

# Development and Use of Certified GMO Reference Materials



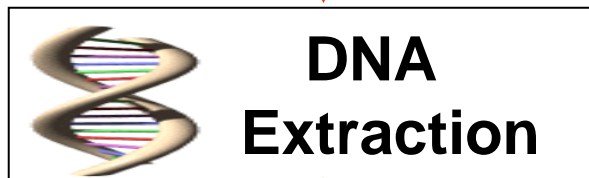
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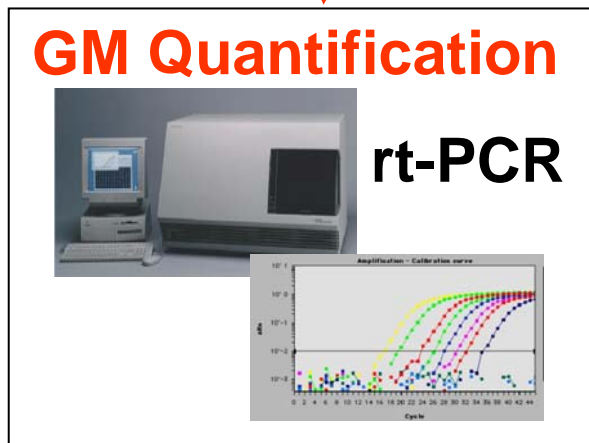
[hendrik.emons@ec.europa.eu](mailto:hendrik.emons@ec.europa.eu)



**sample to be controlled**



**appropriate measurement procedure**



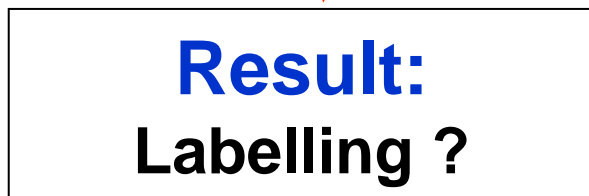
*glassware etc., solvents*

**Competent staff**

*instrumentation*



*pipettes, buffer solutions, reagents  
(DNA primers, fluorescence labels,...)*



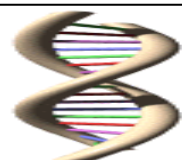
**... and 'Reference Materials' !**



**Food / Feed**



**Identification  
of GM event**



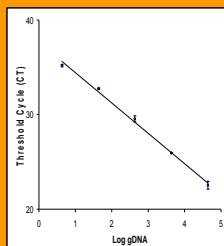
**DNA  
Extraction**

**Quality  
control**

**Control  
of  
correct  
identification**



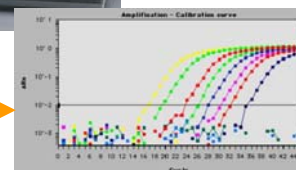
**Calibration**



**GM Quantification**



**rt-PCR**



**matrix CRM**



**'identity'  
CRM**



*independent  
calibrant*



**measurement  
result**



- performance controls (precision, consistency) of methods or labs (internal & external)
- method developments



- calibration
- trueness control
- full method validation
- all QA/QC measures

## non-certified RMs

- homogeneous subsamples
- appropriate stability



- statements on homogeneity & stability

## Certified RMs

- homogeneous subsamples
- appropriate stability

metrologically valid establishment of property value(s)

- property value(s) traceable to adequate reference system
- stated meas. uncertainty
- stated homogeneity & stability
- intended use



**GMO seeds**

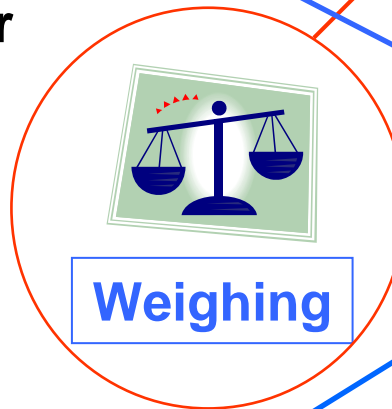


**GMO powder**

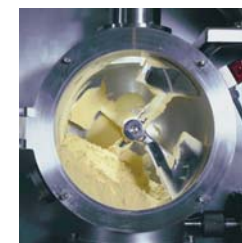


Washing  
Drying  
Milling

**Purity  
control**



**Mass fraction**



**Mixing**



**Bottling  
Labelling**



**Non-GMO powder**



**Non-GMO seeds**



**Production Control**  
Particle size, water determination

**Mass fraction**

**Uncertainty**

Dried maize powder		
	Mass fraction	
	Certified value <sup>1</sup> g / kg	Uncertainty <sup>2</sup> g / kg
MON 810 maize content	10.0	0.5
<p>1) The certified value is based on the mass fraction of dried non-GMO powder and dried GMO powder mixed and corrected for the water content. The value is traceable to the International System of Units (SI).</p>		
<p>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 <math>k = 2</math>.</p>		

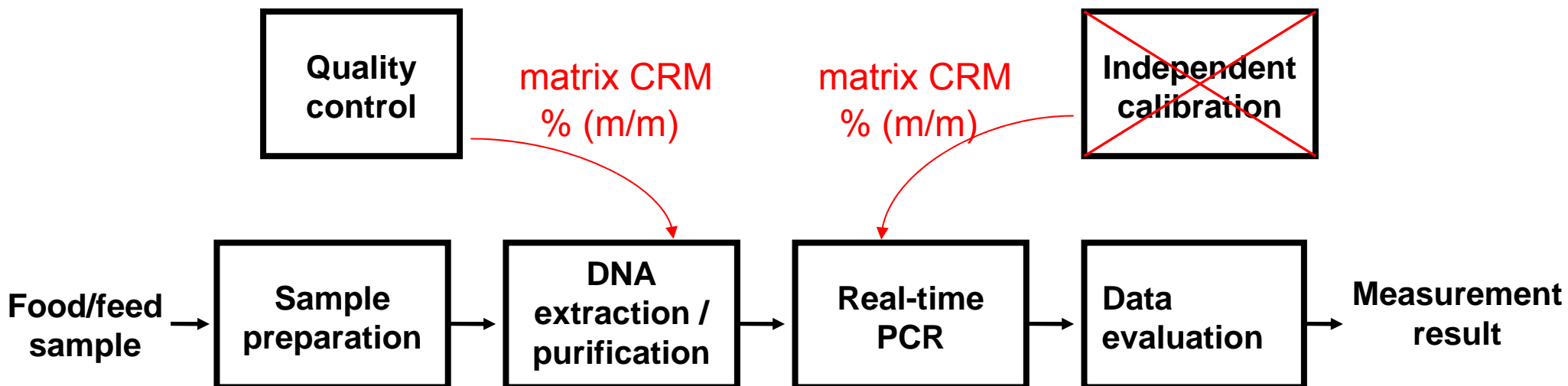
**Metrological Traceability**

## Mass fraction m/m [%]

*Measurement principle*

*Analytical method*

*GM Analytical procedure*



**Regulation (EC) No 1829/2003** specifies that food and feed products containing more than **0.9 %** GMO have to be labelled

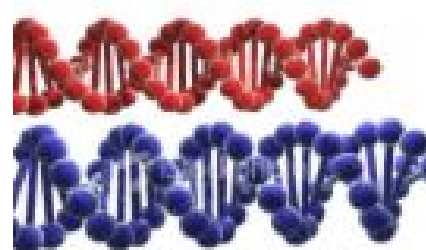
$$\frac{\text{GMO mass}}{\text{total species mass}} \times 100$$

**mass fraction**



$$\frac{\text{copies of transgenic DNA}}{\text{species-specific DNA}} \times 100$$

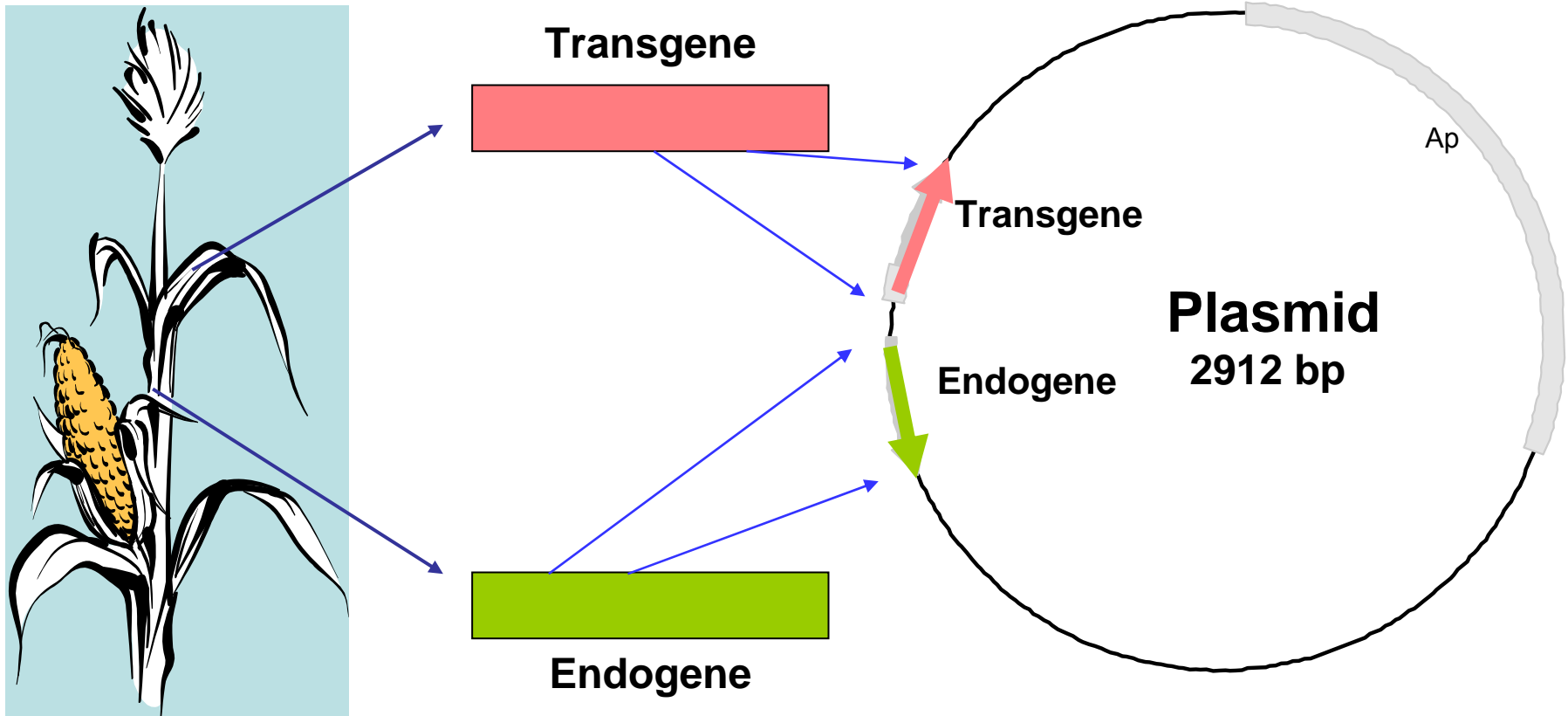
**number fraction**



**Recommendation (EC) 787/2004** recommends to express the GMO content in DNA copy numbers



## Maize Transgenic plant



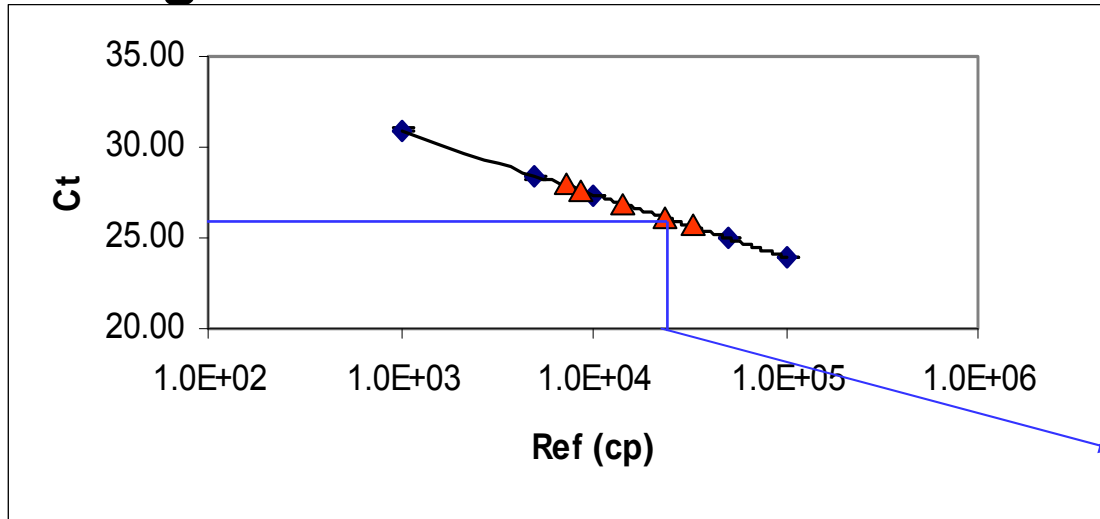
Number of copies per plasmid

Uncertainty

PLASMIDIC DNA CONTAINING MON 810 MAIZE DNA FRAGMENTS		
	Number	
	Certified value	Uncertainty <sup>2)</sup>
Fragment of 5' <i>plant-P35S</i> junction DNA / plasmid <sup>1)</sup>	1	negligible
Fragment of <i>hmg</i> DNA / plasmid <sup>1)</sup>	1	negligible
1) The sequence identity has been confirmed by dye terminator cycle sequencing of the <i>hmg</i> and 5' <i>plant-P35S</i> junction fragments present in <i>Zea mays</i> MON-ØØ81Ø-6.		
2) The uncertainty related to the sequencing method applied is negligible.		

Traceability

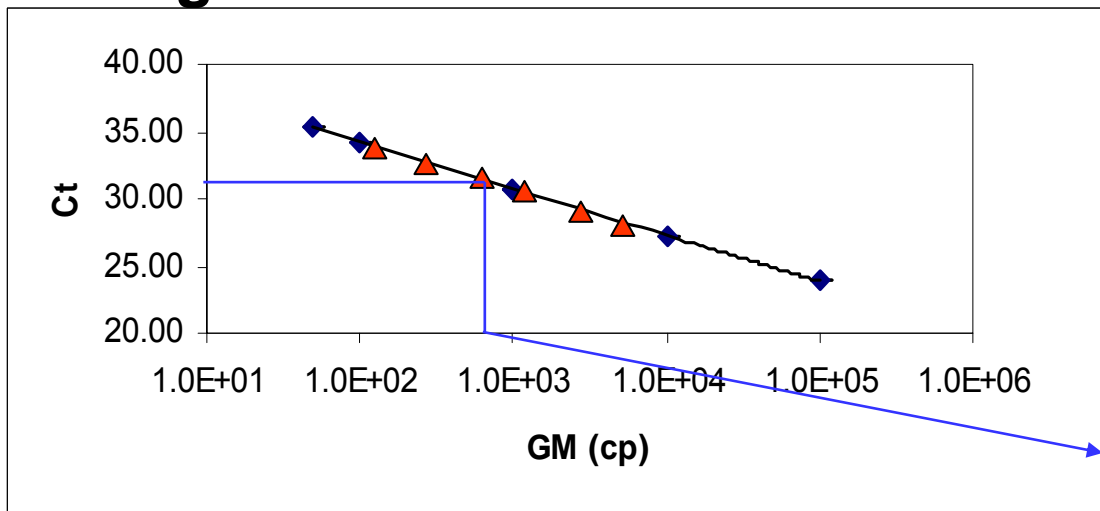
## Endogene



◆ calibrant  
▲ unknown

X cp endogene

## Transgene



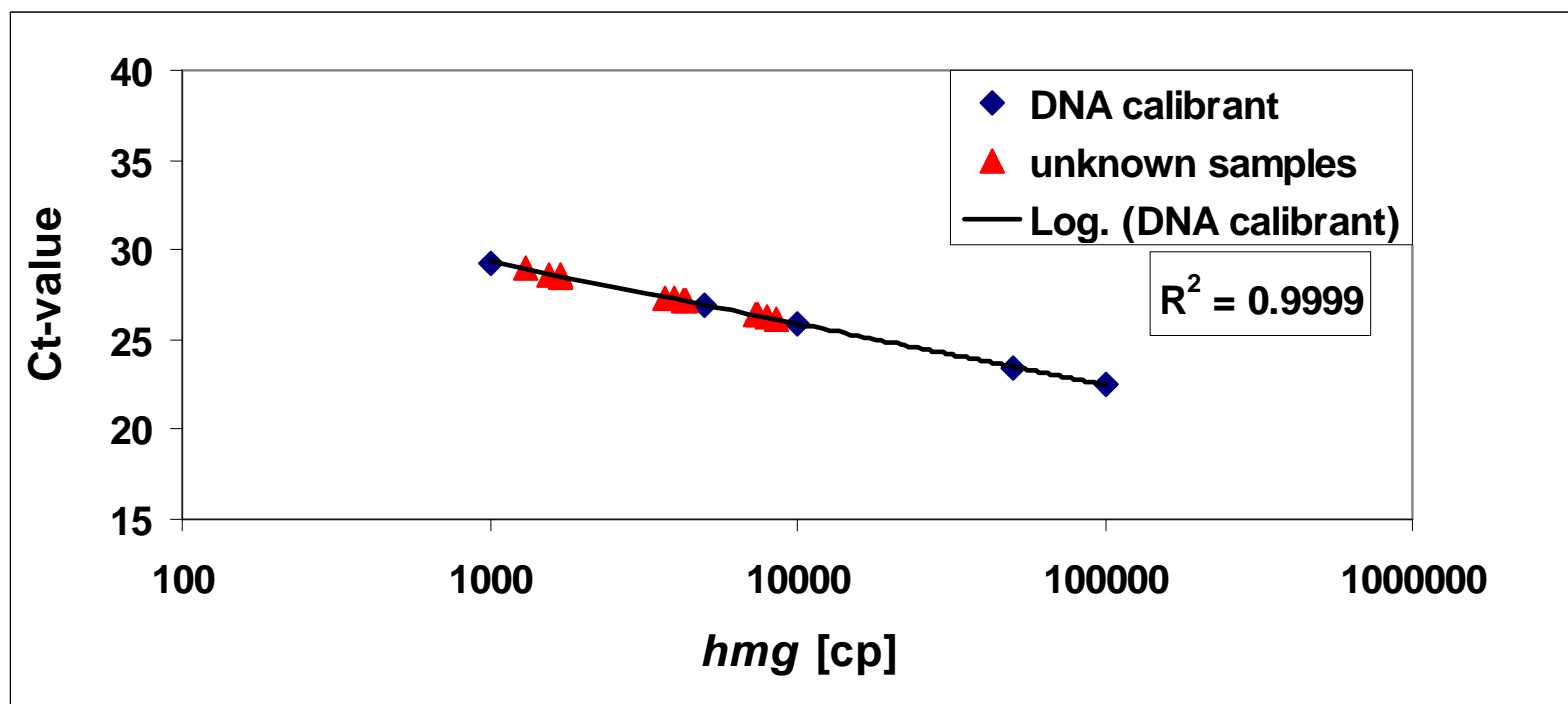
Copy Number  
ratio

Y cp transgene

## Comparison of the calibration curves:

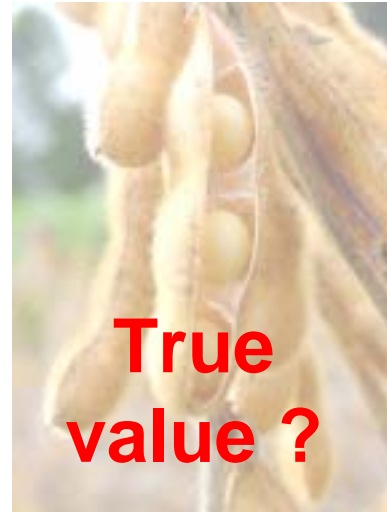
- calibrants (gDNA and pDNA) with gDNA extracted from the powder sample
- amplification efficiency of transgene and endogene & transgene : endogene ratio

**Example:**



## Study of

1.17 %  $\pm$  0.21 %  
using gDNA for  
calibration



## Soybean 356043

0.90 %  $\pm$  0.19 %  
using pDNA for  
calibration

## Considerations:

- homozygous species, should lead to a transgene : endogene ratio of 1 and consequently to 1 % for mass fraction of 10 g/kg
- Extractable DNA content GMO vs. non-GMO powder:  
1.07  $\pm$  0.14 (for N = 9,  $\bar{x} \pm U$ ; k = 2), no significant difference (95 % confidence level)
- Seed size differences, an average GM seed has about 10 % less mass than a non-GM seed of the varieties concerned

**Mass fraction**

**Copy number ratio**

**Uncertainty**

MAIZE SEED POWDER		
	Certified value	Uncertainty <sup>3)</sup>
MON 810 maize mass fraction	10.0 g/kg <sup>1)</sup>	0.5 g/kg
MON 810 maize DNA copy number ratio	0.57 % <sup>2)</sup>	0.17 %
<p><sup>1)</sup> The certified value is based on the masses of mixed dried genetically modified MON 810 maize seed powder and of dried non-modified maize seed powder, taking into account their respective purity with regard to MON 810 maize and their water content. The value is traceable to the International System of Units (SI).</p>		
<p><sup>2)</sup> The certified value is the DNA copy number ratio of the event-specific plant/P35S junction region and a single copy target of the high mobility group gene (<i>hmg</i>) measured by real time Polymerase Chain Reaction, expressed in percent. The certified value is traceable to the real-time Polymerase Chain Reaction detection method ISO 21570:2005, Annex D2 calibrated with the MON 810 maize plasmid DNA Certified Reference Material ERM<sup>®</sup>-AD413.</p>		
<p><sup>3)</sup> 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 <math>k = 2</math>, corresponding to a level of confidence of about 95 %.</p>		

**Metrological Traceability**

❖ **All certificates and reports are available via the web catalogue**

[http://www.irmm.jrc.be/html/reference\\_materials\\_catalogue](http://www.irmm.jrc.be/html/reference_materials_catalogue)

❖ **How to estimate measurement uncertainty for rt-PCR procedures?**

⇒ **Guidance document on [www.irmm.jrc.be](http://www.irmm.jrc.be)** ('reference materials' – 'user support')

❖ **How to use CRMs for GMO quantification in food and feed?**

⇒ **Application notes on [www.irmm.jrc.be](http://www.irmm.jrc.be)** ('reference materials' – 'user support') **in 22 languages**

- Use only sufficiently characterised & documented (C)RMs**
- Use independent RMs for calibration and quality control**
- Check equivalent analytical behaviour of RM & test sample !**
- CRM suitability may be limited when RM was only characterised by real-time PCR methods with 'routine performance'**

## International Conference

### “The Future of Reference Materials – Science and Innovation”



Institute for Reference Materials  
and Measurements

**23-25 November 2010**

**Geel, Belgium**

<http://irmm.jrc.ec.europa.eu/future-rm>

## Acknowledgements

- ***IRMM's RM Unit***
- ***Collaborators all over the world***

**THANK**

**YOU !**