European Commission

Report

15th WORKSHOP OF GMO NRLs

JRC Ispra, 30 September 2019

The European Commission's science and knowledge service

Joint Research Centre

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Session: National Reference Laboratories

1) Welcome, and approval of the agenda
The Chair welcomed the participants (annex 1). The agenda was approved without modifications.

2) Approval of the 14th Workshop report
The report of the last meeting was adopted without amendments.

3) Update on comparative testing activities *(W. Broothaerts, JRC)*: results 2019 and plan for 2020
An update was provided on the proficiency tests (PT) organised in 2019. Each PT consisted of two test items, the preparation of some of them required extensive processing work. The assigned value was determined by two expert laboratories and using different extraction methods (CTAB extraction & Nucleospin food). An adapted extraction method (2% CTAB) was used for the cotton event. The σ_{pt} was chosen as 25% of the assigned value determined on the raw reported results (which corresponds to 0.1 on the log scale).

The performance of the laboratories was evaluated on the raw data (not on the log transformed data as explained in a separate presentation). The *z* and zeta scores were calculated and the uncertainty of the participants' results was evaluated and classified in three performance groups (a, b, c).

In the PT 19/01 some unsatisfactory *z* scores were observed for results on the NK603 maize event in T1 (9). It was found that 13 laboratories still used the *adh1*-70 bp reference gene method, which resulted in four unsatisfactory and two questionable scores, and all values were equal to or above the assigned value. Mismatch priming and allelic variability could explain the bias. As remarked in many ENGL meetings this *adh1* method should not be used for official control. In the analysis of test item T2 only one lab obtained an unsatisfactory *z* score for the result on the maize 4114 event.

In the PT 19/02 two unsatisfactory *z* scores were obtained for results on the soybean event GTS 40-3-2, seven for data on the soybean event MON87708 and one for the result on cotton event GHB119 (but GM cotton was not analysed by 60 % of the laboratories). All results were satisfactory for maize event DAS-44406 in the T2 test item. The results obtained on soybean event MON87708 were plotted against the DNA extraction methods used by the laboratories but there was no clear correlation between the extraction method and the performance scores for measurements of MON87708.
In both PTs between 73% and 81% of the compliance statements were correct.

The speaker informed that in 2020 the test items would consist of bird feed fat balls (T1) and maize flour (GA21, MON88017, T25 maize) (T2) for the first PT and meat paté (frozen) with soybean MON89788 (T1), and rapeseed meal (all GM oilseed rape events to be tested) (T2) for the second PT. The reporting deadlines will be 20/04/2020 and 17/07/2020, respectively.

The speaker raised questions on the variability of results observed for the detection of soybean MON87708 and wondered why many laboratories failed to analyse the test item for GM cotton events. Suggestions were requested for matrices to be used in the PTs of the year 2021.

Follow-up discussion
Some participants commented that GM cotton was not tested by their laboratories because cotton is not used for food or feed in the respective MS. Others appreciated the inclusion of GM cotton in

the test materials because their laboratory had comprised cotton GM events in their accreditation status or because it is usually found in feed or the demand for GM-free cotton clothing is increasing.

Overall, they also appreciated the use of simple and complex matrices and the inclusion of CRMs from AOCS in the PTs, and considered the fast reporting of the EURL GMFF very useful for accreditation.

The speaker clarified that negative results or results below the LOQ of the method should be reported as "below [LOQ]". He also remarked that all GMOs included in the PT test items are authorised or fall under Regulation (EU) No 619/2011, meaning that validated detection methods and CRMs are available. A list of authorised GM events can be retrieved from the EU GMO Register (https://webgate.ec.europa.eu/dyna/gm_register/index_en.cfm).

A participant questioned the calculation of the measurement uncertainty (MU) from the mean values of the expert laboratories. In his opinion, the individual uncertainties from the expert laboratories should be used. Another question concerned the potential use of dPCR for obtaining the assigned values in the future.

Some representatives requested to simplify the PT questionnaire to avoid redundancy in the information requested for different test items.
The Secretariat wondered if the quality of the data using different extractions method had been verified. A participant from Austria remarked that the same results have been obtained for the three different extraction methods used in the PTs except for MON87708 where an absolute difference of 3 m/m % was observed between a standard CTAB method and the automated Maxwell system with CTAB lysis buffer. The speaker pointed to the importance of the lysis step for highly processed PT samples, and commented that commercial kits with columns may not bind small (degraded) DNA fragments.

The Chair requested suggestions for the PT rounds planned for 2021. The participants requested to provide an easy material and mixtures of highly processed matrices containing more than one GM event to mimic real life situations, real samples rather than spikes, including GM events for which the CRMs are provided by AOCS. In addition, they suggested considering events frequently occurring in the market, using fish or pet feed as they contain animal proteins and fat, and GM rice varieties, sugar beet or potato GM events. The Chair commented that there is no GM rice or potato authorised (or falling under Regulation 619/2011) in the EU.

4) Tour de table: issues/opinions/training needs from NRLs

Follow-up discussion

Some participants mentioned the planning of the purchase of new dPCR or NGS instruments or setting them up in their laboratory, while others are already using the technology, mainly for species identification or bacterial genome sequencing.

Several participants requested training on dPCR, NGS sequencing and bioinformatics analysis. Some participants reported problems in extracting DNA from complex matrices and suggested organising another workshop on DNA extraction. Some members would appreciate sharing information on screening approaches, on the detection of new breeding technique products or on sampling and accreditation. Only one participant reported problems in detection of low-level presence of GMOs in feed, or calibration curves or changes of reagents' quality for dPCR. A representative requested training on multiplex PCR.

One participant noted that the concentration reported for the plasmid standards was lower than stated on the label. The EURL GMFF explained that the concentration is only indicative and that the material should not be used for LOD determination but only for qualitative purposes.

A participant from France reported that his Competent Authorities (CA) agreed with the DG SANTE approach of reporting results for stacked events. However, they argued that the decision on conformity should be taken at EU level to avoid different interpretations by Member States. A guidance document at ENGL level on how to report results on stacked events would be useful.

DG SANTE recommended providing as detailed information as possible on the content of the GM events in the sample, but reminded that it is the task of the Competent Authority to decide about

compliance (they have more information to consider) and that EU harmonisation should be achieved at the level of Competent Authorities.

5) Update on NRL Training Workshop Nov. 2019

The JRC informed that a two days training workshop on dPCR will be offered on the 12th-13th of November at JRC Geel. The training is open to 30 participants and will cover two topics:
1) transferring a method from a qPCR into a dPCR format for accreditation. The laboratories preparing for accreditation will share their experiences;
2) conversion of dPCR results from DNA copy number into mass fractions. The workshop will offer hands-on practical examples.

6) AOB and conclusions

The Chair clarified the rules for reimbursing participation to the NRL workshops and the ENGL meetings. He announced that the JRC can only reimburse one NRL participant for each Member State for participation to both meetings and for the ENGL Plenary only one person per ENGL Member institution if this is not already reimbursed as NRL. For the current year, the JRC will be able to support participation for invited speakers. It has to be seen if this option will be available also in the future. The Chair declared to be impressed by the high number of participants and closed the meeting.

Annex 1: agenda

15th Workshop of the GMO NRLs and JRC Ispra, 30 September

Auditorium, Building 58c

30 September 2019

Session: National Reference Laboratories and Official Control Laboratories

AP	Time	Topic	Documents/comments
1 2 3	14:00	▪ Welcome, and approval of the agenda ▪ Approval of the 14th Workshop report ▪ Update on comparative testing activities *(W. Broothaerts, JRC)*: results 2019 and plan for 2020 Follow-up discussion	Draft agenda Report Presentation
	15:30	*Coffee Break*	
4 5 6	16:00 17:00 17:15	▪ Tour de table: issues/opinions/training needs from NRLs (each NRL is invited to report orally) Follow-up discussion ▪ Update on NRL Training Workshop Nov 2019 ▪ AOB and conclusions	Oral input from NRLs
	17:30	*End of day 1*	