



Report of the Training Workshop on DNA Extraction from Food and Feed

Ispra, 7-9 June 2017

Agenda: Annex 1.

Within its mandate under Regulation (EC) No 882/2004 the EURL GMFF organised a training workshop on **DNA extraction from food and feed** that took place at the Ispra site of the JRC (Building 100/Room 1003 - Acqua) from 7th June 2017 at 09:15 h to 9th June 2017 at 12:45 h.

This EURL training workshop (with assistance from LGC, UK) was aimed at providing a forum to discuss and agree best analytical practices in the area of DNA extraction for GMO analysis. The programme included presentations on the selection of suitable DNA extraction methods for a given matrix, assessment of DNA quality and quantity, validation of extraction methods and troubleshooting experiences, and an exhibition from major companies which provide DNA extraction kits/instruments.

Each participant was requested to talk on their own experiences on DNA extraction, on examples of problematic issues, and on future requirements in order to learn from each other and to maximise the utility of the workshop. For this reason every participant was requested to prepare a few slides in line with a provided ppt template and to present them during the event (approx. 10 min).

Participation to the event was open to NRLs and EU official control laboratories under Regulation (EC) No 882/2004. In addition, up to 3 participants from developing or EU neighbouring countries were admitted. The total number of participants was 37, including 4 JRC colleagues from Ispra and Geel (+ some others for specific sessions), and 3 external experts. In addition, company representatives from 3 providers of kits/instruments in the field of the workshop topic joined the first day.

Main messages

1. GMO testing requires two steps: 1. the extraction of DNA, 2. the quantitation of the GM and non-GM DNA. The extracted DNA (for quantitation) has to represent the DNA in the sample as provided to the laboratory, mainly in terms of the ratio of GM copies and non-GM copies of DNA (as a relative, i.e. ratio, result is required). The first step in this analytical process is insufficiently controlled.
2. The modular approach is only valid if the assumption of both representativeness and amplifiability of GM and non-GM DNA in the DNA, extracted by whatever method, is guaranteed.
3. To ensure this, validation of the whole procedure (possibly also step-wise) and quality control need to be done. Minimum performance criteria for extraction methods may be used, and the current MPR document is a starting point for this. Further more detailed guidance may be needed.

4. The EURL GMFF validates the DNA extraction methods provided by Applicants, but these are for ground seeds; other matrices may require modifications or a different extraction method adapted to the matrix. The participants/ENGL agreed to collect and share positive and negative experiences and discussed best practices for DNA extraction from several food and feed matrices. This fostered a better understanding of why a certain method or kit works (or does not work).

Outcome – next actions

1. The workshop has been successful and participants shared their experiences and learned from each other in terms of selection of methods for particular matrices or for matrices sharing similar chemical compositions (fat, carbohydrate, proteins) which result in a similar extraction behaviour for DNA. Tips and tricks have been exchanged and the importance of a thorough quality assessment of the extracted DNA is better understood.

2. The discussions have highlighted the need to set up an ENGL working group on DNA extraction; the mandate of this group may be drafted for further discussion during the upcoming ENGL SC. The outcome of the WG could be a guidance document on 1) selection of a DNA extraction method according to the matrix composition, 2) the validation parameters and approaches for DNA extraction methods and 3) establishing acceptance criteria for DNA extracts for qPCR analysis. A web-based discussion forum and compendium of positive and negative experiences on the ENGLnet may be useful to support the GMO testing community.



Training workshop on DNA EXTRACTION FROM FOOD AND FEED

7-9 June 2017, Ispra, Italy
(Room 1003/Acqua – Building 100)

Organised by
the EU Reference Laboratory for GM Food and Feed, Ispra (W. Broothaerts)
with support of LGC, UK (M. Burns)

Day 1: Wednesday 7 June 2017

Time	Topic	Documents
9:15	Welcome and introduction to the workshop <ul style="list-style-type: none"> ▪ Objectives of the workshop ▪ Expectations: tour de table ▪ Programme ▪ Introduction to DNA extraction principles (M. Burns) ▪ DNA extraction in EU legislation, ISO and Codex and the modular approach (M. Mazzara) 	Programme Presentation Presentation
10:30	<i>Coffee Break</i>	
11:00	<i>Methods for DNA extraction from plants, food and feed (chair: W. Broothaerts)</i> <ul style="list-style-type: none"> ▪ Company presentations on DNA extraction products: Biotecon Diagnostics, Macherey-Nagel, Promega 	Presentations (3)
12:45	Buffet lunch + product exhibitions	
14:00	<i>Selection of a DNA extraction method (1) (chair: Ph. Corbisier)</i> <ul style="list-style-type: none"> ▪ LGC experiences with DNA extraction and quality metrics for extracted DNA (M. Burns) ▪ Different approaches for DNA isolation for different matrices and DNA quantification approaches (Th. Prins) ▪ Survey on DNA extraction methods (W. Broothaerts) ▪ Observations on DNA extraction and DNA quality analysis from comparative testing (W. Broothaerts) ▪ Comparison of 3 DNA extraction methods on a CT test item (R. Hochegger) 	Presentation Presentation Presentation Presentation Presentation
16:00	<i>Coffee Break</i>	
16:30	<i>Selection of a DNA extraction method (2) (chair: Ph. Corbisier)</i> <ul style="list-style-type: none"> ▪ Decision support system NIB, Slovenia (T. Demšar) ▪ Development and use of a selection key for sample preparation and extraction (F. Debode) ▪ Identifying inhibitors/enhancers of qPCR in food samples using a synthetic plasmid (J. Ovesna) ▪ Discussion and wrap up of day 1 	Presentation Presentation Presentation
17:45	End of day 1	
19:30	Social dinner at Restaurant Il Melograno - Angera	

Day 2: Thursday 8 June 2017

Time	Topic	Documents
09:15	<i>Validation of a DNA extraction method (chair: W. Broothaerts)</i> <ul style="list-style-type: none"> ▪ EURL GMFF experience with validation of DNA extraction methods (F. Gatto) ▪ Some suggestions for DNA extraction methods validation/comparison (W. Broothaerts on behalf of P. Philipp) ▪ Guidelines for validation of DNA extraction methods (L. Grohmann) 	Presentation Presentation Presentation
10:30	<i>Coffee Break</i>	
11:00	<i>Participant experiences (1) (chair: M. Burns)</i> <ul style="list-style-type: none"> ▪ DNA extraction methods used ▪ Approach for selection of a method ▪ Issues and troubleshooting 	Short presentations
13:00	Buffet lunch	
14:00	<i>Participant experiences (2) (chair: M. Burns)</i> <ul style="list-style-type: none"> ▪ DNA extraction methods used ▪ Approach for selection of a method ▪ Issues and troubleshooting 	Short presentations
16:00	<i>Coffee Break</i>	
16:30	<i>Participant experiences (3) (chair: M. Burns)</i> <ul style="list-style-type: none"> ▪ Wrap up and trends ▪ Discussion Experiences of the EURL GMFF on the evaluation of the quality of extracted DNA (C. Savini)	Presentation
17:45	End of day 2	
	Free evening	

Day 3: Friday 9 June 2017

Time	Topic	Documents
09:15	<i>DNA extraction and downstream applications (chair: M. Mazzara)</i> <ul style="list-style-type: none"> ▪ DNA extraction for NGS downstream applications (N. Papazova) ▪ DNA extraction in function of ddPCR experiments, compared to qPCR experiments (M. Deloose) ▪ Discussion 	Presentation Presentation
10:45	<i>Coffee Break</i>	
11:15	<i>Minimum performance requirements for DNA extraction and role of EURL/ENGL (chair: M. Mazzara)</i> <ul style="list-style-type: none"> ▪ Discussion ▪ Action points from participant experiences ▪ AOB and wrap-up ▪ Feedback on workshop 	
12:45	Sandwich lunch	