

The EU-RL GMFF

Update on activities in fulfillment of EU legislation

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www.jrc.ec.europa.eu

<http://ihcpnet.jrc.it/>

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Stimulating innovation
Supporting legislation*



European Union Reference Laboratory for Genetically Modified Food and Feed (EU-RL GMFF)



- Labelling of authorised Genetically Modified Organisms (GMOs)
- Prevent un-authorized GMOs on the European market

Two legal mandates:

1. Regulation (EC) No 1829/2003 → validation of methods for detection and quantification of GM events
2. Regulation (EC) No 882/2004 → official controls applied to ensure the verification of compliance with feed and food law





Duties and tasks of the EU-RL GMFF as defined by Reg. (EC) No 1981/2006

- the reception, preparation, storage, maintenance and distribution to the members of the European Network of GMO laboratories and NRLS of the appropriate positive and negative **control samples**
- **evaluating** the data provided by the applicant for authorisation for placing the food or feed on the market, for the purpose of testing and validation of the method for sampling and detection;
- **testing and validating** the method for detection, including sampling and identification of the transformation event and, where applicable, for the detection and identification of the transformation event in the food or feed;
- submitting full evaluation **reports** to the Authority.



The EU-RL GMO: tasks as outlined by Article 32 of Reg. (EC) No 882/2004

- Providing NRL with details of **analytical methods**
- Coordinating application by NRLs of analytical methods, in particular by **organising CT** and ensuring follow-up
- Coordinating application of **new analytical methods**
- Conducting **training** for NRLs and developing countries
- Providing **support to COM** especially in cases of disputes between MS
- Collaborating with labs in **third countries**

Method Validation - Definition

- ISO/IEC 17025:2005
- Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled

Terminology

IUPAC: Appendix 2: METHOD-PERFORMANCE STUDY

“An interlab study in which all laboratories follow the same written protocol and use the same test method to measure a quantity in sets of identical test items. The results are used to estimate the performance characteristics of the method”

SYNONIMS: Collaborative study, validation study, ring-trial, collaborative trial, full validation.....(vs in-house validation)

Terminology

IUPAC Appendix 2: LABORATORY-PERFORMANCE STUDY

“An interlab study that consists of one or more analyses or measurements by a group of laboratories on one or more homogenous, stable test items, by the method selected or used by each laboratory. The results are compared with those or other laboratories or with the known or assigned value, usually with the objective of evaluating or improving laboratory performance”

SYNONIMS: Proficiency testing, Comparative testing

Method Validation

Validation is the conclusion of a long process

Development
of a new
method

Optimization
of the
method

Pre-validation
of the
method

Full
validation of
the method

Method Validation

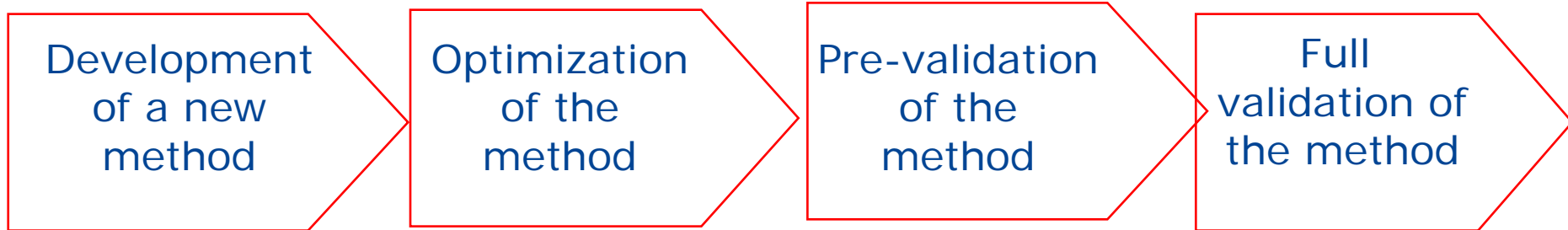
a process, not a result...but....

Validated methods often serve to:

- verify compliance to legislative limits with an acceptable variability
- Support decisions (e.g. authorisation of a product, risk management decisions)

Method Validation

Validation is the conclusion of a long process



**Acceptance criteria
(pre-validation requirements)**



**Performance
requirements**

Methods minimum performance requirements: EU-RL GMFF acceptance criteria and performance requirements

(<http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm>)



Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing European Network of GMO Laboratories (ENGL)

Applicability	Scope of the method, interferences with analytes etc.
Practicability	Equipment, timing, practical difficulties
Specificity	Event-specificity
Dynamic Range	Include the 1/10 and at least 5 times the target concentration
Accuracy	Within $\pm 25\%$ of the reference value
Linearity	≥ 0.98
PCR efficiency	- $3.1 \geq \text{slope} \geq 3.6$
RSDr	Below 25% over the whole dynamic range
LOQ	Less than 1/10th of the value of the target concentration with an RSDr $\leq 25\%$
LOD	Less than 1/20th of the target concentration
Robustness	Deviate not more than $\pm 30\%$
RSDR	Below 35% at the target concentration; < 50% below 0.2%
Trueness	Within ± 25 of the accepted reference value over the whole range

ENGL working groups

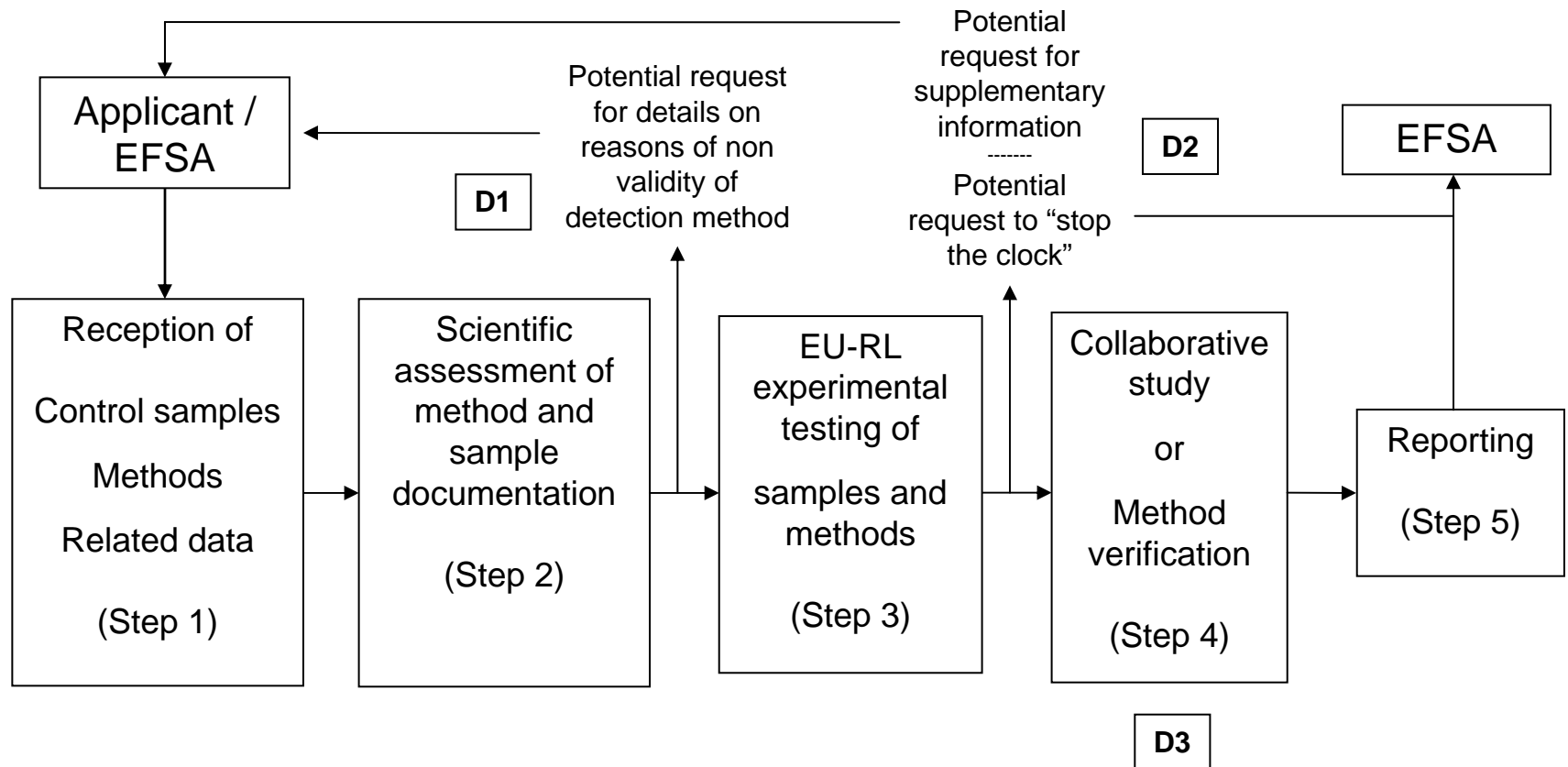
Working Group Method Performance Requirements (MPR)

Mandate:

- Review the current MPR doc, enlarge scope to Reg. (EC) No 882/2004
- Establish MPR for qualitative methods, screening and multiplex methods
- Improve/enlarge MPR for DNA extraction methods

Progress: 2/3 of the doc reviewed; concept of “module”, new definitions, revision of most of the criteria (specificity, range, RSDr%, LOQ, LOD, efficiency, robustness), criteria for qualitative methods (FN and FP).

Under way: DNA extraction, multiplex (duplex)



The process is a step-by-step procedure and can be stopped or re-initiated as required

Status of Applications Reg. (EC) No 1829/2003

115 received

71 completed (method validated)

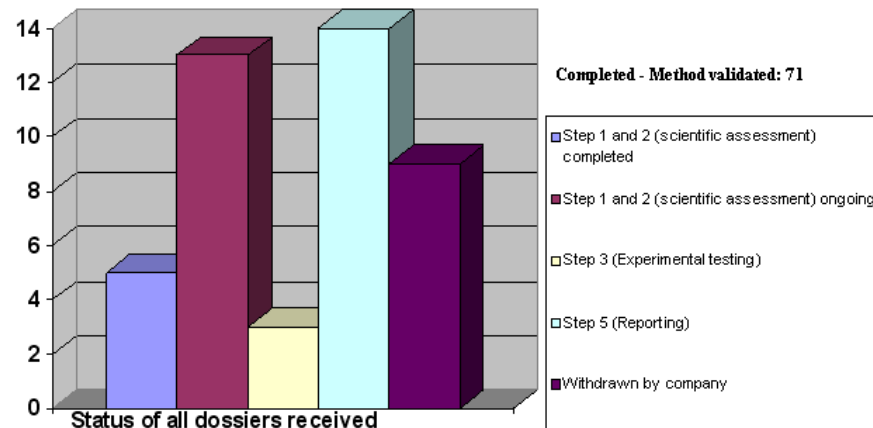
14 reporting (step 5)

3 in the lab (step 3):

5 ready for the lab

13 under assessment (step 2):

9 withdrawn



Status of Applications Reg. (EC) No 1829/2003

reporting:

MON87708; FG72; 40278; AM04-1020; PL73 biomass; GHB614xLLCotton25;
NK603xT25; MON87769xMON89788; Ms8xRf3xGT73;
Bt11xMIR162xMIR604xGA21; Bt11xMIR162xGA21; MON88913xMON15985;
305423x40-3-2

in the lab (step 3):

GHB119; T304-40; 281-24-236x3006-210-23xMON88913

ready for the lab:

MON87705xMON89788; Bt11xMIR162x1507xGA21; GHB614xT304-
40xGHB119; GHB614xLLCotton25xMON15985

under assessment (step 2):

MON87427; 44406; 073496; YC-1214.6; 5307; MON88302;
Bt11x59122xMIR604x1507xGA21; PH048;
Bt11xMIR162xMIR604x1507x5307xGA21; 3272xBt11xMIR604xGA21;
MON87708xMON89788; 1507x59122xMON810xNK603; AV43-6-G7; 68416



Control Samples

- Prepared and distributed for all methods validated
- Regular shipments to 72 laboratories
- Ad-hoc preparation and distribution in support to emergency measures (unauthorised GMOs)
- Distribution to third countries
- 2722 CS aliquots distributed

EU-RL GMFF Comparative testing



Comparative testing (CT)

Article 32: Regulation (EC) No 882/2004

The EU-RLs for feed and food shall be responsible for:

Coordinating application by the NRLs of analytical methods, in particular by organising comparative testing and by ensuring appropriate follow-up of such comparative testing

Proficiency testing (PT) = comparative testing

Comparative testing

- Advisory board (ENGL experts)
- Collaboration with IRMM
- Online submission of results
- ~ 100 participating laboratories
- 5 rounds so far:

Test materials:

1st: two GM levels of maize NK603

2nd: two levels of maize MON810

3rd: two levels of RUR soybean

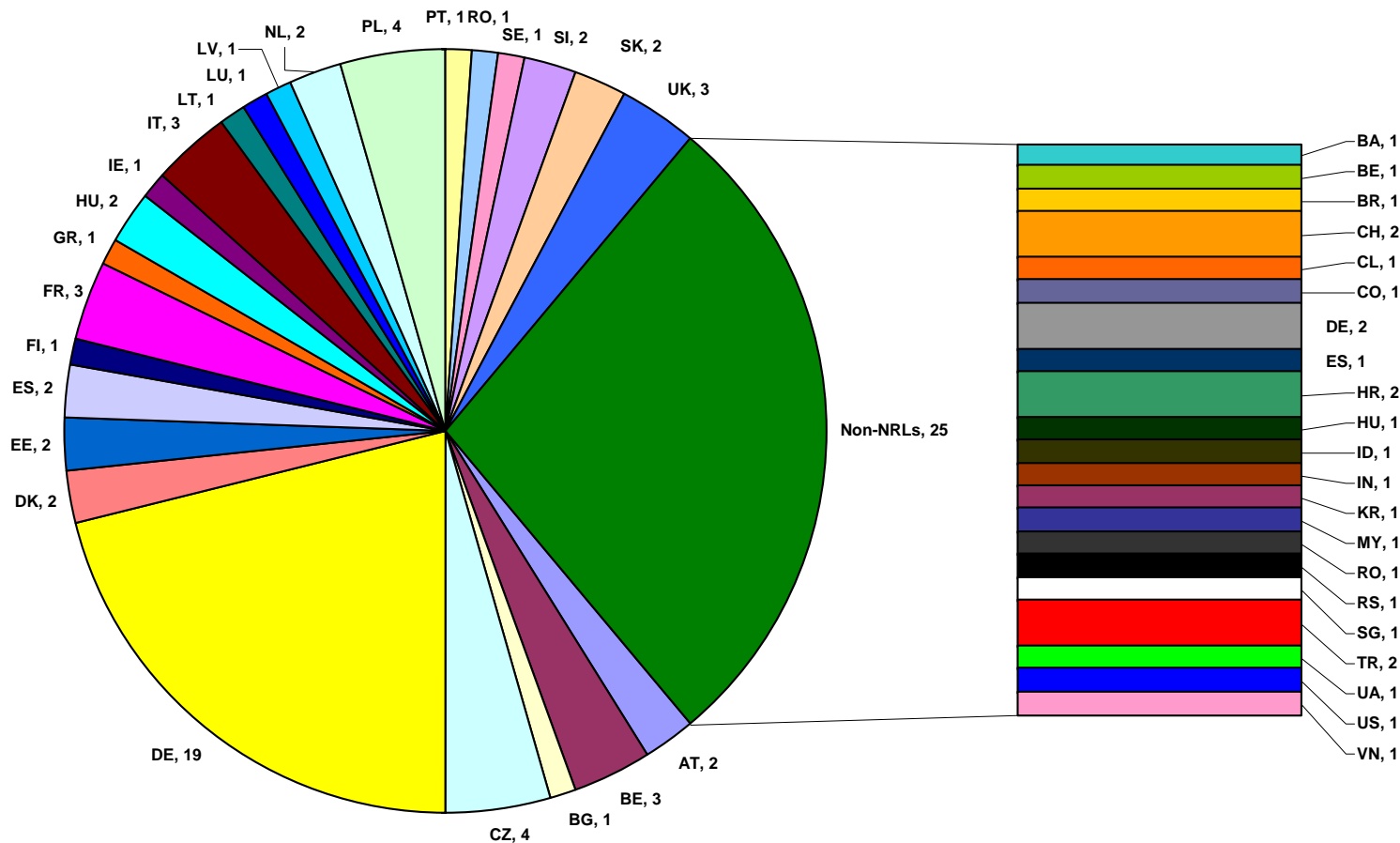
4th: detection and quantification of Maize Events GA21, TC1507 and MIR604;
two test items, two levels; screen for 3272, Bt11, Bt176, 59122, GA21,
MIR604, MON 810, MON 863, NK603 and TC1507; then quantification.

5th: quantification of maize 59122 and rapeseed GT73, two levels each,
genomic DNA



Distribution of participants

ILC-CRL-GMFF-CT-02/10



Comparative testing (CT)

CT01/11: soybean 40-3-2 in soybean powder; two test items, two levels.

- Preliminary report: 1-4% Z-scores outside satisfactory range

CT02/11: Detection and Quantification of Maize Events GA21, TC1507 and MIR604; two test items, two levels; screen for 3272, Bt11, Bt176, 59122, GA21, MIR604, MON 810, MON 863, NK603 and TC1507; then quantification.

- Preliminary report:

- 4-9% Z-scores outside satisfactory range for GA21
- 6-5% Z-scores outside satisfactory range for 1507
- 12-13% Z-scores outside satisfactory range for MIR604

The role of the EU-RL GMFF in response to emergencies regarding un-authorized GMOs



The EU-RL GMFF has a central role in emergency cases for fast validation/verification of detection methods, gathering and provision of specific information to NRLs (e.g. sequence, molecular structure), preparation and distribution of suitable control samples to NRL, preparation of technical guidance documents.

The role of the EU-RL GMFF in response to emergencies regarding unauthorised GMOs

- Decision 2005/317/EC on emergency measures regarding the non-authorized genetically modified organism Bt10 in maize products
- Decision 2006/754/EC on emergency measures regarding the non-authorized genetically modified organism LLRICE601 in rice products
- Decision 2008/289/EC on emergency measures regarding the non-authorized genetically modified organism Bt63 in rice products
- Decision 2011/884/EU on on emergency measures regarding unauthorised genetically modified rice in rice products originating from China
- Other cases, e.g. flax FP967 from Canada, Kefeng6 rice, maize 59132



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

21 December 2011

**Joint Research Centre
Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit**

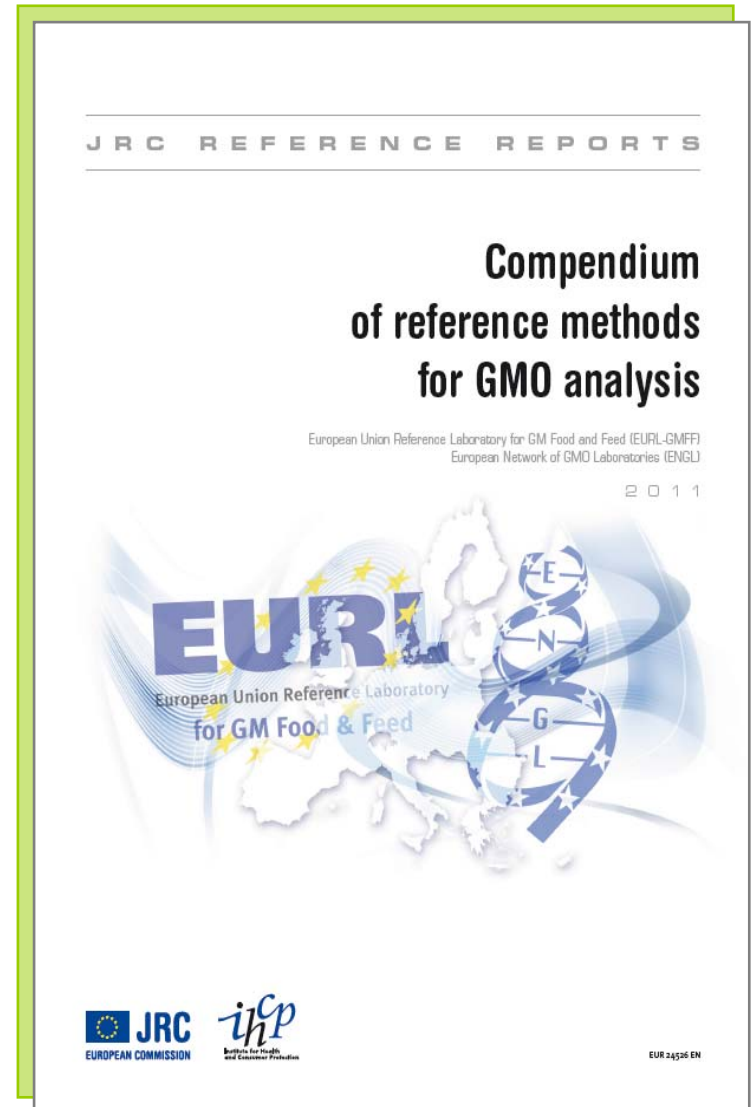
EU Harmonisation : Compendium of reference methods for GMO analysis

(V1 Nov. 2010; V2 April 2011 publicly
available at
[http://gmo-
crl.jrc.ec.europa.eu/gmomethods](http://gmo-crl.jrc.ec.europa.eu/gmomethods))

Aim:

Provide an up-to-date reference
for all collaborative trial validated
methods for the detection of GMO

Concept of Reference Method



WWW-Compendium Dbase (Web application)

GMOMETHODS: EU Database of Reference Methods for GMO Analysis

publicly available at <http://gmo-crl.jrc.ec.europa.eu/gmomethods/>

All info + methods retained in the Compendium

Search functions at various levels
(event, target, crop ...)

Open access

GMOMETHODS: EU Database of Reference Methods for GMO Analysis

<http://gmo-crl.jrc.ec.europa.eu/gmomethods/>

All info + methods retained in the Compendium



EU Database of Reference Methods for GMO Analysis - Joint Research Centre (JRC), European Commission

http://gmo-crl.jrc.ec.europa.eu/gmomethods/

compendium gmo analysis 2011

European Commission
Joint Research Centre
Institute for Health and Consumer Protection

European Commission > JRC > IHCP > EU-RL GMFF > GMOMETHODS

European Union Reference Laboratory for GM Food & Feed

Home Legal basis Guidance documents Status of dossiers Methods database Contacts

Main Search **GMOMETHODS** for Select by GMO Unique Identifier: Search

GMOMETHODS: EU Database of Reference Methods for GMO Analysis

Quantitative GMO detection PCR methods

- GMO specific
 - Event specific
 - Maize
 - Soybean
 - Cotton
 - Oilseed rape
 - Potato
 - Rice
 - Sugar beet
 - Construct specific
 - Element specific
- Taxon specific
 - Validated independently
 - Validated in combination with other method(s)

Qualitative GMO detection PCR methods

- GMO specific
 - Event-specific
 - Construct-specific
 - Element-specific
 - Cauliflower Mosaic Virus 35S promoter
 - Figwort Mosaic Virus 35S promoter
 - Neomycin phosphotransferase II gene
 - Nopaline synthase terminator
 - Cauliflower Mosaic Virus 35S promoter and nopaline synthase terminator (partim T-nos)
 - Phosphinothricin N-acetyltransferase gene
- Taxon specific
 - Validated independently
 - Validated in combination with other method(s)
 - Plant-specific

Released the GMOMethods app for iPad on 20-12-2011.



Last update

Date	ID	Description
15/12/2011	QL-EVE-ZM-002	Qualitative PCR method for detection of maize event Bt10 (verified by the EU-RL GMFF in the context of Commission Decision 317/2005/EC)
13/12/2011	QL-EVE-OS-001	Qualitative PCR method for detection of rice event LLRICE601 (verified by the EU-RL GMFF in the context of Commission Decision 2006/578/EC)

GMOMETHODS is the EU Database of Reference Methods for GMO Analysis based on the "Compendium of Reference Methods for GMO Analysis" assembled



Bablok case

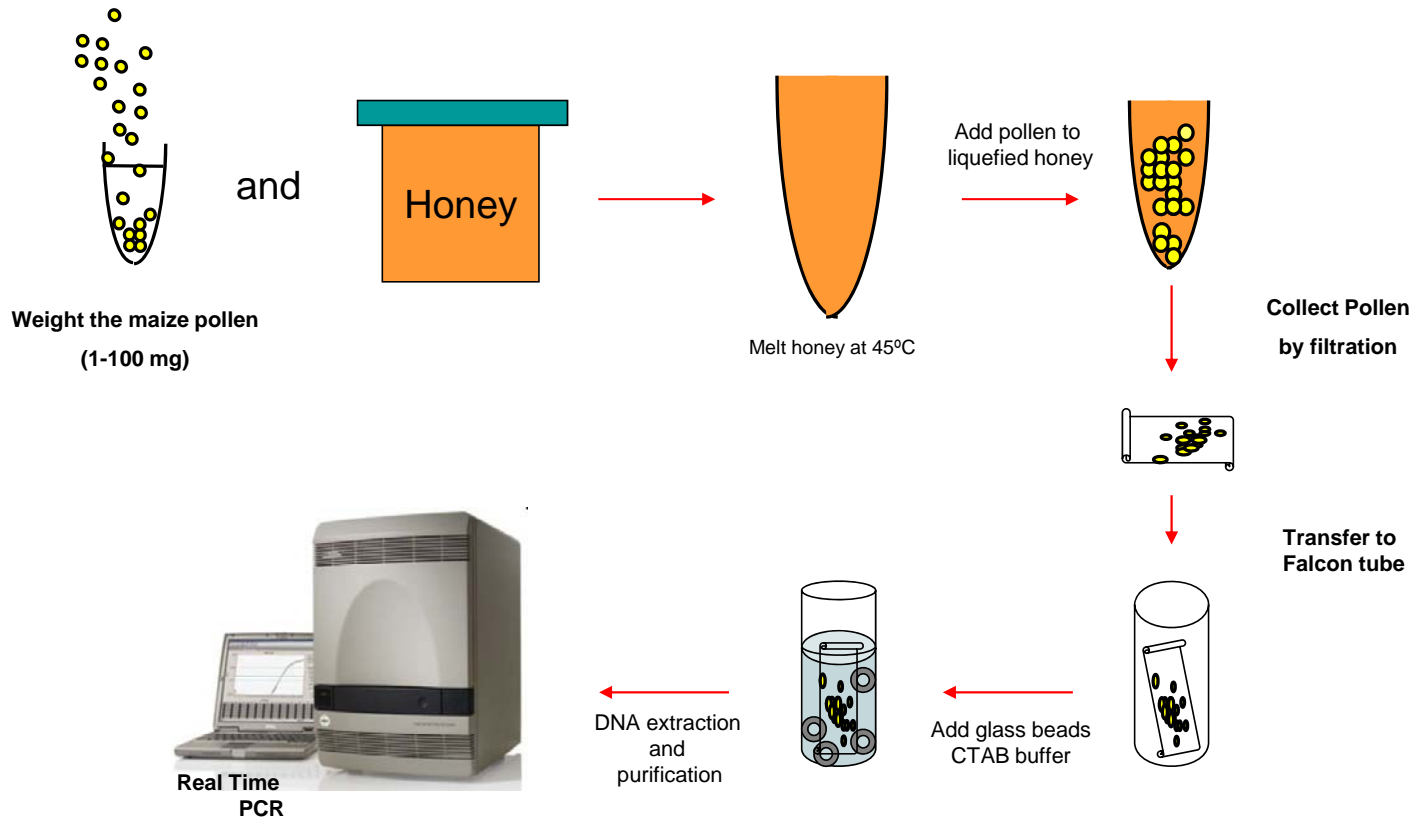
Issues:

- DNA extraction from honey
- method of quantification of pollen in honey
- how to express the GM %
- how to label

Overview of the honey samples used in the study

Sample name	Country of origin	Produced by	Period of Harvest	Honey type	Colour	Physical state
SP0311_HM-ROM-01	Romania	Private production	June 2011	Polyfloral	pale yellow	Liquid
SP0311_HM-HU-01	Hungary	Private production	September 2011	Polyfloral	amber	Liquid
SP0311_HM-BIH-01	Bosnia and Herzegovina	Private production	May-August 2011	Polyfloral	light beige	Crystallized
SP0311_HM-ROM 02	Romania	Private production	June 2011	Monofloral (Tilia)	bright yellow	Liquid
SP0311_HM-ROM 03	Romania	Private production	September 2011	Monofloral (Rape)	white	Crystallized
SP0311_HM-HU-02	Hungary	Private production	September 2011	Monofloral (Rape)	white	Crystallized
SP0311_HM-ITHU-01	Italy, Hungary	G.B. Ambrosoli S.p.A , Ronago (CO) IT	Not indicated	Polyfloral	light brown	Liquid
SP0311_HM-ITHUARG-01	Argentina, Italy, Hungary	Carrefour, Milano IT	Not indicated	Polyfloral	light brown	Liquid
SP0311_HM-ITHUARG-02	Argentina, Italy, Hungary	Cavallo srl, Zafferana Etnea (CT) IT	Not indicated	Polyfloral	light beige	Crystallized
SP0311_HM-FR-01	France	Famille Michaud for Luna di Miele srl, Milano, IT	Not indicated	Monofloral (Acacia)	very pale yellow	Liquid

Extraction of total DNA of GM pollen from honey



PCR analysis on the commercial honey extracts

Honey	Detection Method	Tested Species	# extractions	DNA yield	Measured Ct-value	Measured T _m - value*	Remarks**
SP0311_HM-BIH-01	Alcohol dehydrogenase 1 SYBR@Green	Maize	4	4,73±1,91	36,09 ±0,29	75,8	Low amounts of maize pollen detected
	Cruciferin SYBR@Green	Rapeseed			32,52±0,2	81,1	Low amounts of rapeseed pollen detected
SP0311_HM-HU-02	Cruciferin SYBR@Green	Rapeseed	2	23,3±2,5	22,63±0,06	80,7	High amounts of rapeseed pollen detected
SP0311_HM-HU-01	Cruciferin SYBR@Green	Rapeseed	2	15,5±0,5	27,68±0,72	80,3	Moderate amounts of rapeseed pollen detected
SP0311_HM-ROM 03	Cruciferin SYBR@Green	Rapeseed	2	36,5±2,8	22,69±0,1	80,6	High amounts of rapeseed pollen detected
SP0311_HM-ROM 01	Alcohol dehydrogenase 1 SYBR@Green	Maize	2	1,1	37,19±0,24	75,9	Low amounts of maize pollen detected
	Cruciferin SYBR@Green	Rapeseed			36,62±2,08	80,3	Low amounts of rapeseed pollen detected
SP0311_HM-ROM 02	Cruciferin SYBR@Green	Rapeseed	2	2,3±1,7	29,7±0,2	81	Moderate amounts of rapeseed pollen detected
SP311_HM-FR-01	Cruciferin SYBR@Green	Rapeseed	2	5,45±3	30,44±0,2	81	Moderate amounts of rapeseed pollen detected
SP0311_HM-ITHUARG-01	Alcohol dehydrogenase 1 SYBR@Green	Maize	2	15,4±0,1	39,32 ±4,28	75,9	Trace amounts of maize pollen detected
	Cruciferin SYBR@Green	Rapeseed			29,44±0,21	80,6	Moderate amounts of rapeseed pollen detected
	Lectin SYBR@Green	Soy			35,06±0,74	80,6	Low amounts of soy pollen detected
SP0311_HM-ITHUARG-02	Alcohol dehydrogenase 1 SYBR@Green	Maize	2	8,25±1,25	38,30±1,69	76	Trace amounts of maize pollen detected
	Cruciferin SYBR@Green	Rapeseed			27,70±0,4	80,6	Moderate amounts of rapeseed pollen detected
SP0311_HM-ITHU-01	Cruciferin SYBR@Green	Rapeseed	2	7,05±1,48	32,40±0,33	80,7	Moderate amounts of maize pollen detected

Rt-PCR analysis on the GM MON810 spiked honey extracts

Honey	Spike	# extractions	DNA yield	% PCR inhibition	HMG	MON 810	Conclusion
SP0311_HM-BIH-01	450 mg maize pollen (100% GM in mass) 0.9% pollen mass in honey (50g)	4	202,2 ± 66	acceptable	26,72 ± 0,4	28,57 ± 0,4	GMO detected at lower estimated GM ratio
SP0311_HM-BIH-01	50 mg maize pollen (100% GM in mass) 0.1% pollen mass in honey (50g)	4	9,45 ± 1,73	not tested	26,19 ± 0,4	27,79 ± 0,28	GMO detected at correct estimated GM ratio
SP0311_HM-FR-01	100 mg maize pollen (1% GM in mass) 1% pollen mass in honey (10g)	4	12,12 ± 3,15	not tested	30,55 ± 0,62	37,95 ± 0,72	GMO detected at lower estimated GM ratio
SP0311_HM-FR-01	100 mg maize pollen (10% GM in mass) 1% pollen mass in honey (10g)	1	34,7	not tested	24,58 ± 0,008	29,32 ± 0,09	GMO detected at correct estimated GM ratio
SP0311_HM-FR-01	10 mg maize pollen (10% GM in mass) 0.1% pollen mass in honey (10g)	1	3,4	not tested	29,86 ± 0,08	34,24 ± 0,22	GMO detected at correct estimated GM ratio
SP0311_HM-ITHU-01	100 mg maize pollen (0,2% GM in mass) 1% pollen mass in honey (10g)	1	7,3	not tested	27,94 ± 0,4	36,82 ± 0,09	GMO detected at correct estimated GM ratio

**PCR inhibition tested on the SP0311_HM-BIH-01:
1.1% and 5.1% for *hmg* and MON810 PCR method respectively**



Golden Rice 2

Development of a new Real Time PCR method



Golden Rice 2

- In collaboration with IRRI
- Real-time PCR event-specific method
- New PLD method
- Validation in 2012

Kernel Lot Distribution Assessment (KeLDA): a Comparative Study of Protein and DNA-Based Detection Methods for GMO Testing

**Marco Mazzara • Claudia Paoletti • Philippe Corbisier •
Emanuele Grazioli • Sara Larcher • Gilbert Berben •
Marc De Loose • Imma Folch • Christine Henry •
Norbert Hess • Lotte Hougs • Eric Janssen •
Gillian Moran • Roberta Onori • Guy Van den Eede**

Food Anal. Methods

DOI 10.1007/s12161-012-9445-z

Testing the Robustness of Validated Methods for Quantitative Detection of GMOs Across qPCR Instruments

**E. Luque-Perez • M. Mazzara • T. P. Weber • N. Foti •
E. Grazioli • B. Munaro • G. Pinski • G. Bellocchi •
G. Van den Eede • C. Savini**

Thank you for your attention!

