"The experience of the EC Joint Research Centre (Ispra) on the validation of molecular methods"

4th Annual workshop of the National Reference Laboratories for E. coli

Rome, 30 October 2009

The Joint Research Centre (JRC):

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Molecular Biology and Genomics Unit:

- **Biotechnology Research** & **Development**:
  - Sampling
  - Method development & validation
  - Mol. characterization & stability studies
  - Bioinformatics & information systems in support to regulatory processes
  - Training and capacity building

- Mandate of **Community Reference Laboratory for GM Food & Feed (CRL-GMFF)** and **Community Reference Laboratory for GMOs (CRL-GMO)**

- Management & Coordination of the **European Network of GMO Laboratories (*ENGL*)**

The Community Reference Laboratory for GM Food and Feed: two legal mandates

1) Community Reference Laboratory for GM Food and Feed (CRL-GMFF) under **Regulation (EC) 1829/2003**.

2) Community Reference Laboratory for Genetically Modified Organisms (CRL-GMO) under **Regulation (EC) No 882/2004** on “official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules”.

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

• **Assisting the National Reference Laboratories** (NRLs) in their duties to monitoring the European market in a context of health and consumer protection with three main objectives:

• Solving scientific issues related to **harmonisation and communication** of scientific data among laboratories;

• **Monitoring** the quality levels of the analytical laboratories for GMO detection;

• **Building capacities** through **training**, workshops and any common scientific normative tool available.

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

a) the reception, preparation, storage, maintenance and distribution to the members of the European Network of GMO laboratories of the appropriate positive and negative **control samples**….

(b) without prejudice to the responsibilities of the Community reference laboratories laid down in Article 32 of Regulation (EC) No 882/2004… the **distribution** to national reference laboratories within the meaning of Article 33 of that Regulation of **the appropriate positive and negative control samples**….

(c) **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**;

(d) **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;

(e) submitting **full evaluation reports** to the Authority.

Why Validation Studies ?

We need to get information about a food/feed item by submitting the sample to analysis, applying a specific method

- The analytical problem defines the *purpose* of the method
- Conducting a validation study is a tool to check whether the method is *fit for the purpose*
- The validation study delivers *performance characteristics*

How to validate the analytical method?

- By performing an in-house validation
- By conducting a collaborative study

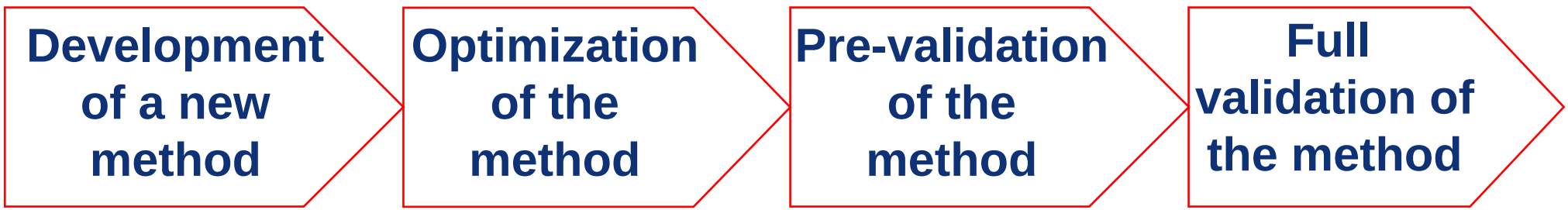
Method Validation

Validation is a confirmation of the EXPECTED performance indices in a multilaboratory study

validation is a process NOT a result…

Method Validation

Validation is the conclusion of a long process



Acceptance criteria

(pre-validation requirements)

Performance requirements

Method Modularity
A model that allows flexibility and enforcement

The whole process from extraction up to the PCR-technique (or equivalent) constitutes a method.

The PCR “module”, as fully described, assessed against performance criteria and validated, is applicable to any DNA template containing a given GMO; provided that the template DNA is prepared by suitable methods, and fully characterised in terms of quality/quantity prior to use in PCR;

The CRL-GMFF provides guidance on minimum quality criteria for DNA extraction methods;

The model as described allows flexibility to enforcement laboratories in using validated modules adapted to real needs; the CRL-GMFF provided guidance on introduction of validated methods in individual laboratories (*Žel et al., 2008. Food Anal. Methods (2008) 1:61–72).*

Modularity in practice

MATRIX	Sample preparation	DNA extraction	Real-time PCR analysis		
			Screening	**Identification**	**Quantification**
crop A matrix A	x	x	element 1	A event 1	A event 1
			element 2	A event 2	A event 2
			element 3	A event 3	A event 3
				A event 4	A event 4
crop B **matrix B**	**y**	**y**		B event 1	B event 1
			element 2	B event 2	B event 2
			element 3	B event 3	B event 3
			element 4		

new sample prep and DNA extraction, same PCR(s)

Holst-Jensen & Berdal, 2004. Journal of AOAC International Vol. 87, No 4, 927-936

GMO Standards for Method Validation

- ENGL: Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing – Version 13/10/2008
- ISO 5725 – Accuracy (trueness and precision) of measurements methods and results
- IUPAC, 1995 – Protocol for the Design, Conduct and Interpretation of Method-Performance Studies
- Codex Alimentarius Commission - Consideration of the methods for the detection and identification of foods derived from biotechnology general approach and criteria for the methods. Accepted 2008.
- Codex Alimentarius Commission – Single Laboratory Validation – Consideration of Harmonized IUPAC guidelines for Single-Laboratory Validation of Methods of Analysis

Regulation (EC) No 641/2004 of 6 April 2004 on detailed rules for GM food and feed legislation

Annex 1: Method Validation

- **Method acceptance criteria and method performance requirements**: ENGL/CRL guidance document "Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing"
- Information about the method: **event-specificity**, applicability, detailed description of the methods etc.
- Information about method testing carried out by the applicant: method optimisation, inter-lab transferability, stability, specificity, limit of detection (LOD), limit of quantification (LOQ), testing report
- **Full sequence of the insert(s) + flanking sequences**
- Control samples and samples of food and feed

In conclusion:

- The CRL-GMFF is legally mandated by the EC Regulatory system on GMO to conduct method assessment and validation;
- Its activities span beyond this original core business, as a result of a continuous acquisition of expertise and recognition of its broader role in the area of GMO detection;
- Method validation is conducted according to worldwide recognised standards (i.e ISO, IUPAC) and taking into account the real needs of control laboratories, necessity of harmonisation, evolving science and translating applied research into an (hopefully!) improved model.

Thank you for your attention

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