GMO analysis and Measurement of Uncertainty – critical aspects and specific requirements

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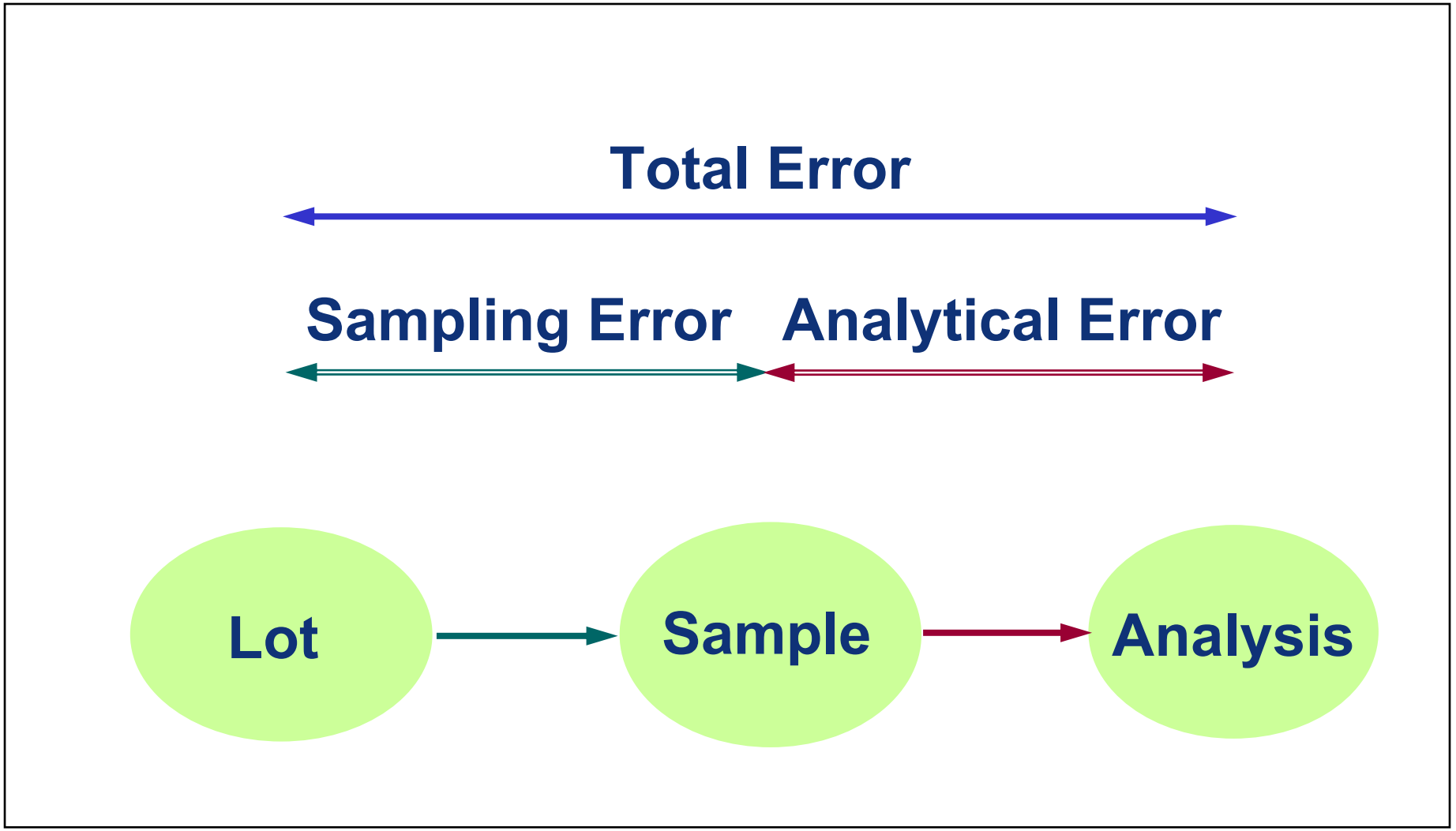
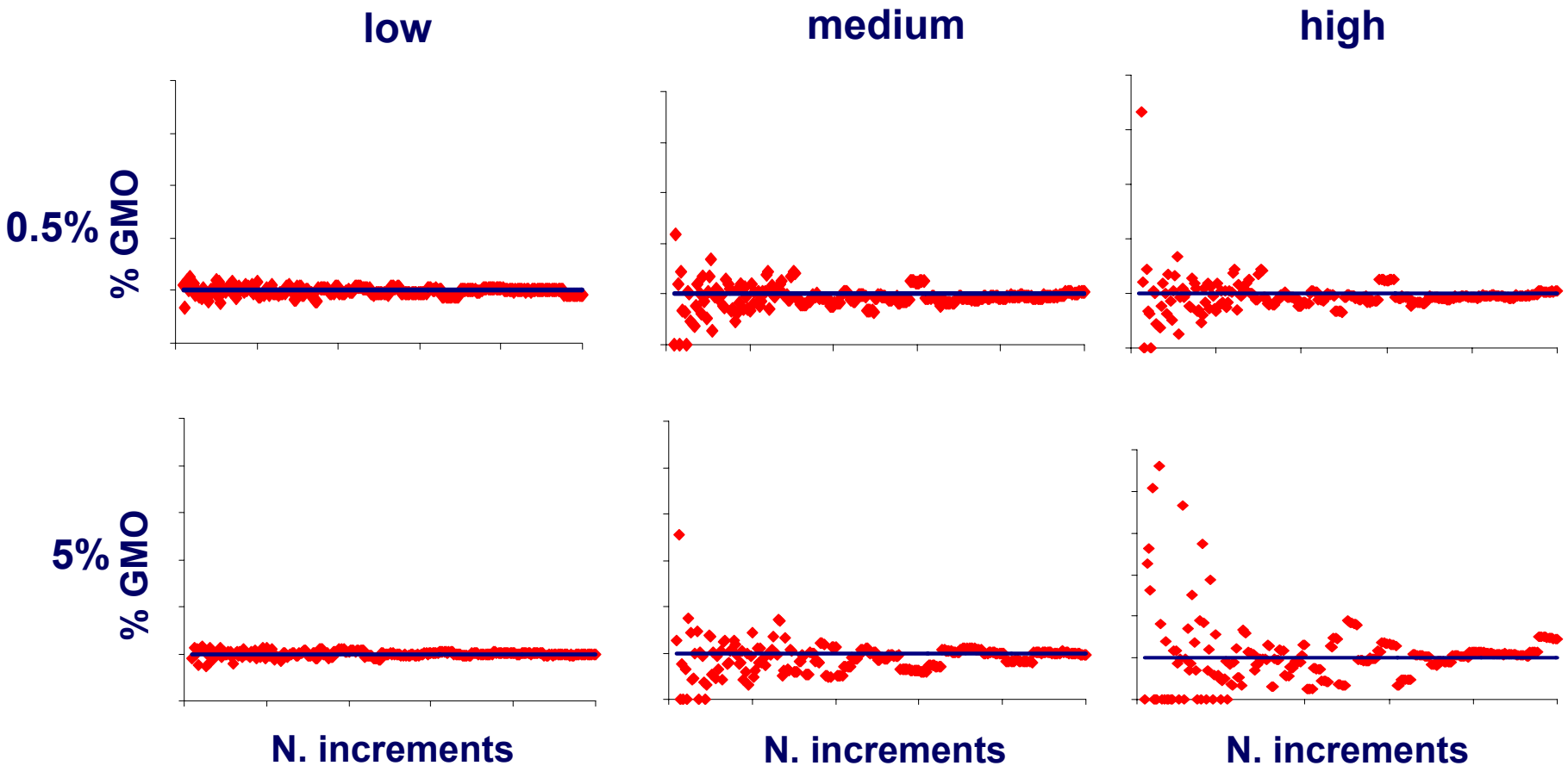
Labelling

- Labelled products that contain more than 0.9% GMO
- Enforcement shall be sure that a product must be labelled where result minus uncertainty is greater than 0.9%
- Producer shall be sure that a product does not require labelling where result plus uncertainty is less than 0.9%
- Results of different laboratories' results have to be considered for legal disputes

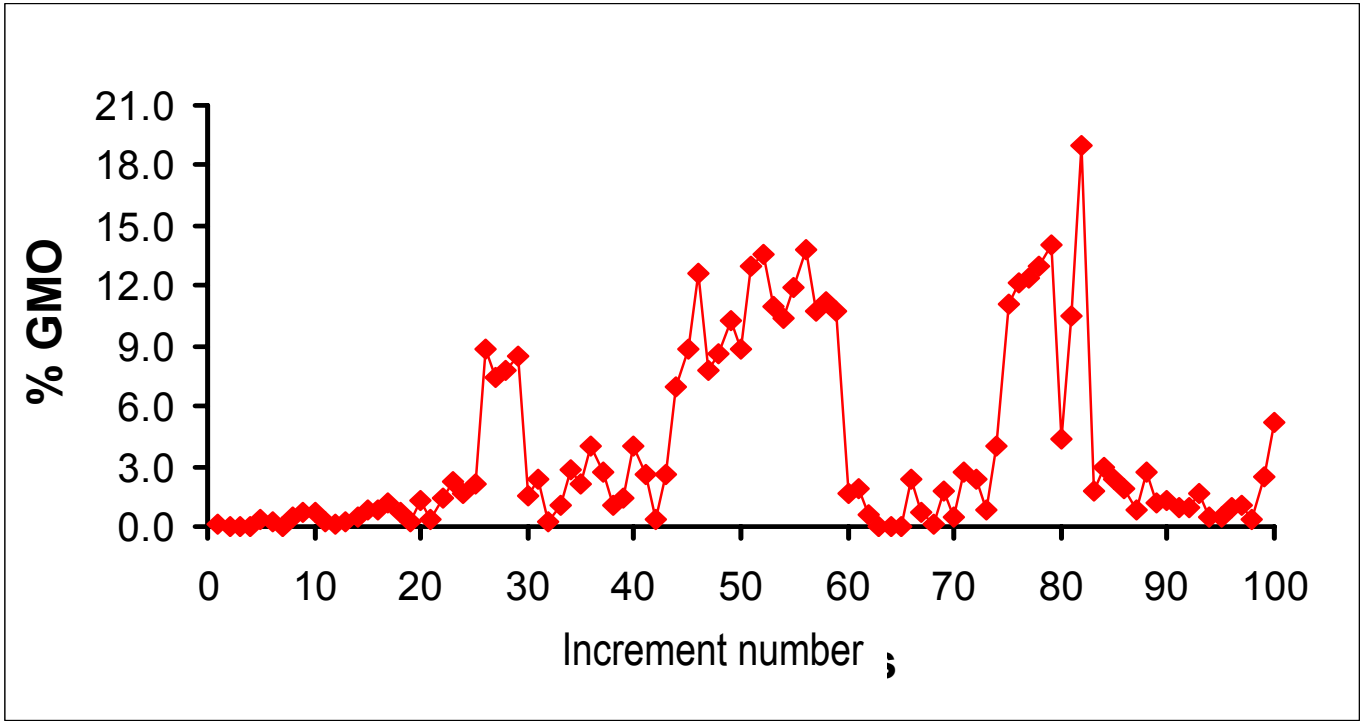
GMO analysis: sources of errors



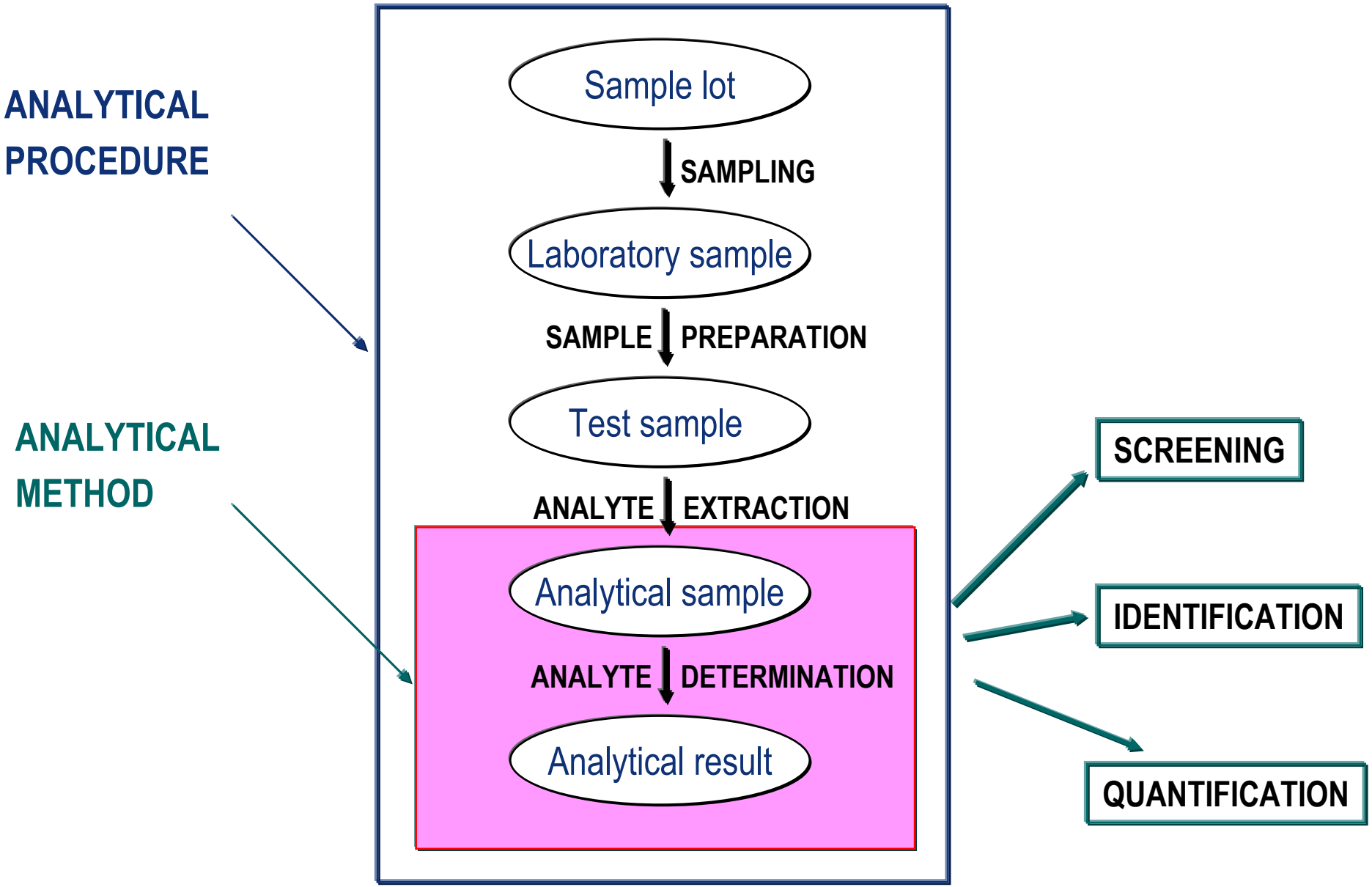
…the pattern of convergence to the "true" value is the same for all content and heterogeneity levels



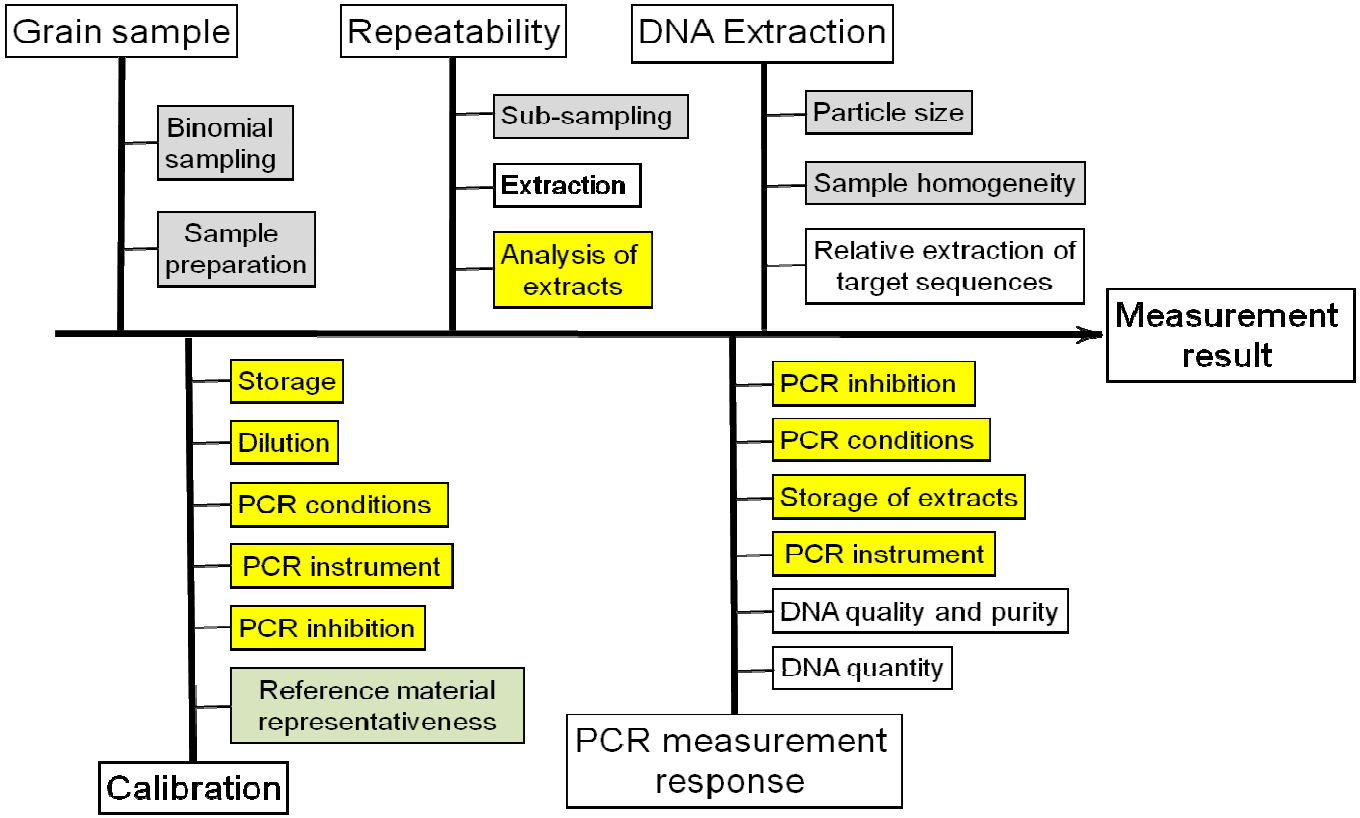
The Reality…


% GMO
21.0
18.0
15.0
12.0
9.0
6.0
3.0
0.0
0
10
20
30
40
50
60
70
80
90
100
Increment number

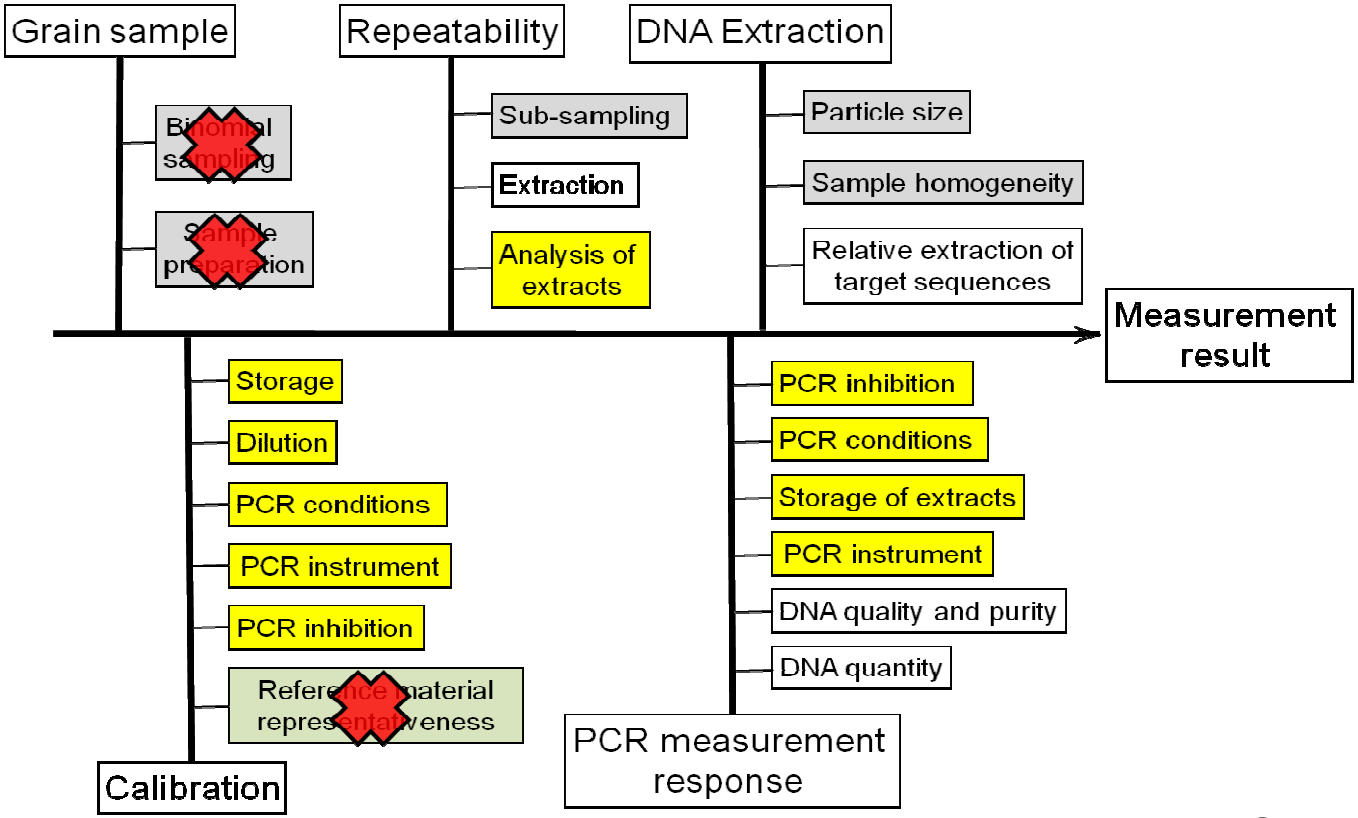
Modularity of DNA-based analysis



Sources of uncertainty



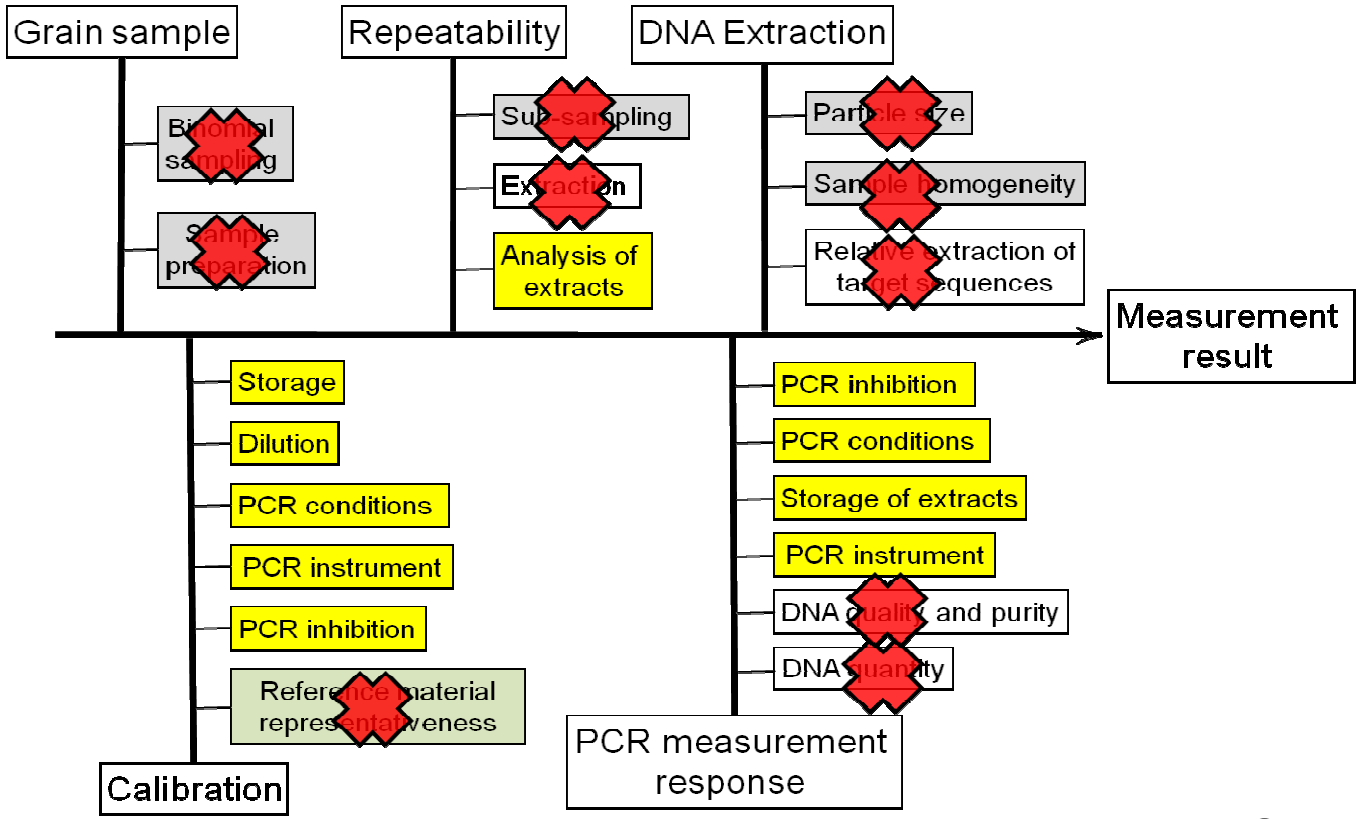
Whole method validation



RSD_R > 35%

Uncertainty is a **factor** of app. 3

Validation of PCR module



$RSD_R < 35\%$

Uncertainty is a **factor** of app. 2

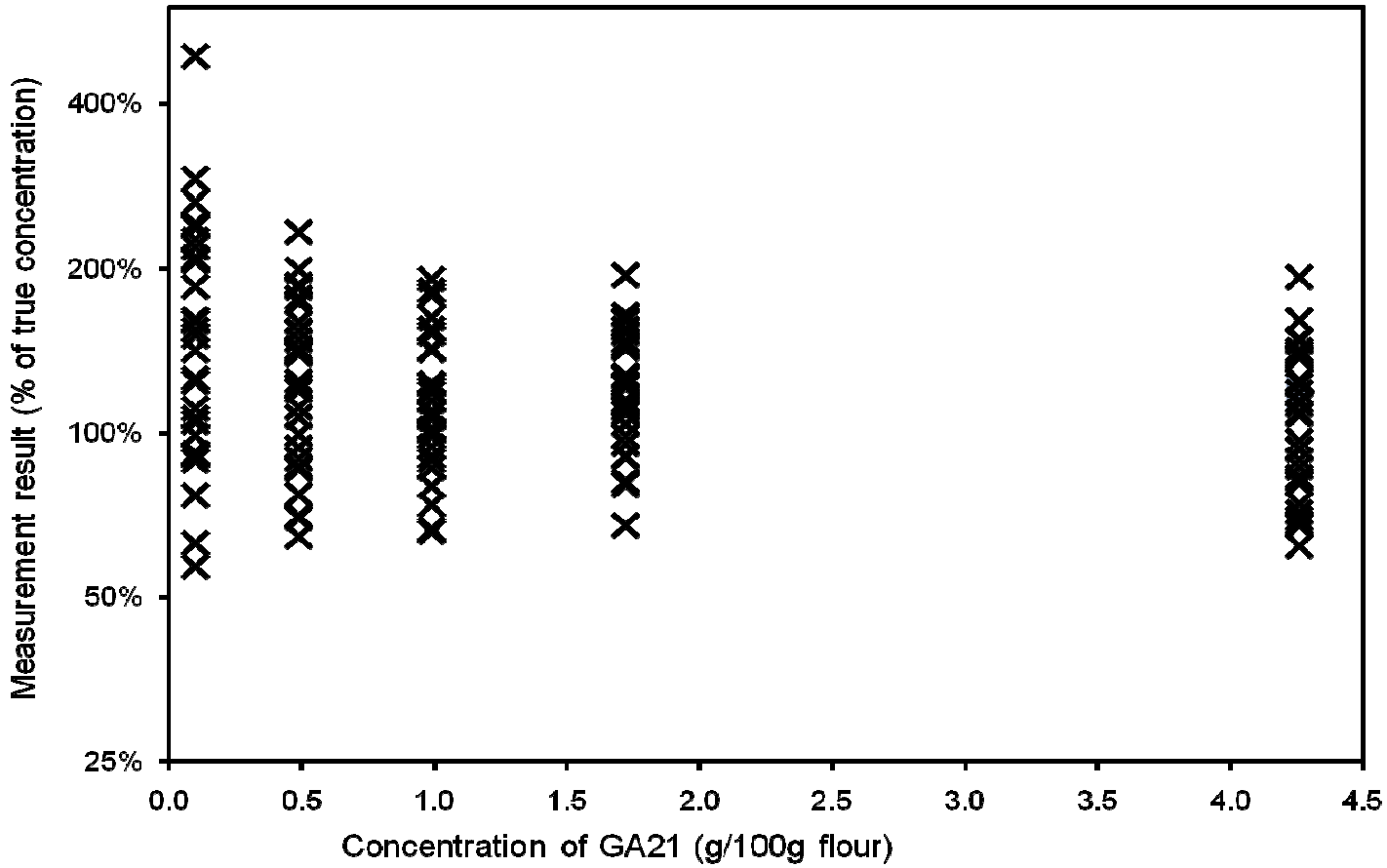
General guidance on uncertainty

- Where nothing is known about the analytical method of the whole analytical process:
  - Proficiency tests are a good model
- Results show a log-normal distribution
- Uncertainty is a **factor** of app. 3 (RSD_R > 35%)
- Result > 2.7% demonstrates need for labelling
- Result < 0.3% proves no need for labelling

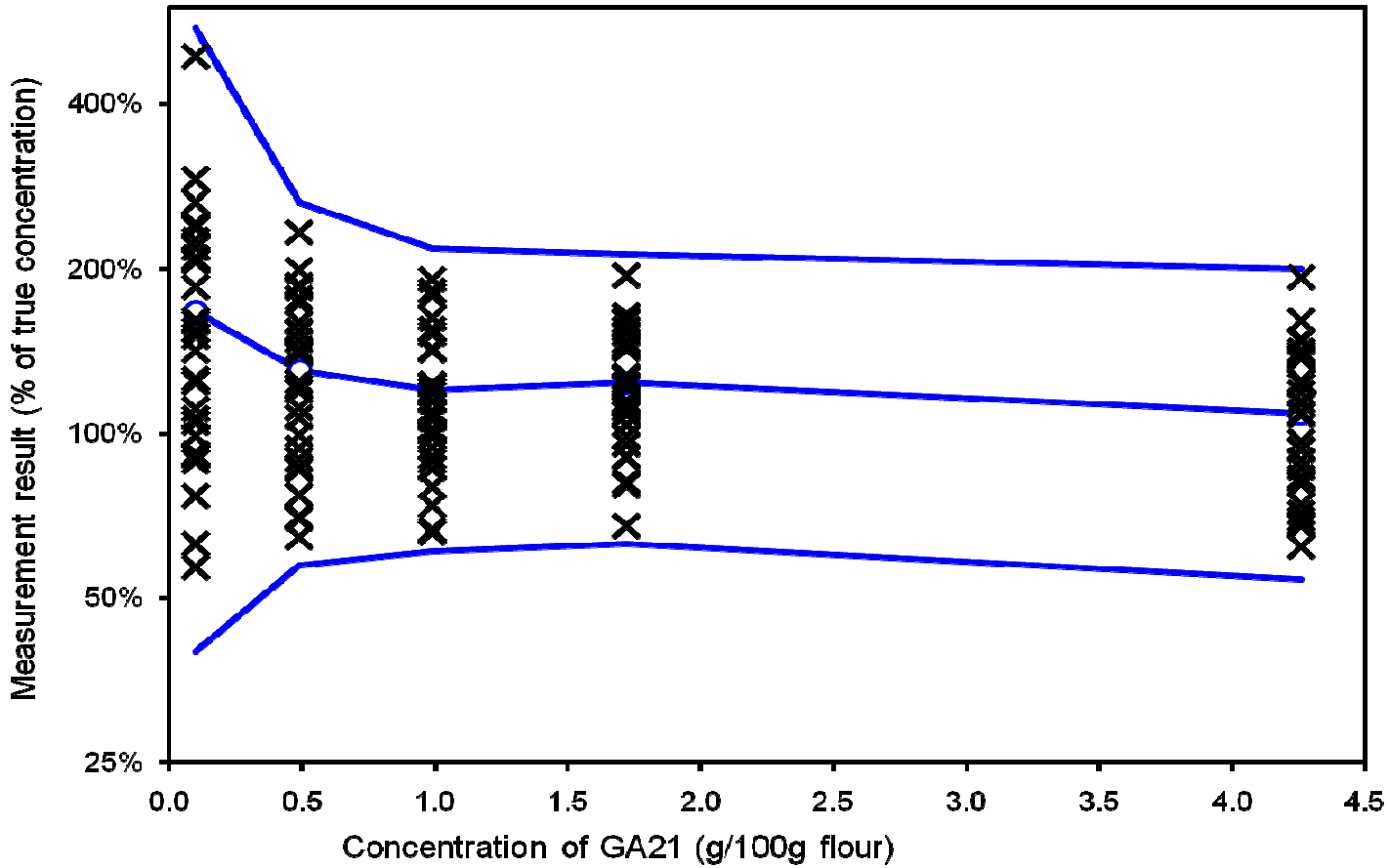
General guidance on uncertainty

- For CRL validated PCR modules
- Result display log-normal distribution
- Uncertainty is a **factor** of app. 2 (RSD_R < 35%)
- Result > 1.8% demonstrates need for labelling
- Result < 0.45% proves no need for labelling

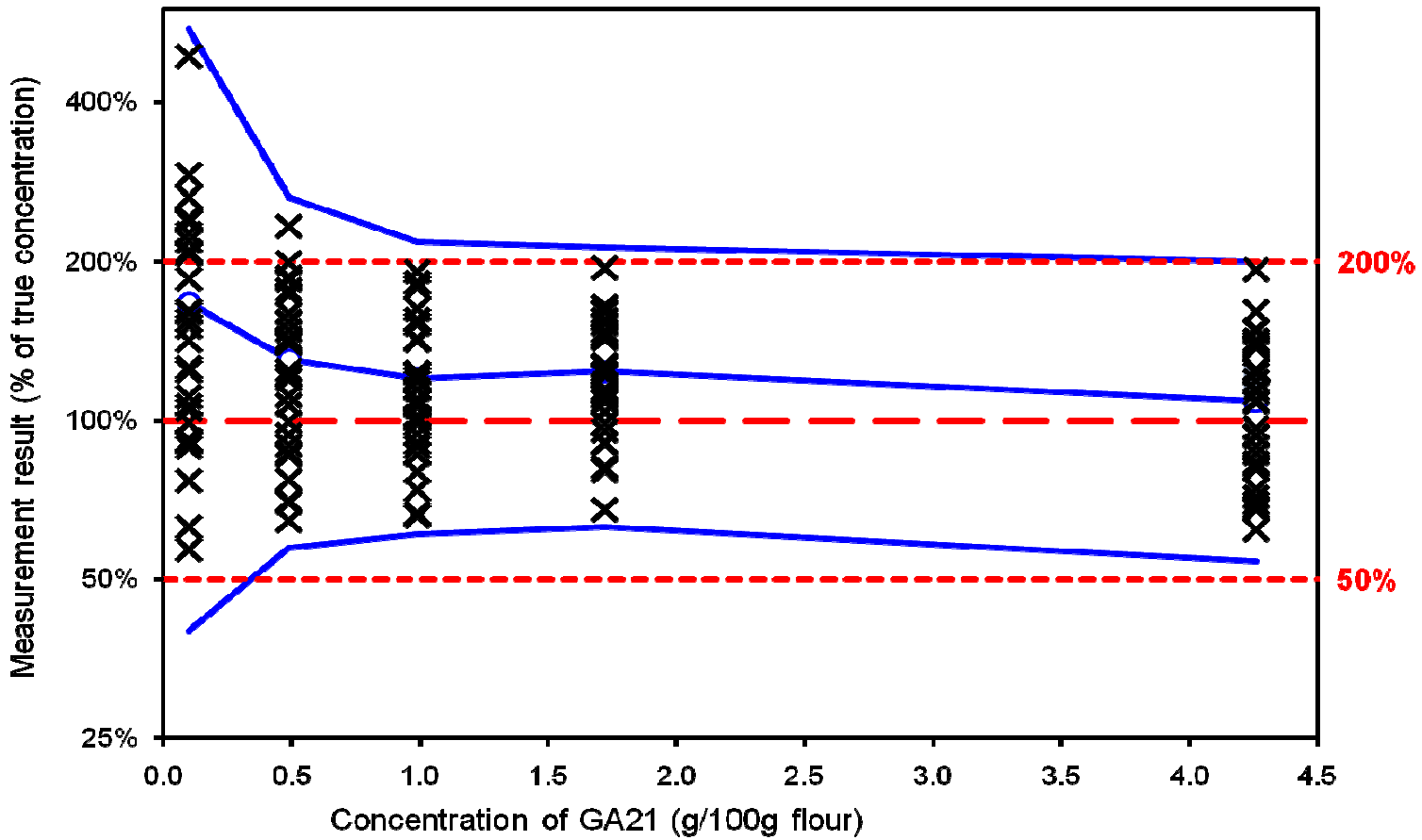
CRL validation GA21



Uncertainty profile

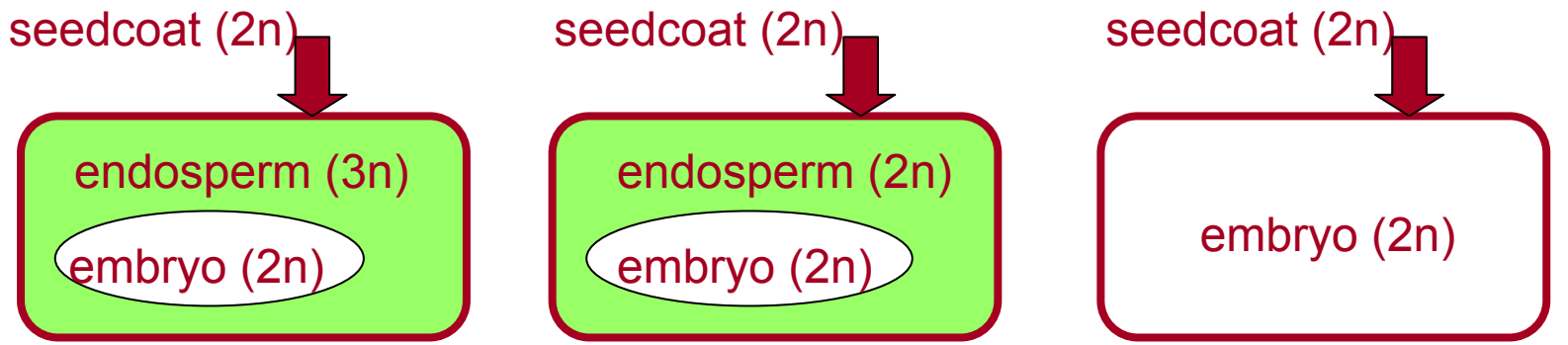


Uncertainty profile



Genetics of plant seeds – relative parent contributions

monocot (maize): Chenopodiaceae (beet) Fabaceae (soya)



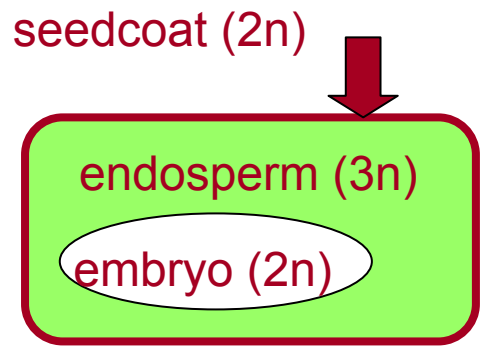
	♀	♂
embryo	1	1
endosperm	2	1
seedcoat	2	0

	♀	♂
embryo	1	1
endosperm	2	0
seedcoat	2	0

	♀	♂
embryo	1	1
endosperm	-	-
seedcoat	2	0

Taken from A.-H. Jensen

Effect of parental contributions : the concept of "biological uncertainty"



	♀	♂
embryo	1	1
endosperm	2	1
seedcoat	2	0

Maize:
Embryo ≈ 48 % of total DNA?
Endosperm ≈ 49 % of total DNA?
Seedcoat ≈ 3 % of total DNA?

From Trifa & Zhang
J. Agric. Food Chem.
52: 1044-1048 (2004)

GM ♀ X non-GM ♂
GM = 24 + 33 + 3 % = 60 % of total DNA

Non-GM ♀ X GM ♂
GM = 24 + 16 + 0 % = 40 % of total DNA

GM ♀ X GM ♂
GM = 48 + 49 + 3 % = 100 % of total DNA

What does 0.7 % GMO mean in Qn analysis?

Taken from A.-H. Jensen

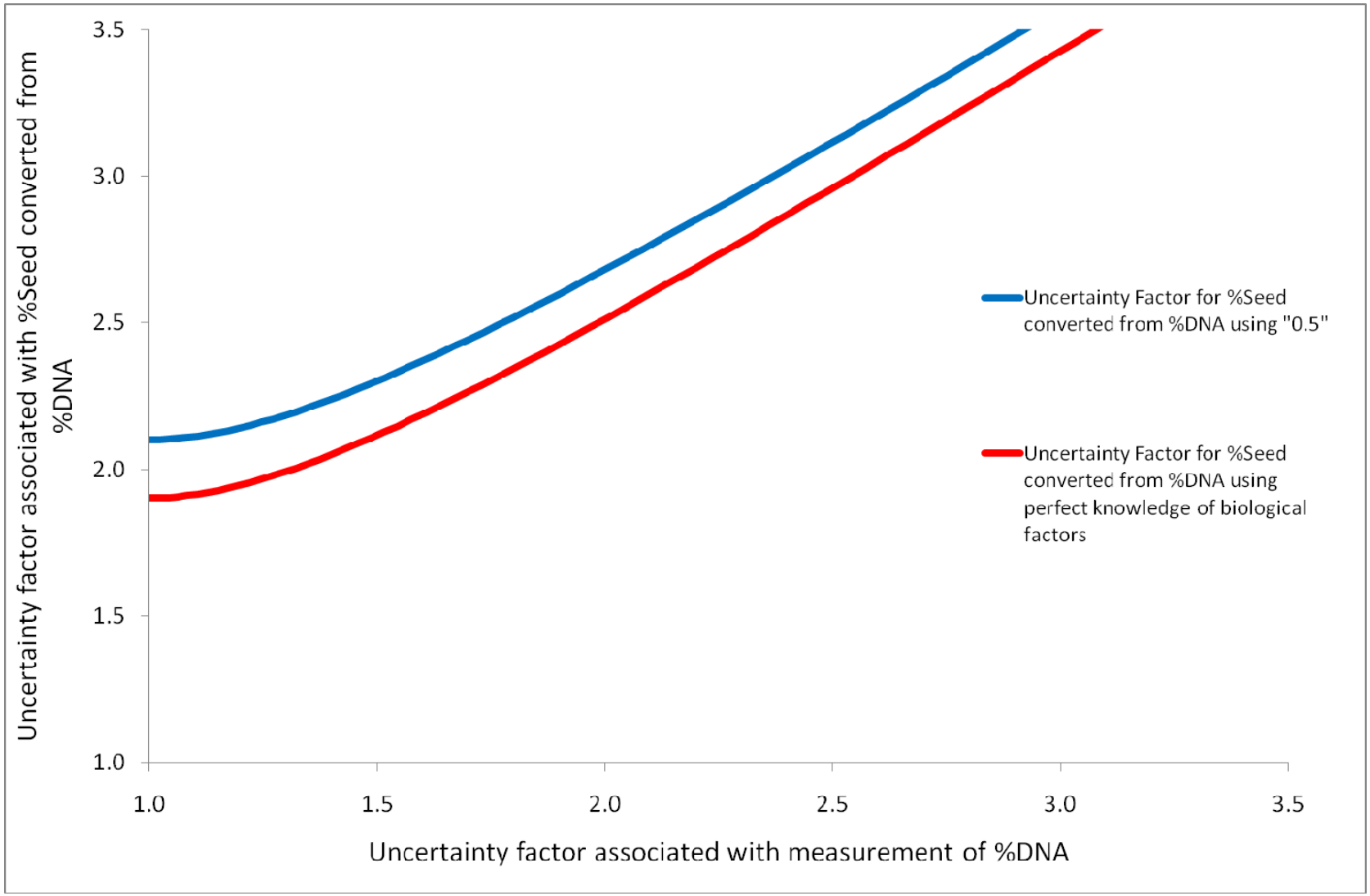
Uncertainty associated with estimates of GM content on %seed and %DNA scales

Scenario	Relative standard uncertainty	Factor for 95% confidence interval
%DNA, direct measurement (based on CRL validation data)	0.35	2.0
%Seed, direct measurement (based on results of the last 3 ISTA proficiency test)	0.38	2.1
%DNA from seed	0.54	2.8
%Seed from DNA	0.52	2.7
%DNA from seed, with perfect knowledge of biological factors	0.51	2.6
%Seed from DNA, with perfect knowledge of biological factors	0.49	2.5

Conclusion for seed analysis

The analytical uncertainty (expressed as a 95% confidence interval) is approximately a factor of 2 (whichever unit of measurement used) for samples that contain approximately 1% of GM (and the analytical uncertainty would be larger for samples that contain lower concentrations).

Relation between the uncertainty (at 95% confidence) associated with a converted quantity of GM in seeds and the uncertainty associated with the directly measured quantity



- For a crop like maize, the GM content in seeds will be composed of GM seeds heterozygous for the transgenic locus since commercial maize seeds are hybrids and are resulting from cross-pollination between two different parents (one of them carrying the relevant transgene).

- For a crop like soya, the GM content in seeds will be composed of GM seeds, homozygous for the transgenic loci, since commercial soya seeds are not hybrids and are resulting from self-pollination.

Possible conversion factor in seeds

For all crops, the GM content expressed in percentage of seeds can be considered roughly equivalent to GM content expressed in percentage of mass (in other words, the percentage in weight of GM seeds/weight of conventional seeds can be considered roughly equivalent to number of GM seeds/total number of seeds)

% seed ≈ % mass

Possible conversion factor in seeds

For crops like **maize**, where the GM content will be composed of heterozygous GM seeds, a conversion factor of 0.5 can be used between measurements based on DNA copies and measurements based on number of seeds:

% DNA ≈ % seed x 0.5 x stacking level

[i.e. number of inserts per seed]

Possible conversion factor in seeds

For crops like **soya**, where the GM content will be composed of homozygous GM seeds, a conversion factor of 1 can be used between measurements based on DNA copies and measurements based on number of seeds:

% DNA ≈ % seed x stacking level

[i.e. number of inserts per seed]

JRC Scientific and Technical Reports

Guidance Document on Measurement Uncertainty for GMO Testing Laboratories

S. Trapman, M. Burns, H. Broll, R. Macarthur,
R. Wood, J. Zel

Muito Obrigado!