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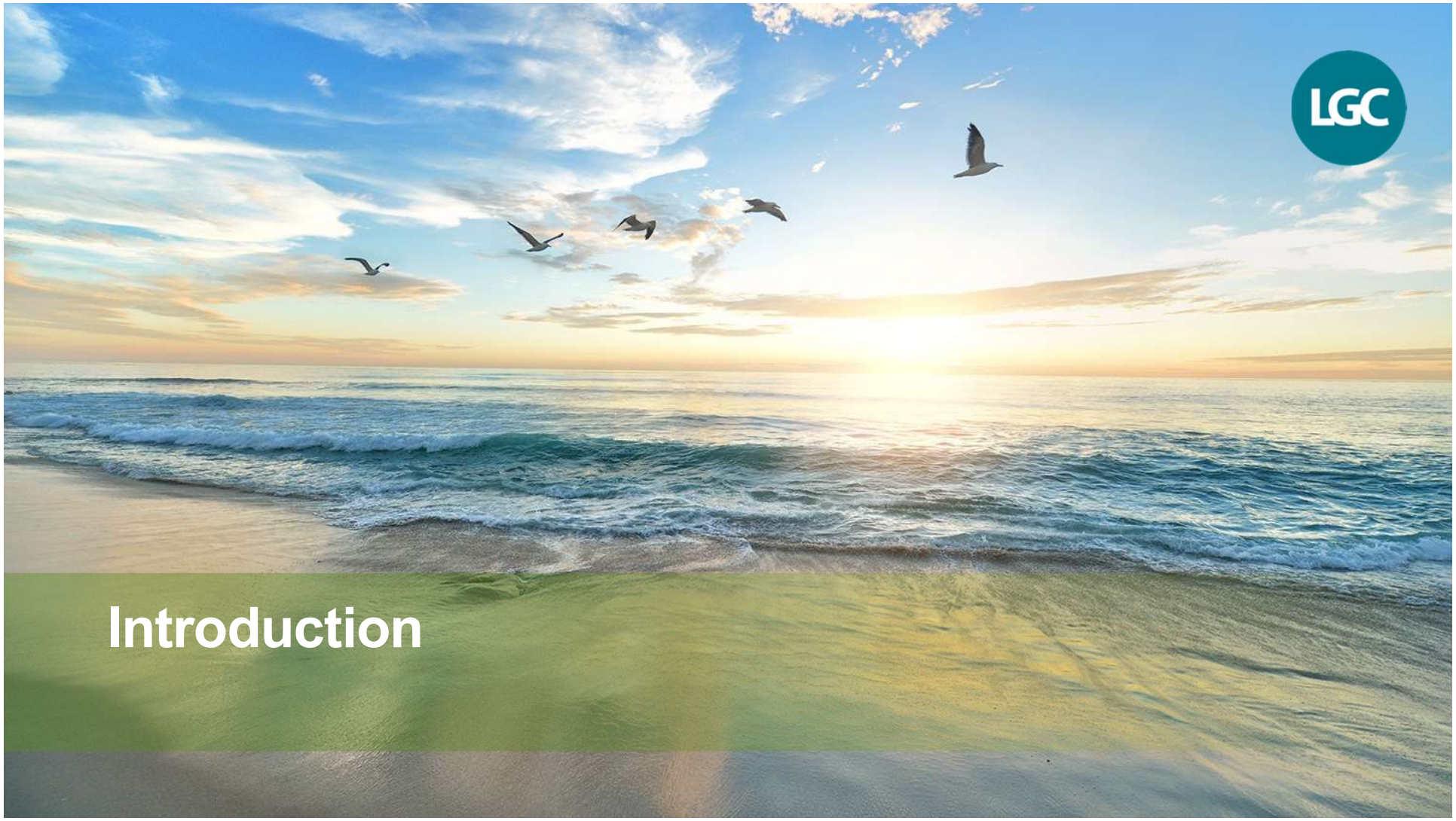
Towards effective solutions for GMO analysis

Thursday 24th June 2021

Presentation overview

- **Introduction**
  - GMO controls
- **GMO analytical challenges**
  - Past, present and future perspectives
- **GC work in support of effective solutions for GMO analysis**
- **Summary**
  - Conclusion and key take home messages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Introduction

Introduction

- **What do we mean by the term GMO?**
  - GMO: Genetically Modified Organism
  - Based on (retained) EU legislation (Directive 2001/18/EC):
    - "*an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination*"
  - Prior to ECJ (2018) ruling on products of gene editing:
    - Organism formed from association of 2+ DNA sequences from different species, using molecular techniques
- **Supporting and affording consumer choice:**
  - Consumers may not want to purchase food which contain or consist of GMOs
- **Sampling and controls associated with GMOs are subject to a number of regulatory texts**

Introduction

- **Legislation: products containing GM material must be clearly labelled**
- **For those GMOs authorised for use (placing on the market) in the UK/EU:**
  - 0.9% m/m of a particular ingredient
- **Successful labelling of food produce: dependent upon reliable, stringent, efficient way of quantifying GM at very low levels**
- **Target analyte of choice: DNA**
  - Ubiquitous; resistant to degradation; specific; qualitative/quantitative
- **Quantitative PCR (qPCR)-based analyses are the current preferred DNA-based method for routine GMO analysis**
- **EURL validated protocols for event specific detection of GM varieties provide unequivocal target identification ***

** https://gmo-crl.jrc.ec.europa.eu/gmomethods/*


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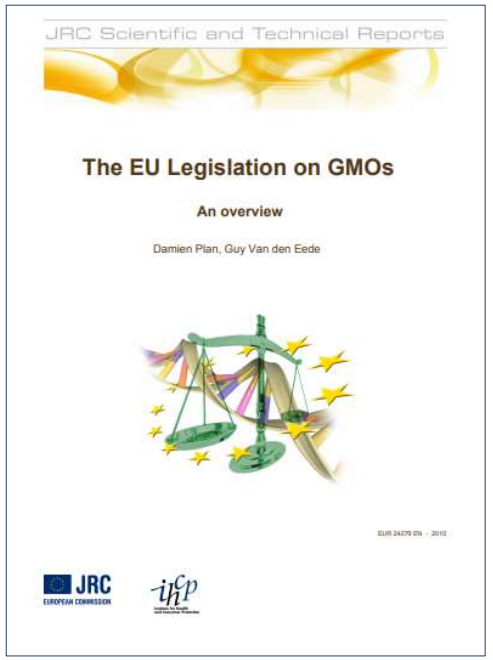
GMO analytical challenges
Past, present and future perspectives

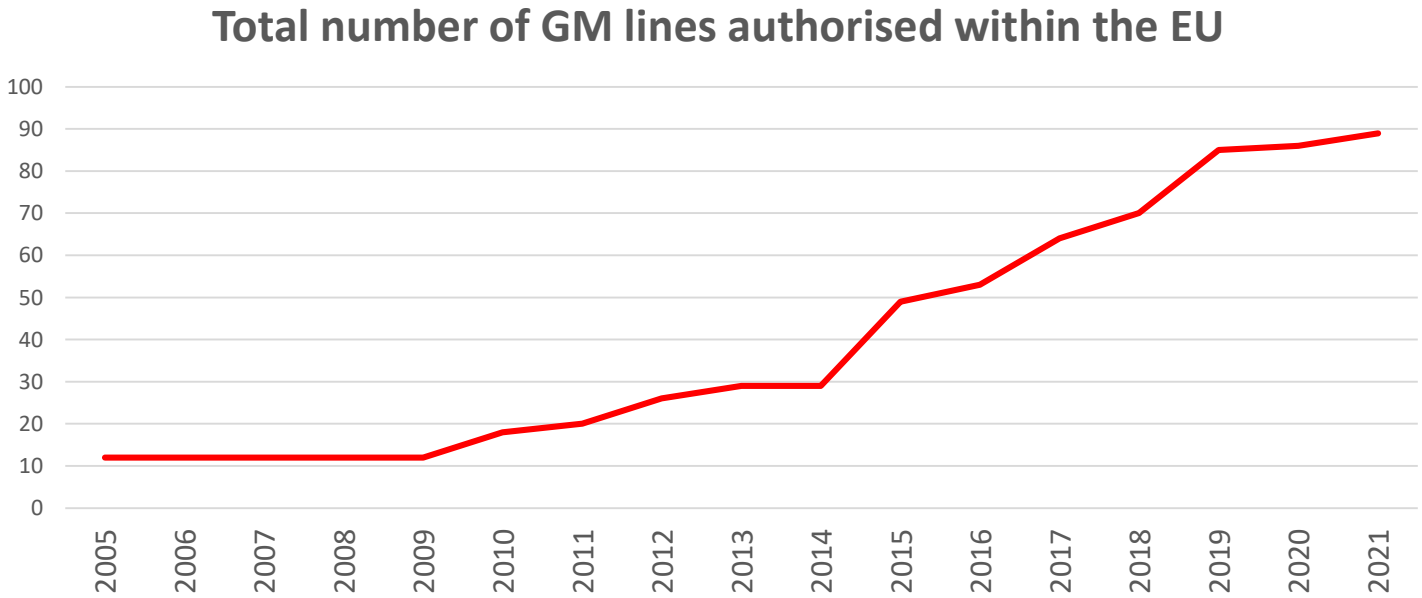
GMO analysis - past

- **2001**
  - Directive 2001/18/EC
    - Deliberate release of GMOs into the environment
  - EU: moratorium on authorisation of new GMOs (1998 - 2006)
- **2003**
  - Regulation (EC) No. 1829/2003 and 1830/2003
    - Placing on the market of GMOs
    - Traceability/labelling of GMOs
  - Two main legal mandates for GMO controls which continue to operate within UK as retained legislation following EU exit
- **2008**
  - Total number of 12 GM lines authorised within the EU
    - Identifiable using a small number of tests
    - No immediate requirement to adopt a universal GM screening approach



* ***https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/eu-legislation-gmos-overview***

GMO analysis - present



Source: EU register

- ***Based on total number of individual lines approved***
  - *Includes stacked events*
  - *Does not include different combinations of single events within a stacked event*
  - *Does not include pending applications subject to Regulation (EU) 619/2011*

GMO analysis - future

Issues
Complexity of analyses: • Number of authorised/unauthorised GM lines • Range of food/feed matrices to extract from
New techniques: - e.g. Genome editing (NGT) New GMOs: - GM animals e.g. AquAdvantage® Salmon, GloFish® New technologies: - e.g. digital PCR and NGS
Dependency on reference materials and databases
UK withdrawal from the EU

GMO analysis - future

Issues	GC approaches to providing solutions
Complexity of analyses: • Number of authorised/unauthorised GM lines • Range of food/feed matrices to extract from	• A review of harmonised **screening approaches** • **GC interpretative guidance** on testing for GM rice • Practical evaluation of **DNA extraction approaches**
New techniques: - e.g. Genome editing (NGT) New GMOs: - GM animals e.g. AquAdvantage® Salmon, GloFish® New technologies: - e.g. digital PCR and NGS	Involvement in national/international expert working groups aimed at providing harmonised and **published guidance** - e.g. European Network of GMO Laboratories (ENGL) - GE, GMM, GM animals, NGS, dPCR
Dependency on reference materials and databases	Continued close **networking** with CA's (FSA/Defra) and Official Control Laboratories on projects, support and advice
UK withdrawal from the EU	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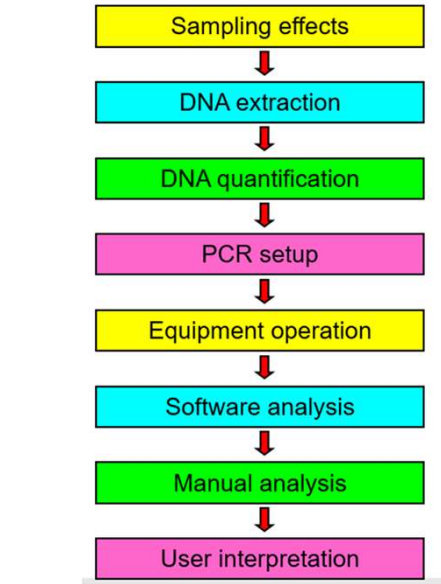
GC work in support of effective GMO analyses

ISO 17025 accreditation: automated DNA extraction

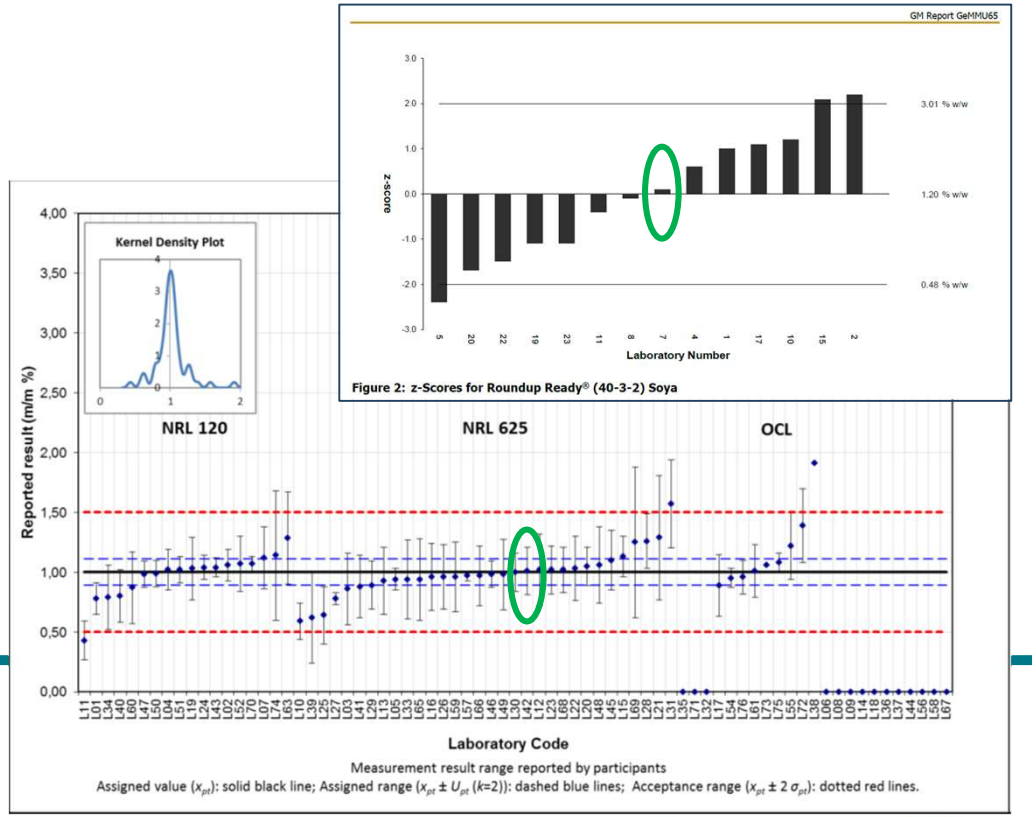
- **DNA extraction**
  - Fundamental upstream procedure prior to GMO analysis using PCR
  - Obtaining DNA of sufficient quantity and quality is critical in ensuring the success of the downstream PCR
- **Issues:**
  - Complexity of matrices being extracted from
  - Demand for increased throughput
- **Maxwell® RSC instrument**
  - Automated DNA extraction instrument
  - Increased efficiency and throughput
- **Extension to scope for ISO 17025 flexible scope of accreditation for GMO analysis:**
  - Maxwell® RSC instrument
  - Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System (augment the Applied Biosystems™ 7900HT Fast Real-Time PCR System)



Maxwell ® RSC Instrument (© Promega)

Adherence to quality management systems

- **Proficiency Test rounds**
  - Demonstrable evidence of EQA
  - Mandatory part of ISO 17025 accreditation[Δ]
  - Qualification of fitness for purpose with different DNA extraction approaches, qPCR instruments and different GM events/matrices
- **LGC participation in 59 GMO PT rounds**
  - GeMMA and EURL Comparative Tests (NRL*)
  - 95 separate GM events quantified (soya, maize, oilseed rape, cotton, rice)
  - z<[2]



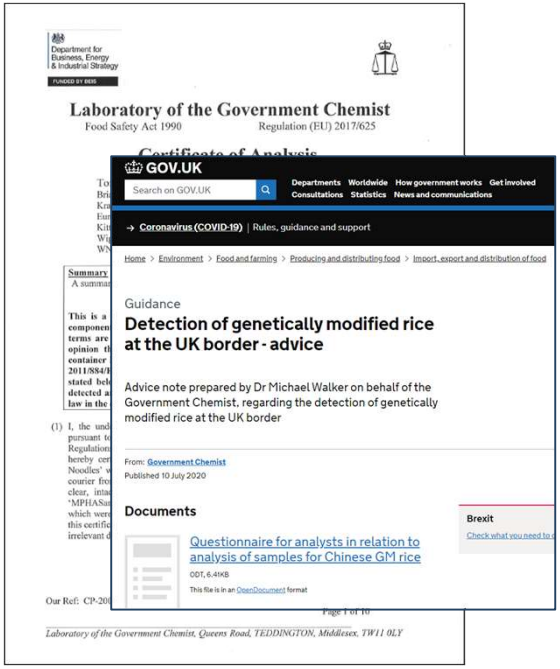
[Δ] *Jane White (President Association of Public Analysts)*

* *synergy with NRL function*

Advice on complex analyses

- **GM rice products originating from China**
  - *Paul Hancock (LGC, Referee Analyst) - Overview of recent referee cases*

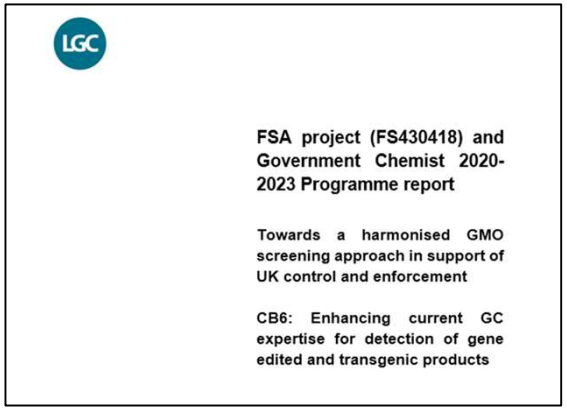
- **Previous GC programme: 6 referrals received (≈ 20% of referee cases)**
  - Ranked 2nd in terms of type of referee samples received
- **Current GC programme: ≈ 40% of the samples submitted for referee analysis are GMO related**
  - Successfully delivered and resolved disputes regarding four samples submitted to the GC function
    - COVID-19 restrictions
- **EU guidance: includes use of SYBR® Green and melt-curve analysis**
- **GC advice and published guidance**
  - https://www.gov.uk/government/publications/detection-of-genetically-modified-rice-at-the-uk-border-advice



GMO screening and DSS

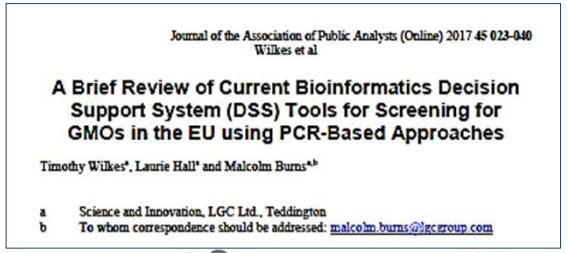
- **GMO screening review**
- **Provision of a review for effective GM screening approaches in the UK following EU exit**
- **Collaboration with the FSA**
- **Key points:**
  - No harmonised approach for screening within EU/UK
  - Challenge/opportunity presented by EU exit - withdrawal of access to some EURL services
  - Examines criteria for effective GMO screening approach
  - Provides recommendations on screening approaches to adopt to promote harmonisation

- **Decision Support System (DSS) tools**
- **Bioinformatics approaches**
- **Facilitates informed decisions regarding the least number of assays needed to provide the greatest likelihood of successfully identifying a GMO event**
  - A range of different DSS tools available to assist with PCR-based GMO analysis
  - Unclear on which DSS to use in different circumstances
- **GC publication providing guidance on which DSS tools to adopt ***


FSA project (FS430418) and Government Chemist 2020-2023 Programme report

Towards a harmonised GMO screening approach in support of UK control and enforcement

CB6: Enhancing current GC expertise for detection of gene edited and transgenic products


Journal of the Association of Public Analysts (Online) 2017 45 023-040
Wilkes et al

A Brief Review of Current Bioinformatics Decision Support System (DSS) Tools for Screening for GMOs in the EU using PCR-Based Approaches

Timothy Wilkes[a], Laurie Hall[a] and Malcolm Burns[a,b]

a Science and Innovation, LGC Ltd., Teddington
b To whom correspondence should be addressed: malcolm.burns@lgcgroup.com

* *Wilkes T., Hall L., and Burns, M. Journal of the Association of Public Analysts (Online) 2017 45 023-040*

Validation of reference materials

- **Certified Reference Materials (CRMs)**
  - *"Reference material accompanied by a certificate and authenticated by a recognised governing body to a high level of traceability and to a given estimate of uncertainty"*

- **Certification of ERM-BF411g**
  - European certified reference material for GM maize
- **GC assisted the JRC in the certification of the above reference material**
  - DNA extraction from multiple samples
  - DNA and PCR quality metrics
  - Quantitative data used to ascribe measurement uncertainty estimates

- **EC ring-trial using dPCR to value assign copy numbers to a maize MON810 CRM**
  - One of the first ever digital PCR method validation exercise as part of the EU authorisation for GMO events
  - As part of the official EU authorisation process for placing on the market of GMOs, applicants need to propose a method for detection of those GMOs for control purposes
  - Digital PCR is gaining increasing favour as a reliable, accurate and sensitive method for detection of GMOs

JOINT RESEARCH CENTRE
Directorate F – Health, Consumers and Reference Materials

REFERENCE MATERIAL CERTIFICATE

ERM®- BF411g

DRIED MAIZE POWDER		
Mass Fraction		
	Certified value [2] [g/kg]	Uncertainty [3] [g/kg]
Bt-176 maize [1]	1000	+ 0 - 53

1) Bt-716 maize is genetically modified and corresponds to the unique identifier SYN-EV176-9.

2) This certified reference material has been produced from genetically modified Bt-176 maize seeds. The certified value is based on the genetic purity of the maize seed powder with regard to Bt-176. In total 56 seeds from the whole batch were grown and tested individually for the presence of the Bt-176 genetic modification. All seeds tested positive. With 95 % confidence, the true Bt-176 maize mass fraction of the material is above 947 g/kg. The certified value is traceable to the International System of Units (SI).

3) The asymmetric uncertainty is based on the 95 % confidence interval from the Poisson distribution of the tested seeds. With 95 % confidence, the true Bt-176 maize mass fraction of the material is therefore between 947 and 1000 g/kg. If using ERM-BF411g for calibration the value 1000 g/kg with an expanded uncertainty of + 0 / - 53 g/kg should be used. The 53 g/kg corresponds to a standard uncertainty of 53 g/kg / √3 = 31 g/kg.

This certificate is valid for one year after purchase.

Sales date:

The minimum amount of sample to be used is 100 mg.

Geel, August 2020

Signed:

Dr Robert Koeber
Head of Unit Reference Materials
European Commission, Joint Research Centre
Directorate F – Health, Consumers and Reference Materials
Retieseweg 111
B-2440 Geel, Belgium

Registration No. 268-RM
ISO 17034 for the production of reference materials

All following pages are an integral part of the certificate.

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

EURL
European Union Reference Laboratory
for GM Food & Feed

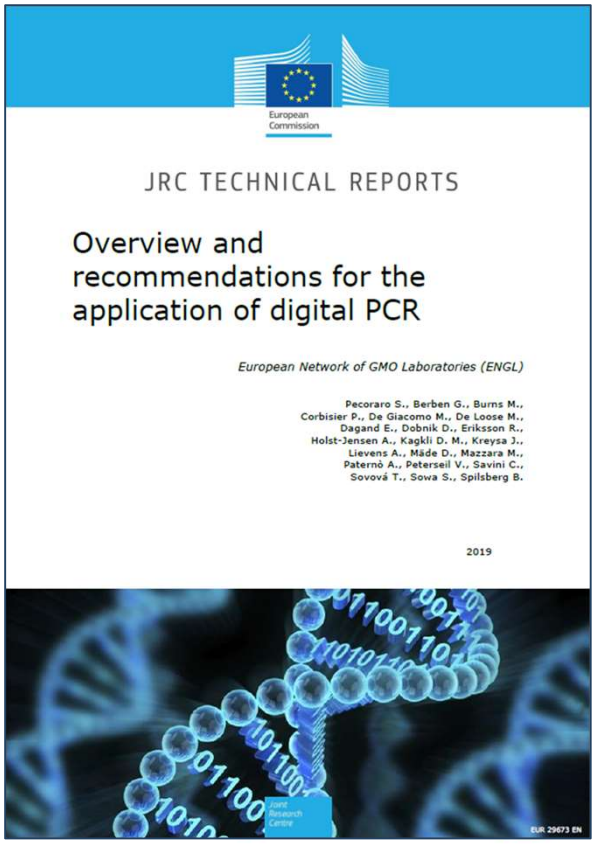
Published guidance documents

- **JRC (EC) guidance document: "*Overview and recommendations for the application of digital PCR*"**

- **dPCR utilises high numbers of partitioned, small volume, replicate PCR reactions**
  - Provides absolute quantification without need for a standard curve (mitigates matrix differences)
  - High precision (number of partitions)
  - Ideally suited for the detection of minority targets

- **Awareness of scope:**
  - e.g. Sample throughput, multiplexing, etc.,

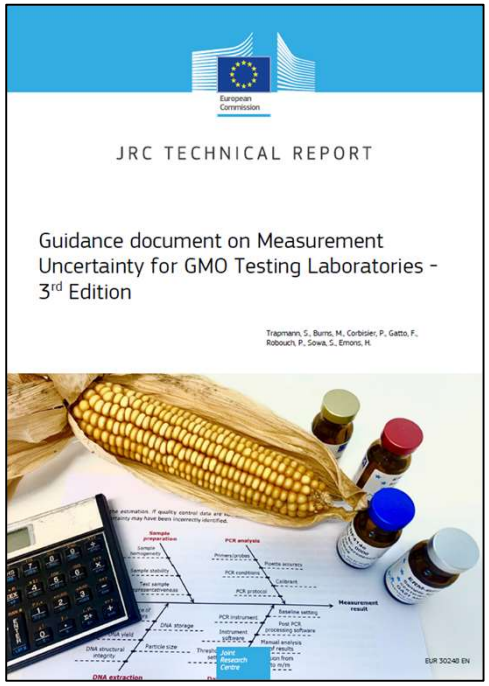
- **Guidance document provides advice and recommendations for establishing dPCR capability in an analytical testing environment, with a focus on quantitative analysis of GMOs**
  - https://gmo-crl.jrc.ec.europa.eu/ENGL/docs/WG-dPCR-Report.pdf



**synergy with NRL function*

Published guidance documents

- **Author on the 3rd edition of the EC Guidance document on MU estimation in GMO testing labs**
  - 1st edition (2007): provided a benchmark on how to estimate measurement uncertainty (MU) in analytical laboratories, aimed at supporting the enforcement of EU food and feed labelling legislation in line with ISO/IEC 17025
  - 3rd edition (2020): updated to take into account current EU legislation, availability of certified reference materials (CRMs) and associated validated quantification methods *
  - Synergy with the NRL function



* **https://publications.jrc.ec.europa.eu/repository/handle/JRC120898**

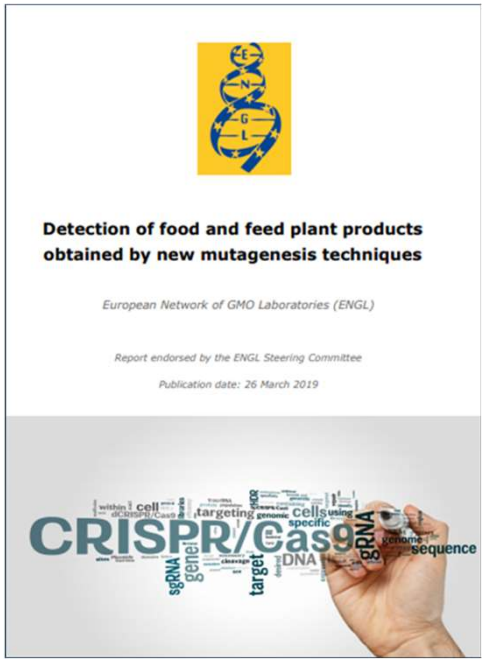
Published guidance documents

- **Gene editing**
  - *Gideon Henderson (Defra, Chief Scientific Officer) – Keynote speech*

- **A group of new directed mutagenesis techniques that facilitate addition, removal, or alteration of DNA sequences at a specific location in the genome**
- **Example modifications: SNPs, InDels, etc.,**
- **Example techniques: CRISPR-CAS nucleases, etc.,**
- **July 2018: European Court of Justice ruled that products of gene editing be regarded as GMOs and fall under the pre-existing legislation for detection and labelling**
- **Main issue:**
  - Changes introduced by gene editing may be genetically indistinguishable from changes due to natural variation, breeding or conventional mutagenesis approaches
  - Analytical challenge in identifying products as a result of gene editing

- *https://gmo-crl.jrc.ec.europa.eu/doc/JRC116289-GE-report-ENGL.pdf*

**synergy with NRL function*



Gene editing consultation

Department for Environment Food & Rural Affairs


EUROPEAN COMMISSION

Brussels, 29.4.2021
SWD(2021) 92 final

COMMISSION STAFF WORKING DOCUMENT

Study on the status of new genomic techniques under Union law and in light of the Court of Justice ruling in Case C-528/16

- **Defra consultation**
  - Early 2021, Defra opened consultation regarding UK definition of products of gene editing and GMOs
  - Results of consultation have potential to impact upon UK legislation with respect to GMO definitions and controls
  - GC provided response to consultation, focussing on measurement issues associated with detecting products as a result of gene editing
- **29th April 2021: EC published the long-anticipated study on new genomic techniques**
- **Original request from the European Council in 2019**
- **Key elements:**
  - Study confirmed NGT products have potential to contribute to sustainable agri-food systems
  - Study identified limitations to the capacity of the legislation to keep pace with scientific developments; these cause implementation challenges and legal uncertainties
  - Informative, but does not provide a recommended policy course of action
- **Council has asked the Commission to perform a follow-up study/proposal as a result of the report, to help inform the Council on possible policy actions**

NML UK NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIO-MEASUREMENT HOSTED AT LGC

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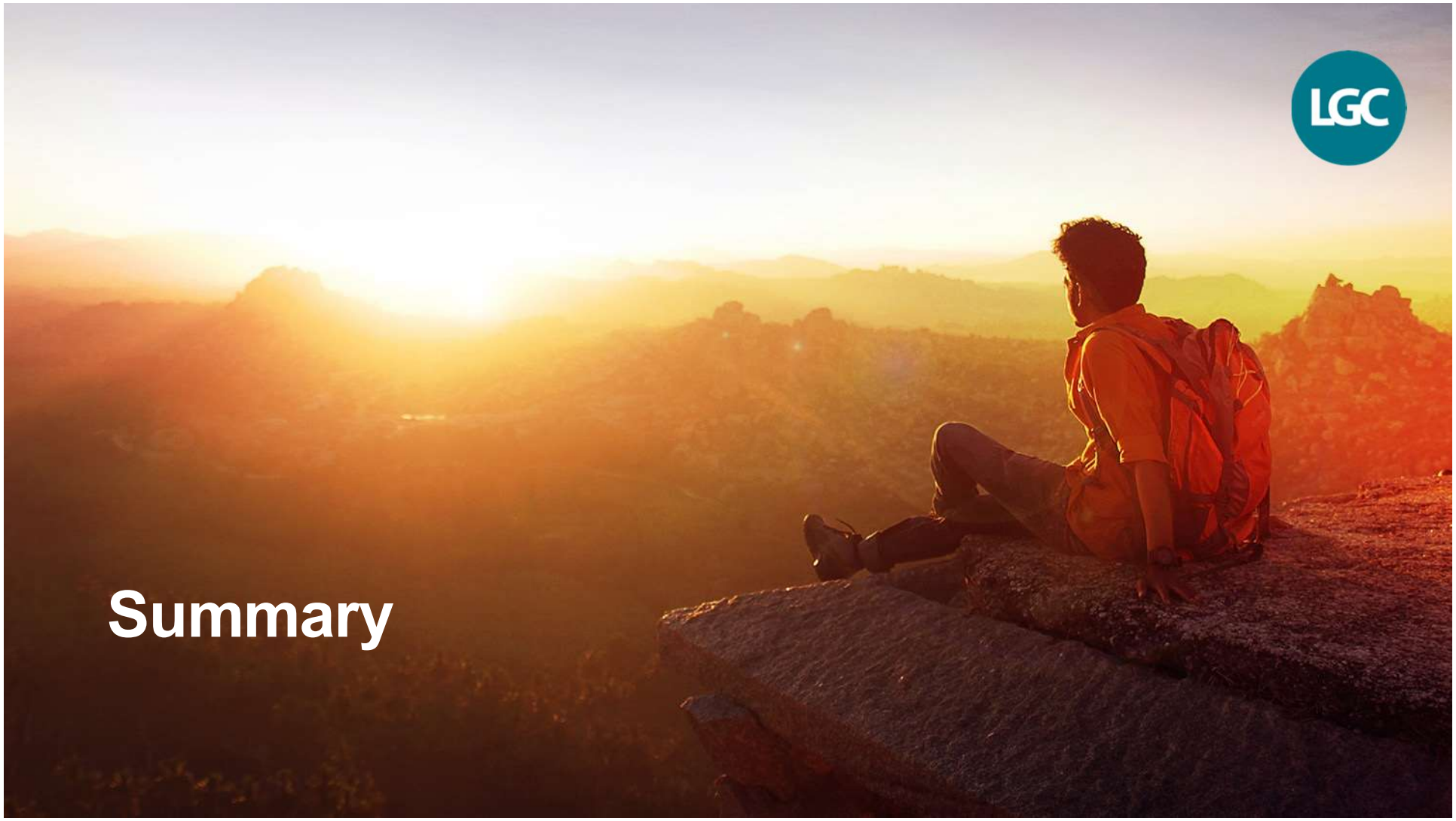
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Summary

Summary

- **"*Towards effective solutions for GMO analysis*"**
- **Presentation has reviewed a number of barriers which provide analytical challenges:**
  - Increasing complexity of GMO analyses
  - New techniques and technologies to produce and analyse GMOs (GE, dPCR, NGS, etc.)
  - EU exit
- **GC programme input towards providing effective solutions:**
  - Incorporation into Quality Management systems
    - ISO 17025, PT rounds, etc.
  - Provision of advice and informative guidance documents
    - Testing for GM rice, GMO screening, Decision Support Systems, dPCR, uncertainty estimation, etc.
  - Reference materials
  - Participation and networking with expert Working Groups
- **Towards developing and then maintaining effective solutions for GMO analysis:**
  - Continued engagement and networking with stakeholder community (FSA, Defra, official control laboratories, industry, producers, retailers, consumers, and national/international expert groups and regulatory bodies)
  - Further supported by the application of sound analytical measurement science

Acknowledgements

- **BEIS**
- **FSA**
  - Bhavna Parmar
  - David Franklin
- **Defra**
- **GC (LGC/NML)**
  - Claire Bushell
  - Gavin Nixon
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  - Ana Fernandez-Gonzalez

Department for Environment Food & Rural Affairs

Food Standards Agency food.gov.uk

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