WETENSCHAPPELIJK INSTITUUT VOLKSGEZONDHEID
INSTITUT SCIENTIFIQUE DE SANTÉ PUBLIQUE

Validation and Measurement Uncertainty for GMO analysis

Broeders Sylvia and Roosens Nancy

Scientific Institute of Public Health, Department Expertise,Service Provision and Consumer Relations , Unit Platform Biotechnology and Molecular Biology, Juliette Wytsmanstreet 14, 1050 Brussels, Belgium

ABSTRACT

❖ Strict EU regulation for GMO commercialization: Directive 2001/18/EC[1] and Regulations (EC) N° 1829/2003[2] and (EC) N° 1830/2003[3] ⇒ detection and **quantification of GM events** in food and feed samples
❖ Quantitative **real-time PCR methods** validated by the EU-RL[4] to be adopted by enforcement laboratories ⇒ **in-house validation**
❖ Assessment of a number of **method acceptance parameters**
❖ Calculation of **measurement uncertainty** for each method ⇒ estimate of the variability of the quantitative analytical results
❖ Establishment of a **validation dossier** ⇒ method fit for purpose
❖ In routine analysis: testing of **real-life food and feed samples** for the presence of GM events ⇒ outcome: measurement result and its uncertainty[6]

⇒ **Steps of the validation process and the calculation of the measurement uncertainty for maize MON 89034**

APPLICABILITY
❖ Validation of the method: gDNA extracted from the CRM
❖ Different GM% as quality control samples analysed
❖ Method tested on gDNA extracted from real-life samples such as maize and soybean shreet in routine GMO analysis

→ **The method can be applied to different matrices and GM%**

PRACTICABILITY
❖ Same conditions as other validated methods
❖ No special equipment or reagents
❖ Implementation in routine lab

→ **The method can be used in routine analysis along with previously validated methods**

PCR EFFICIENCY (ε)
❖ PCR efficiency over the different runs transgene: 97.2% - 100.0%
❖ PCR efficiency over the different runs endogene: 106% - 113%

→ **The PCR efficiencies are in the criteria set by the ENGL[5]**

LINEARITY (R^2)
❖ Linearity for transgene runs ≥ 0.98
❖ Linearity for transgene runs ≥ 0.98

→ **The linearity of the method is in the criteria set by the ENGL[5]**

DYNAMIC RANGE
❖ Need: The target concentration of a quantification method is the labeling threshold (0.9%): 1/10 and at least 5 times the target concentration to test (i.e. 0.09 – 4.5%)
❖ Validation: quality control samples ranging from 0.1% till 8%
❖ Results within the acceptable level of trueness (25%)

→ **Dynamic range necessary is covered**

LIMIT OF QUANTIFICATION (LOQ)
❖ Method performs well down to 0.1% GM
❖ Accordance with the labeling threshold
❖ Importance considering the LLP regulation

→ **Limit of quantification = 0.1%**

RELATIVE REPEATBILITY STANDARD DEVIATION (RSDr)
❖ Four independent runs
❖ Repeatability conditions: same method, same sample, same lab, same operator, same equipment, short time interval
❖ ≤ 25% over dynamic range

→

GM%	RSDr (%)
0.1	21.03
0.4	17.78
1.0	12.54
2.0	10.01
5.0	8.56
8.0	10.75

MEASUREMENT UNCERTAINTY (MU)
❖ Measure for variability of analytical result
❖ Top-down approach
❖ Also to be calculated for sample: Nordtest procedure

→

GM%	Expanded uncertainty (%)
0.1	27.62
0.4	16.15
1.0	13.5
2.0	9.79
5.0	10.09
8.0	11.98

REFERENCES
1. Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities (2001) L106: 1-38.
2. Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Communities (2003) L268: 1-23.
3. Regulation (EC) N° 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labeling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC. Official Journal of the European Communities (2003) L268: 24-28.
4. European Union Reference Laboratory for GM food and feed: http://gmo-crl.jrc.ec.europa.eu/
5. European network of GMO laboratories (ENGL): Definition on Minimum Performance Requirements for Analytical Methods of GMO Testing. European Commission - European Union Reference Laboratoria for GM food and feed (2008) p 1-7.
6. S. Trapman, M. Burns, H. Broll, R. Macarthur, R. Wood, J. Zel: Guidance Document on Measurement Uncertainty for GMO Testing Laboratories. European Commission – Joint Research Centre – Institute for Reference Materials and Measurements (2009) p 1-41 (ISBN 978-92-79-11228-7).1111

ISP-WIV | Rue Juliette Wytsmanstraat 14 | 1050 Brussels | Belgium
T +32 2 642 54 25 | F +32 2 642 52 92 | e-mail: sylvia.broeders@wiv-isp.be | site web: www.wiv-isp.be