validation, a flat rate contribution (as for single events) and an additional contribution for each validation are required.

For what concerns Annex I to the regulation, the requirement for laboratories to be accredited under ISO 17025 will remain the same, while the current provision of 'being in the process of accreditation' will be clarified with a time limit.

It was clarified that NRLs should be accredited specifically for GMO testing.

Concerning Annex II to the regulation, it was noted that there are currently 72 laboratories in the list of NRLs, but some have merged or no longer operate. As a result, the EU-RL GMFF currently has 62 laboratories in the list of NRLs. This list will be revised by SANCO, for which the contact point is Mr. Joachim Bollman

The revision of the Regulation is foreseen for June 2013. Laboratories that are not accredited under ISO 17025 and those losing the accreditation for a short period cannot be on the list of NRLs. The expiration of an accreditation should however be foreseen and prevented.

No increase is foreseen of the amount of the financial contribution paid to laboratories that participate in a validation study. The current amount was calculated on the basis of the results of a survey performed two years ago. A new survey could however be performed.

4. Participation of 1981/2006 NRLs to Comparative Testing organised by the EU-RL, and skills to be tested

The EU-RL GMFF introduced the topic by clarifying that the participation to comparative tests (CT) organised by the EU-RL GMFF in the context of Regulation (EC) NO 882/2004 is not obligatory by law; however, the important element is the ability to implement new methods, not normally in use.

The possibility to participate to other proficiency tests instead of to the (CT) of the EU-RL GMFF was discussed. The advantage to participate to EU-RL GMFF CT is that, unlike other proficiency testing schemes, all NRLs are compared to each other, in a clearer and more comprehensive situation.

A question was raised on the link between validations and CT schemes. In CT the whole procedure from DNA extraction to GM quantification is tested, while validation studies normally cover only the PCR module. It was noted that Regulation (EC) No 1829/2003 provides that the method includes the whole analytical procedure; the fact that the EU-RL GMFF mainly validates only the PCR method is linked to the kind of applications received, but it does not exclude that validation of the entire procedure, including the DNA extraction method, is needed, at the discretion of the EU-RL GMFF and on case-by-case basis.

It was underlined that the results of the CTs are not reported to SANCO, but they are useful to increase the efficiency of the laboratories.

Some NRLs argued that unsatisfactory results in the CT could have a negative impact on ISO 17025 accreditation during audits.

5. Invoicing the EU-RL for validation studies and other financial/administrative arrangements

The EU-RL GMFF informed that, according to a new procedure, invoices for the financial contribution due for the participation to a validation study will have to be sent within maximum four weeks after the completion of the validation study. Invoices sent later than four weeks after the completion of the validation study will not be paid.

6. Update from the EU-RL on validation studies

The EU-RL GMFF updated the participants on its activity on validation studies. During 2012 six validation reports have been published, one on a stack events, two on maize single lines and three on soybean single lines. Four more reports are planned be published by the end of the year 2012.

The contents of the validation report have been extensively modified, and include:

- data on optimisation of the method by the applicant
- data on performance of the method during the EU-RL GMFF in-house testing (step 3 of the validation flow)
- list of PCR instruments used
- data of repeatability (RSDr %) at 0.1 % concentration, implementing the LLP requirements

During 2012 six validation studies were performed and two more are scheduled for December 2012, after the meeting.

It was reminded that the NRLs participating to a validation study should always compile the report of deviations and provide it to the EU-RL GMFF, also in case no deviations are reported.

It was conformed that the selection of laboratories is done randomly; however, a mechanism to correct the pure randomness is in place, ensuring that laboratories are selected at least once every five times they have responded positively to an invitation to participate.

It was highlighted that together with the expression of interest in response to an invitation, the EU-RL GMFF needs to receive the following information:

- acceptance of deadline
- agreement with sum of 2400 €
- acceptance of the technical annex

Finally, an update on the distribution of plasmid control samples was provided; the plasmids control samples have been delivered according to the requests and for each validated method newly published.

7. Feedback from NRLs to the EU-RL on collaborative trials (improvement, changes, etc.)

The topics of this section have already been discussed under 'Participation of 1981/2006 NRLs to Comparative Testing organised by the EU-RL and skills to be tested' and during the tour de table.

8. Training needs for NRLs

The EU-RL GMFF announced that a GMO detection hands-on training for two NRLs (one person per NRL) can be organised in. A total of five-six persons can participate to this laboratory training, but the costs can be entirely covered only for two.

Other possible topics for specific training are sample preparation for comparative testing and organisation of CT studies (including accreditation requirements) new technologies and flexible scope of accreditation.

9. AOB and conclusions

The meeting was closed without additional points.

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RIKILT report 2013.006



RIKILT Wageningen UR is part of the international knowledge organisation Wageningen University & Research centre. RIKILT conducts independent research into the safety and quality of food. The institute is specialised in detecting and identifying substances in food and animal feed and determining the functionality and effect of those substances.

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