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

Report of the

Hands-on Training on digital PCR

Ljubljana, 29 November to 1 December 2017

Programme: See Annex 1.

In line with its tasks under the Official Control legislation, the EURL GMFF organised a training for NRLs on digital PCR (dPCR) in Ljubljana, Slovenia, from 29 November to 1 December 2017. This was the second training organised by the EURL GMFF in 2017, and the third training or workshop for NRLs on the topic of digital PCR organised since 2014. The first training workshop in 2014 was both theoretical and practical and included invited presentations from two commercial companies selling dPCR instruments (RainDance and Life Technologies). The second training workshop in 2015 dealt with both dPCR and Next Generation Sequencing (NGS), and included company presentations on the QuantStudio 3D (Applied BioSystems) and Naica instruments (Stilla), and laboratory demonstrations on both systems. The current training was directed particularly to laboratory scientists or technicians and combined an introduction to the basics of the technology and the data evaluation with extensive hands-on work on different dPCR instruments, including the evaluation of the data (Annex 1).

The training was co-organised by the JRC and the National Institute of Biology (NIB) in Ljubljana, the NRL for GMO Control in Slovenia, who offered to host the event at their institute. NIB had organised a similar dPCR training in 2016, and possessed both the necessary expertise and laboratory facilities to act as the host for this training. NIB colleagues have published several papers on the optimisation of dPCR methods and their application to GMO analysis, including on multiplexing approaches. The laboratory has several dPCR instruments, including a QX100, QX200, BioMark HD and (temporarily) a Naica system from Stilla. The JRC managed the overall design and the invitation, registration and reimbursements, and provided additional expertise related to method validation and measurement uncertainty estimation and the statistics behind the technology, and a demonstration of a data analysis excel template.

A maximum of 20 participants from 20 NRLs in 15 EU Member States were allowed to participate to the training. Experts from the local organiser and from the JRC (Ispra and Geel) provided the training. A questionnaire distributed a few weeks before the start of the training collected information on the experience of the participants with qPCR and dPCR, the kind of instrument(s) in the laboratory, the expectations for the training and topics to be addressed. The presentations during the training were adapted to address most of the questions and interests of the participants as indicated in the survey. The majority of participants was active in the laboratory, and had between 6 months and 15 years of experience on qPCR, mainly on GMOs. Twelve out of 20 participants had no experience with dPCR, 4 had some experience, and 4 had between 3 and 5 years of experience. The expectations of the group varied between learning the basics of the technology and the advantages and disadvantages of

different platforms to knowing the critical performance parameters for data evaluation and validation of methods, and experiencing the setup and required handlings on the different instruments, as well as how to evaluate and interpret the data obtained.

After introductory presentations on the JRC and the mandate of the EURL GMFF to organise training for NRLs (JRC) and on the organisation and activities of NIB (NIB), the participants presented themselves and clarified their experience. A NIB colleague explained the basic concepts of dPCR, the underlying assumptions, and how it evolved from previously developed PCR principles. Some hints were given to optimise methods transferred from qPCR to dPCR. The next presentation (NIB) presented the different digital droplet and digital chamber platforms available on the market and their advantages and disadvantages. Variables to consider when buying a dPCR instrument are the total number of partitions (droplets or microchambers), the number of reactions per run, the number of fluorescence channels, the need for use of a proprietary master mix, the optimal concentration range (copies per µL), and the total time required for a certain number of samples. The speaker also discussed a number of general recommendations when setting up of a dPCR experiment.

The success of a training also depends on the interactions among and with the participants. To stimulate this, the participants were asked on beforehand to present some of their own experiences to the group. Four participants accepted to provide a short presentation. A speaker from ILVO, Belgium reviewed their laboratory activities involving use of dPCR. dPCR is used for the absolute quantification of micro-organisms in cattle faeces, GMO detection and quantification, and mainly for food allergen detection and quantification. She presented the results of an experimental comparison between qPCR and ddPCR, using three different master mixes, and reviewed the parameters to consider when transferring a qPCR method into a ddPCR method.

A speaker from NIB presented the Metrofood project on Research Infrastructures aimed at promoting metrology in research on food and nutrition. This initiative involves 40 partners from 18 EU Member States and has links to many other countries.

The second day of the training was mainly devoted to the hands-on part of the training. The group was split into two groups, where each followed parallel sessions in the laboratory, then switched. The experimental setting up of experiments on either the QX100/200 or the BioMark HD was explained, then carried out by each subgroup. This was followed by an introduction to the data analysis and the actual data analysis and interpretation of the results obtained. The samples were DNA extracts from an animal feed containing 40-3-2 soybean, extracted by either NucleoSpin or CTAB. Both extraction methods were compared, as well as the set up of simplex or duplex reactions (GM and reference gene in the same reaction). A demonstration was additionally given on the use of the Naica ddPCR instrument. A speaker from NIB presented the experience of NIB with the Naica instrument versus the QX100/200. A colleague showed the possibilities for multiplexing dPCR reactions, which is possible on all platforms, and referred to his publications on this topic.

Several other participants presented their work. A speaker from IZSLT (NRL, Italy) reviewed their experiences with ddPCR for GMOs, mostly using multiplexing. They had studied the stability of endogenous reference genes among several varieties. Validation of ddPCR methods was done based on the acceptance criteria of the MPR document. A colleague from LGC (NRL, UK) explained that they have several different dPCR instruments in the laboratory, which are used for GMOs, food authenticity testing and allergen detection. An example was given on the use of ddPCR on meat samples, showing that qPCR and ddPCR were comparable, except on canned meat, where ddPCR had

a lower CV and was more accurate at low levels. A speaker from BVL (NRL, DE) referred in his presentation to the detection of SNPs using ddPCR and how the assay was optimised for discrimination of the mutant from the wildtype sequence.

On day 3, a JRC colleague first explained the use of the JRC excel sheets for dPCR data analysis under the ISO 17025 quality system. He then presented the requirements for method validation, the parameters to consider and how to measure themAnother colleague (JRC) presented some statistical considerations related to dPCR and provided guidelines for acceptability of dPCR results and how to validate such methods. He also mentioned that often qPCR methods can be transferred to dPCR methods without major changes (except master mix brand), and that for a few methods further optimisation is necessary. It was suggested to share such information within ENGL. He also provided a short overview of an application of dPCR to authenticate grapevine varieties in wine.

The training was closed by wrapping up the knowledge shared and hands-on experiences learned, particularly also through the little tips and tricks shared among the audience.

Next actions

The presentations given during the training will be shared among the participants. Also the Excel file for data analysis from JRC, as well as the file with the results of the experiments conducted during the training, will be distributed to the participants. The outcome of the training will be presented during an upcoming ENGL meeting.

Annex 1. Programme

HANDS-ON TRAINING ON DIGITAL PCR

for National Reference Laboratories (NRLs)
assigned under Regulation (EC) No 882/2004

29 November – 01 December 2017
Ljubljana, Slovenia

PROGRAMME

Day 1		
08:30	Arrival & registration	
09:00	**Opening comments & objectives & introduction**	
09:30	**Basic concepts of dPCR**	
10:15	**dPCR principles, chemistries & platforms**	
11:00	Coffee break	
11:30	**Overview on hands-on QX100/QX200**	
12:00	**Experiment setup QX100/QX200**	
13:00	Lunch	
14:00	**Hands-on QX100/QX200**	
17:00	Coffee break	
17:30	**Experiences of the participants I**	**All**
18:15	**Metrofood – infrastructure for promoting metrology in food and nutrition**	
18:30	End of day 1	

Day 2

	Group 1		Group 2	
09:00	**Introduction to data analysis QX100/QX200**		**Overview on hands-on BioMark**	
09:30	**Practical work: Data analysis and interpretation**		**Experiment setup BioMark**	
10:00			**Hands-on BioMark**	
11:30	Coffee break			
12:00	**Experiences of the participants II**			**All**
13:00	Lunch			
	Group 1		Group 2	
14:00	**Overview on hands-on BioMark**		**Introduction to data analysis QX100/QX200**	
14:30	**Experiment setup BioMark**		**Practical work: Data analysis and interpretation**	
15:00	**Hands-on BioMark**			
17:00	Coffee break			
17:30	**BioMark analysis - demo**			
18:00	End of day 2			
19:30	Dinner at Castle			

Day 3		
09:00	**MS Excel worksheet for calculation of copy number concentration**	
09:45	**Measurement uncertainties associated to digital PCR**	
10:30	**Statistics, performance parameters, and their optimization**	
11:15	Coffee break	
11:30	**Multiplex**	
12:00	**Wrap-up**	
12:30	Sandwich lunch and coffee	