

Use of Certified Reference Materials for the quantification of GMO in food and feed

This application note provides guidance on the correct use of IRMM's Reference Materials certified for their GM (genetically modified) mass fraction of a specific GM event.

The details given below refer particularly to the use of the CRMs ERM-BF410, ERM-BF411, ERM-BF412, ERM-BF413, ERM-BF414, ERM-BF415, ERM-BF416, ERM-BF417, ERM-BF418 and ERM-BF423.

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INTRODUCTION

Legislation (EC) No 1830/2003 demands the labelling of food and feed products consisting of or containing more than 0.9 % genetically modified organisms (GMOs), provided the GMO has been placed on the European market in accordance with Community legislation. Therefore, quantification of GM in such products has to be performed in a reliable manner. Appropriate Certified Reference Materials (CRMs) are indispensable quality assurance tools for this.

GMO CRM CHARACTERISTICS

The certified values of the CRMs listed above are based on the masses of dried genetically modified seed powder and dried non-genetically modified seed powder used in the gravimetric preparation. The masses are corrected for their water content and the purity estimates. The GM mass fraction is calculated as:

$$\frac{\text{corrected mass GM powder}}{\text{corrected mass GM powder} + \text{corrected mass non - GM powder}}$$

Each GMO CRM is certified for a mass fraction of a specific genetic modification event (as stated on the certificate). Consequently, the CRM can only be used to quantify the event indicated on the certificate and the corresponding blank material can only be used to prove the absence of this event below the threshold given on the certificate.

ERM® - BF418c

DRIED MAIZE POWDER		
	Mass Fraction	
	Certified value ¹⁾ [g / kg]	Uncertainty ²⁾ [g / kg]
1507 maize	9.9	-0.6 ; +0.8

1) The certified value is based on the mass fraction of dried non-genetically modified powder and dried genetically modified powder mixed and corrected for the water content. The certified value is traceable to the SI.

2) The certified uncertainty is the expanded uncertainty estimated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM) with a coverage factor $k = 2$, corresponding to a level of confidence of about 95 %.

Figure 1: Part of the certificate of GM CRM ERM-BF418c.

Recently released GMO CRMs of IRMM have been certified with an asymmetric uncertainty range. If such a CRM is used for bias control (see

ERM Application Note 1), the 'plus uncertainty' has to be used in the case that the average measurement result exceeds the certified value and the 'minus uncertainty' has to be used in the case that the average measurement result is lower than the certified value.

USING REAL-TIME PCR

Real-time Polymerase Chain Reaction (rt-PCR) is commonly used to quantify GM fractions in food and feed samples. This DNA-based quantification technique measures the ratio between transgenic deoxyribonucleic acid (DNA), i.e. derived from the genetic modification, and endogenous DNA, which is specific for the biological species.

Because of the differing genetic composition of different parts of the seeds of monocotyledons (e.g. maize endosperm, seed coat and embryo), the value of the DNA ratio in the reference material may be not the same as the value of the certified powder mass fraction. Hence, the ratio of extractable transgenic DNA / extracted endogenous DNA is not necessarily equal to the ratio GM maize mass / total maize mass, even if both DNA species have comparable extraction yields.

During the preparation of GMO CRMs, special care was taken to ensure that the GM and non-GMO powders are similar with respect to their particle size distribution. This is particularly important regarding the amount of extractable DNA in both powders. Different DNA extraction efficiencies of the GM and non-GM powder would influence the GM concentration value measured by rt-PCR. Therefore, only extraction methods which were validated to fulfil this requirement should be used.

During certification the GM mass fraction of the CRM is verified using an event-specific rt-PCR method. However, one has to be careful to draw quantitative conclusions from measurements of unknown samples, as the DNA-based GM quantification may vary with the particular variety tested. If not available elsewhere, it is advisable to investigate the impact of different varieties on the rt-PCR results during an in-house validation [1].

Real-time PCR detection methods submitted and validated under the provision of Regulation (EC) No 1829/2003 are accessible for the public via the homepage of the Community Reference Laboratory for GM Food and Feed (<http://gmo-crl.jrc.it/detectionmethods.htm>).

EXPRESSING RESULTS IN RELATIVE DNA COPY NUMBERS

In Europe the most often used methodology for GMO quantification is rt-PCR, therefore a recent Commission Recommendation (2004/787/EC) proposes to express measurement results on GM samples in DNA copy numbers. If one is using GMO CRMs certified for their GM mass fraction for the calibration of measurements and expresses the final result in relative copy numbers, one should be aware that the maize CRMs have been produced using GMO maize being heterozygous for the transgene. Information about the zygosity of the seed materials used for the production of the CRMs can be found in the corresponding certification report. Furthermore

one has to take into account that the relative GM copy numbers for maize are influenced by the way the GMO hybrid variety was produced and by the endoreduplication status of the seeds, which increases the impact of genomic distribution present in the endosperm tissue. Considering the extreme cases the relative GM copy numbers can either be 33 % (transgenic event originating from the father used to cross the heterozygous seed) or 66 % (transgenic event originating from the mother plant used to cross the heterozygous seed) under the assumption that endoreduplication is so intense that the impact of the endosperm is close to 100 %. All other cases (less impact of endoreduplication and bigger impact of the embryo tissue) will lead to values between 33 and 66 %, based on the assumption that in comparison to these effects the impact of the seed coat can be neglected.

An example for the transformation of a measurement result and its uncertainty obtained in g/kg into relative copy numbers is given below.

EXAMPLE

By using for calibration CRMs certified for their GM mass fraction, a maize sample was found to contain 65 ± 20 g/kg of maize event 1507. The expanded measurement uncertainty of 20 g/kg was calculated using a coverage factor of 2 and a measurement uncertainty of the quantification method of 15 %, estimated during in-house validation. In order to transform the result obtained for the GM mass fraction into a copy number ratio, the result in g/kg needs to be transformed into percent by dividing by 10. It has to be taken into consideration that the maize GMO CRMs used for calibration have been produced from heterozygous maize seeds, the results need to be divided by 2:

$$\frac{\bar{x}}{10 \times 2} = \frac{65}{20} = 3.3 \quad \text{for } \bar{x} = \text{average of the GM content found in g/kg}$$

Note, that in cases where a different DNA extractability has been observed for the non-GMO and GMO base materials used to produce the CRMs, a correction factor needs to be applied. Information about the DNA extractability can be found in the certification report. Using CTAB a ratio of 0.7 ± 0.3 was found for the DNA extractability of the GMO powder divided by the non-GMO powder. Hence, the true GM content of the sample under investigation is overestimated in terms of copy numbers and needs to be corrected:

$$3.3 * f = 3.3 * 0.7 = 2.3 \quad \text{for } f = \text{correction factor related to the different DNA extractability of the GMO and non-GMO powder used as CRM}$$

The effect of endoreduplication and the breeding of the heterozygous maize event need to be considered in the uncertainty of the measurement results. The uncertainty needs to cover the range of 33 % (66 % minus 33%) and the value measured might differ with 16.5 %. For the estimation of the copy number ratio the DNA extracted from the maize sample under investigation needs to be quantified and the number of maize genomes estimated. For this estimation the DNA concentration is divided by the genome size of maize. Consequently, the uncertainty related to the DNA quantification and the uncertainty related to the genome size estimation need to be considered in the uncertainty calculation. According to literature maize genome sizes are known to vary up to 36 % [2]. During in-house validation the reproducibility of the quantification method was established to be 22 %. Consequently, the expanded combined uncertainty for the result expressed in copy number ratios (U_{cc}) can be calculated as:

$$U_{cc} = k \sqrt{u_m^2 + u_{gs}^2 + u_{Dq}^2 + u_e^2}$$

$$U_{cc} = 2 \sqrt{15^2 + \left(\frac{18}{\sqrt{3}}\right)^2 + 22^2 + \left(\frac{16.5}{\sqrt{3}}\right)^2} \% = 60\%$$

for k = coverage factor

u_m = uncertainty contribution related to the copy number quantification method

u_{gs} = uncertainty contribution related to the genome size estimation

u_{Dq} = uncertainty contribution related to the DNA quantification

u_e = uncertainty contribution related to the breeding and endoreduplication

Expressed in copy numbers the maize sample contains 2.3 ± 1.4 transgenic sequences of event 1507 per 100 endogenous sequence. Compared to the result expressed in 1507 mass fractions of 65 ± 20 g/kg the relative expanded uncertainty increased from 30 to 60 %.

[1] IUPAC Technical Report (2002): Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis

[2] Poggio et al., Annals of Botany 85 (1998), 107-115.