European Commission

Institute for Health and Consumer Protection

Comparative testing: 4th EU-ASIA regional network meeting

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Serving society
Stimulating innovation
Supporting legislation

European Union Reference Laboratory for Genetically Modified Food and Feed (EU-RL GMFF)

Labelling of authorised Genetically Modified Organisms (GMOs)

Prevent unauthorised GMOs on the European market

Two legal mandates:

1. Regulation (EC) No 1829/2003 → validation of methods for detection and quantification of GM events
2. Regulation (EC) No 882/2004 → official controls applied to ensure the verification of compliance with feed and food law

Comparative testing (CT)

Article 32: Regulation (EC) No 882/2004

The EURLs for feed and food shall be responsible for:

Coordinating application by the NRLs of analytical methods, in particular by organising comparative testing and by ensuring appropriate follow-up of such comparative testing.

Proficiency testing (PT) = comparative testing

Aim of proficiency testing

For participating laboratories:

- Evaluation of laboratory performance
- Identification of problems in laboratories
- Education of participating laboratories

For customers, regulators and accreditation bodies:

- Ongoing confidence in laboratory performance
- ISO 17025 accredited laboratories need to show proof of participation in proficiency testing schemes to accreditation body

Launch of CT activities - 2010

- Two CT rounds per year
- On average 100 participants per round
- Each CT round: two test items containing different concentration levels of one or more GM events
- Six weeks to conduct analyses

Preparation of test items

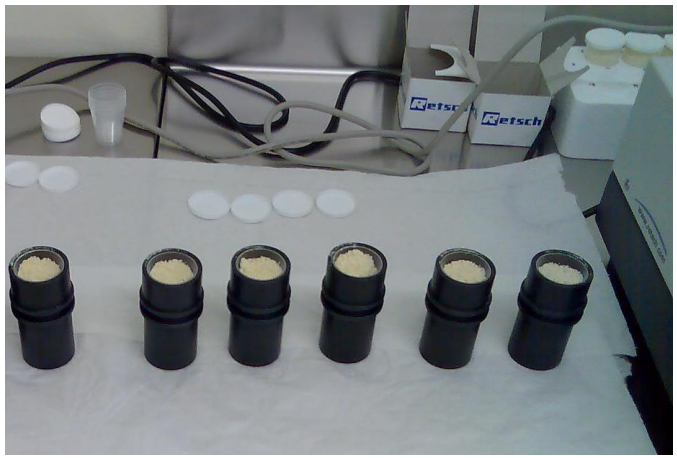
Raw material

Pre-grinding

Post-grinding

Preparation of test items (continued)

Bottling



Final product

Data analysis

- ISO 13528: Statistical methods for use in proficiency testing by interlaboratory comparisons
- Statistician
- Assigned value in-house versus calculation of robust mean
- z-scores

Data analysis (continued)

- Aim: obtain a simple and transparent result
- Submitted results are log-transformed
- Standard deviation for proficiency assessment $\hat{\sigma}$
- Calculation of z-scores: reference value or consensus value from participants (i.e. robust mean $\hat{\mu}$)

$$z_i = \left(\log_{10} x_i - \hat{\mu}\right) / \hat{\sigma}$$

- Where z_i = z-score of participant i
  x_i = results of participant i

Participation of EU-ASIA in previous comparative testing rounds

- ILC-CRL-GMFF-CT-01/10: 5 participants (China, India, Malaysia, Republic of Korea, Singapore)
- ILC-CRL-GMFF-CT-02/10: 6 participants (India, Indonesia, Malaysia, Republic of Korea, Singapore, Vietnam)
- ILC-EURL-GMFF-CT-01/11: 5 participants (India, Malaysia, Republic of Korea, Singapore, Vietnam)

EURL-GMFF-CT-02/11

- Two maize test items containing different GM %ages
- List of 10 GM maize events: 3272, Bt11, Bt176, DAS59122, GA21, MIR604, MON 810, MON 863, NK603, TC 1507
- Qualitative PCR
- Detection of certain GM event → quantify GM content
- Shipment: 24 October 2011
- Deadline submission results: 9 December 2011
- Test items: produced in-house by Marko Maras

Participants

- 158 laboratories invited
- 105 registered laboratories including also double registrations
- 15 laboratories of EU-ASIA network invited
- 8 participants of the EU-ASIA network: China, India, Indonesia, Malaysia, Singapore, Thailand, The Philippines, Vietnam

Distribution of participants

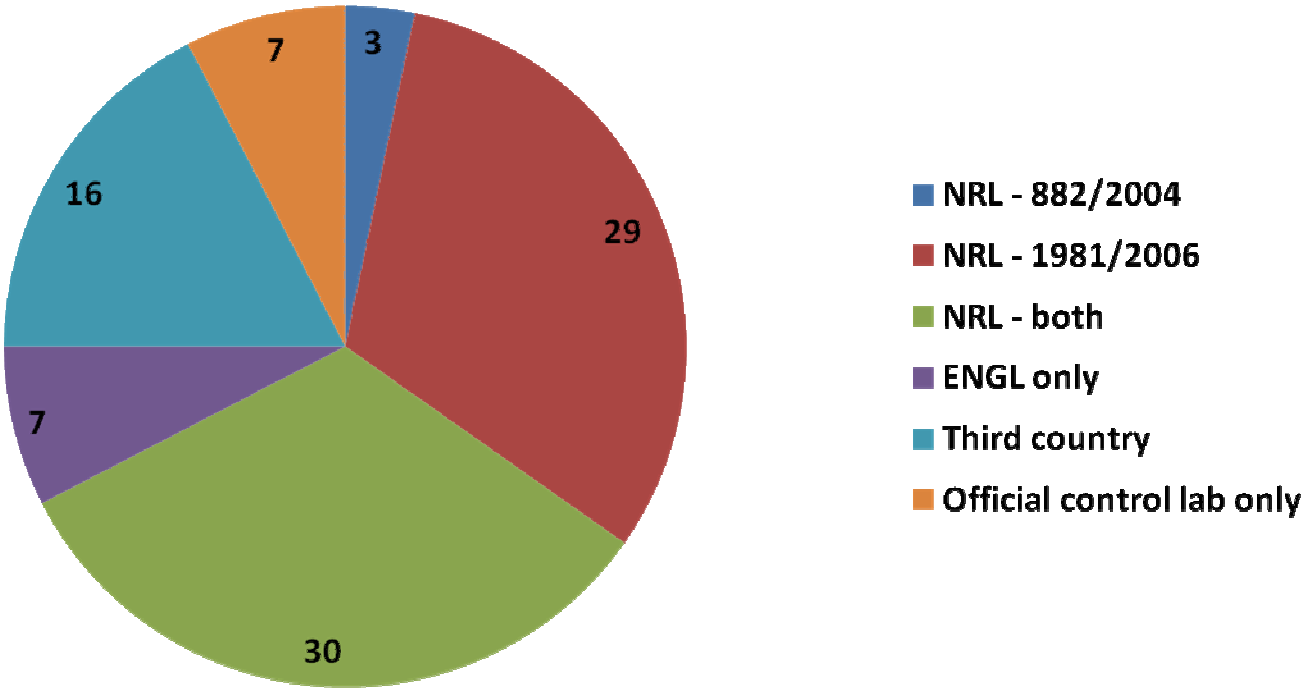


Table 1: Overview of GM events present in maize powder levels 1 and 2.

GM event	Maize powder level 1	Maize powder level 2
GA21	+	+
TC1507	+	+
MIR 604	+	+
NK603	Adv	Adv
59122	Adv	Adv
MON 810	Adv	Adv
MON 863	-	-
3272	-	-
Bt11	-	-
Bt176	-	-

+ indicates that the GM event was added to the test items,
-indicates that the GM event was not added to the test items,
-Adv: adventitious presence

Participants' performance

Quantification of GA21, 1507 and MIR604

- Maize event GA21:
  1. NRLs: 9
  2. ENGL only: 3
  3. Labs from 3rd countries: 2
  4. Total: 14
- Maize event 1507:
  1. NRLs: 5
  2. ENGL only: 2
  3. Labs from 3rd countries: 3
  4. Total: 10
- Maize event MIR604:
  1. NRLs: 8
  2. ENGL only: 3
  3. Labs from 3rd countries: 1
  4. Total: 12

Participants' performance

EU-ASIA network

- Six out of eight participants: qualitative analyses
- Two participants: maize events 1507 and MIR604
- Certificates of successful participation

Repetition of experimental work

- Shipment of test items to 18 laboratories of which 2 double sets
- Deadline for submission of results: 23 May 2012

European Commission

Certificate of successful participation

Comparative Testing Certificate

The European Commission/ DG Joint Research Centre

certify that

Bureau of Plant Industry, Plant Quarantine Service

BPI Compound, Economic Garden 4030 Los Banos

has successfully participated in the comparative test : **EURL-CT-02/11**

on material(s): **1507 GA21 MIR604**

launched in: **June-2011**

The laboratory has correctly detected the GM events GA21 and 1507

organised by the European Union Reference Laboratory for Genetically Modified Food and Feed

The EU-RL GMFF is accredited under ISO 17043 (Comparative Test Providers) by DAKKS (Accreditation D-EP-14322-01-00)

Reasons outlying z-scores

- Problems with calibration curve:
  $R^2 < 0.98$ and/or $-3.6 \leq$ slope ≤ -3.1

Calibrant	Ct-values
3 % NK603	33.40
3 % NK603	33.01
5 % NK603	33.27
5 % NK603	32.90

Dilution factor	Theoretical ΔCt	Actual ΔCt	Theor. – actual ΔCt
2	1	1.6	-0.6
10	3.322	2.322	1

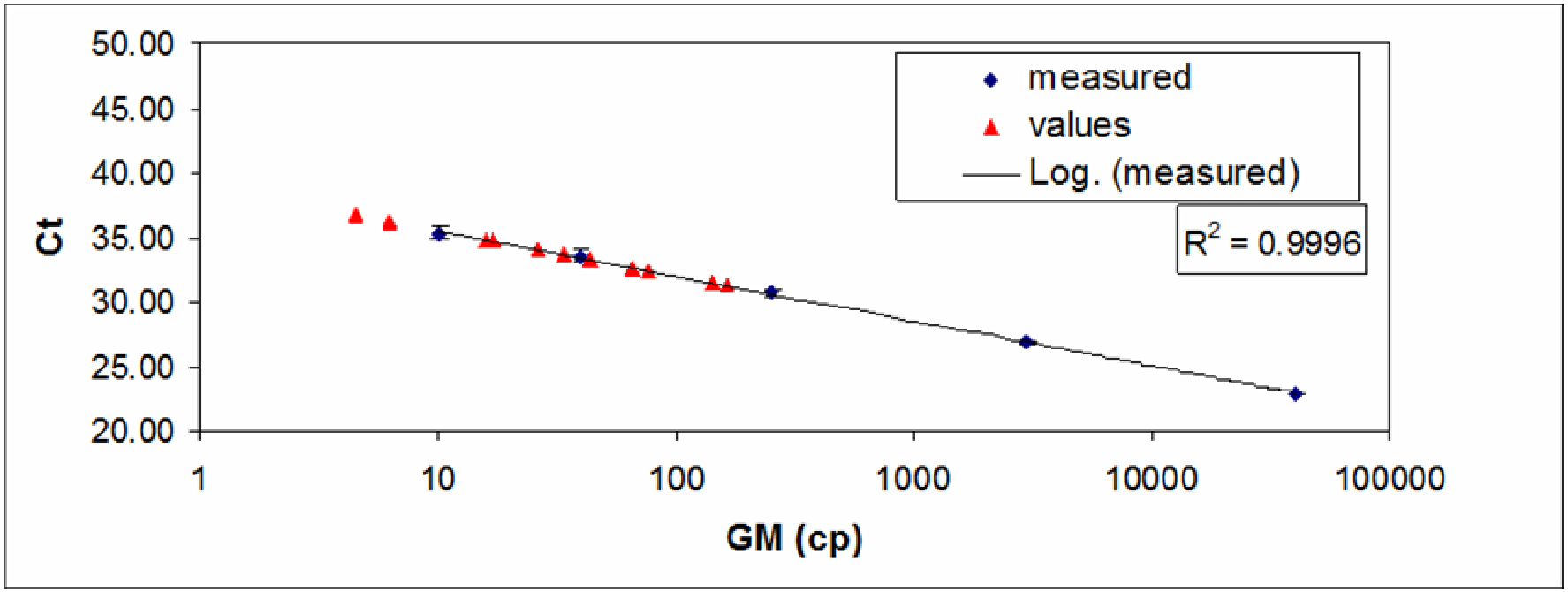
Reasons outlying z-scores (continued)

- DNA quantity loaded on real-time plate = max. 200 ng

  Overloading plate → inhibition
- Overestimation of DNA concentration: (UV spectrophotometer)

  Not enough DNA loaded on plate → Ct values outside linear working range of calibration curve
- Swapping test items
- Use of one PCR replicate (i.e. one sample per well)

Calibration curve



Tips for CT participants

- Ensure labelling and traceability of test items → avoid swapping of test items
- Calibration curve:
  - Dilution of standards
  - Quality criteria of calibration curve
  - Dilution of test items → within linear working range of calibration curve
- Good communication

Tips for CT participants (continued)

- Include controls:
  - Negative control → contaminations
  - Positive control or quality control material → check of quantification
- Reporting → check:
  - Reported values
  - Measurement unit and conversion factors if applicable

Trick

- To avoid Ct values outside the linear working range of the calibration curve:
  - Concentration = 40 ng/µL for standards calibration curve
  - Concentration = 35 ng/µL for unknown samples and quality control materials

Final report

- Drafted after repetition of experimental work
- Results of repeated experiments are reported in a separate section → to see the effect on the z-score
- Draft report submitted to Advisory Board for comparative testing
- Meeting with Advisory Board to discuss report
- Finalisation of report
- Submission to PUBSY
- Each participant receives personal laboratory code along with report
- DG SANCO:
  - Only laboratory codes of NRLs are disclosed
  - Separate letter commenting on NRLs' performance

Team

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