Quality assurance in GMO analysis

A practical approach for estimation of measurement uncertainty and visualisation of quantitative real-time PCR data

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GMO quantification and quality control

- Quantification of genetically modified organisms (GMO) in food or feed samples is primarily done by relating the DNA copy number for a GMO-specific inserted gene (transgene) with the copy number of a species-specific reference gene. This ratio represents a copy-based percentage which is then converted into a weight-based percentage of GMO contents provided that the zygosity of the analysed plant material is known. The copy numbers for GMO and reference gene are determined by two separate quantitative real-time PCR assays with standard curves using TaqMan chemistry [1].
- For calculation of relative GMO content we developed a MS Excel master (Fig. 1) that is semi-automatically combined with a set of control charts (Fig. 2) visualising essential reaction parameters for quality control purposes.
- The approaches presented here have become valuable tools in order to monitor and maintain the quality standards at the Bavarian Health and Food Safety Authority (LGL), assured by accreditation according to DIN EN ISO /IEC 17025:2005.

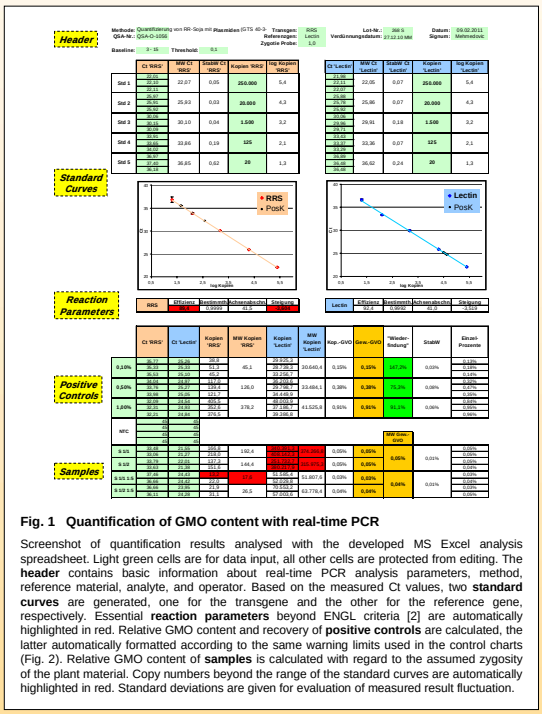


Fig. 1 Quantification of GMO content with real-time PCR

Screenshot of quantification results analysed with the developed MS Excel analysis spreadsheet. Light green cells are for data input, all other cells are protected from editing. The **header** contains basic information about real-time PCR analysis parameters, method, reference material, analyte, and operator. Based on the measured Ct values, two **standard curves** are generated, one for the transgene and the other for the reference gene, respectively. Essential **reaction parameters** beyond ENGL criteria [2] are automatically highlighted in red. Relative GMO content and recovery of **positive controls** are calculated, the latter automatically formatted according to the same warning limits used in the control charts (Fig. 2). Relative GMO content of **samples** is calculated with regard to the assumed zygosity of the plant material. Copy numbers beyond the range of the standard curves are automatically highlighted in red. Standard deviations are given for evaluation of measured result fluctuation.

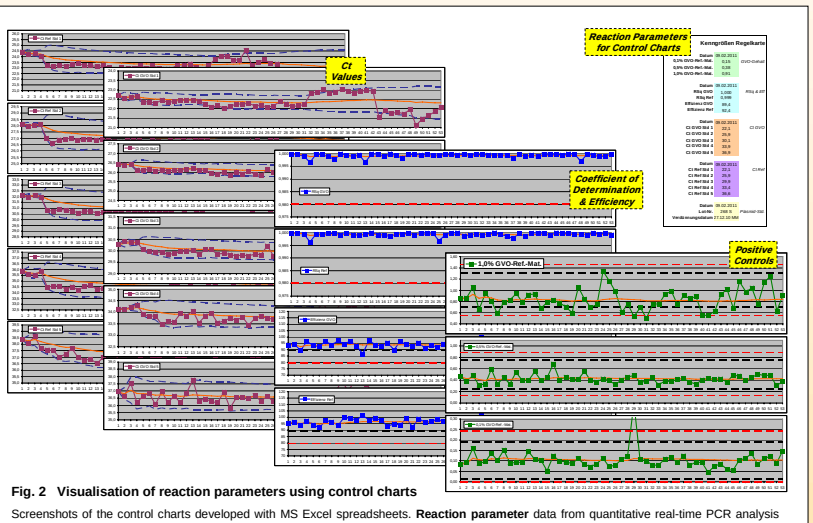


Fig. 2 Visualisation of reaction parameters using control charts

Screenshots of the control charts developed with MS Excel spreadsheets. **Reaction parameter** data from quantitative real-time PCR analysis (Fig. 1) is semi-automatically prepared for insertion into the corresponding control charts. The charts visualise the parameter values from consecutive runs, allowing to spot changes like outliers or trends. Upper and lower warning and control limits are depicted as dashed black and red lines, respectively. Moving average of the parameters is depicted in orange. The measured GMO contents of **positive controls** with three different known GMO percentages give valuable hints about measurement quality in the low percentage range most critical for GMO analysis (2s warning, 3s control limits, based on data from a set of appr. 20 analytical runs). **Coefficient of determination and efficiency** data are extracted from standard curves and allow monitoring of assay performance (ENGL criteria [2]: R^2>0.98, $Eff_{warning}$>89.6; empirically: $Eff_{control}$>79.1). Visualisation of **Ct values** is of minor importance, as changes are mostly irrelevant for relative quantification results and due to mostly uncontrollable small effects (moving 2s limit). All data is available in box plot format as well (data not shown).

Measurement uncertainty

- There are many comprehensive guides for estimation of measurement uncertainty (MU) available [e.g. 3, 4]. Unfortunately, many of these guidelines have a rather chemistry background and may not therefore be reliably applied to real-time PCR measurements.
- Few publications exist that deal especially with MU in GMO analysis [e.g. 5, 6, 7] but unfortunately do not cover the situation in our laboratory adequately.
- We therefore developed a practical approach based on available data from control samples, reference material, and samples (Fig. 3).

Fig. 3 Estimation of MU for quantitative GMO analysis

Our approach on estimation of MU is based on routinely measured positive controls (average value of triplicates; 0.1%, 0.5%, 1.0% w/w certified GMO fraction) and on GMO-positive samples (two isolates; average value of duplicates each).

Accuracy is calculated as standard deviation (SD) of measured positive controls (reference material).

Recovery is calculated as SD of absolute difference between certified value μ and average measured values.

Reference Material has a certified u.

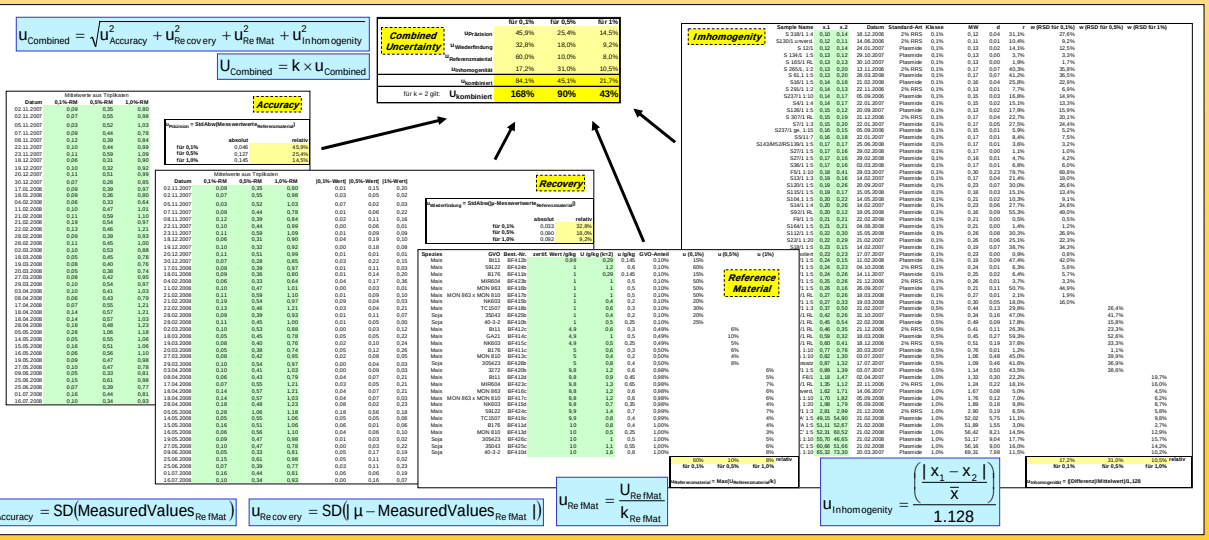
Inhomogenity is calculated according to [7] as absolute difference of both isolates divided by average value of sample, and divided by 1.128 (for n=2).

All four uncertainties (u) are combined according to the rules of error propagation. This **combined uncertainty** is multiplied by a factor k=2 (for 95% confidence interval) to give the expanded uncertainty U.

All uncertainties are estimated separately for 0.1%, 0.5%, and 1.0% GMO.

$$u_{Combined} = \sqrt{u_{Accuracy}^2 + u_{Recovery}^2 + u_{ReMat}^2 + u_{Inhomogenity}^2}$$

$$U_{Combined} = k \times u_{Combined}$$



$$u_{Accuracy} = SD(MeasuredValues_{ReMat})$$

$$u_{Recovery} = SD(|\mu - MeasuredValues_{ReMat}|)$$

$$u_{ReMat} = \frac{U_{ReMat}}{k_{ReMat}}$$

$$u_{Inhomogenity} = \frac{\left(\frac{|x_1 - x_2|}{\bar{x}}\right)}{1.128}$$

References

[1] *European Union Reference Laboratory for GM Food and Feed (EURL-GMFF).* http://gmo-crl.jrc.ec.europa.eu/.

[2] ENGL (2005) *Definition of minimum performance requirements for analytical methods of GMO testing.* http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requirements_Analytical_methods.pdf.

[3] JCGM, *Evaluation of measurement data - Guide to the expression of uncertainty in measurement (GUM 1995 with minor corrections)*, JCGM 100:2008, 2010

[4] ISO, *ISO/TS 21748: Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty estimation*, ISO, 2004. 1-30.

[5] Burns, M. and H. Valdivia, *A procedural approach for the identification of sources of uncertainty associated with GM quantification and real-time quantitative PCR measurements.* Eur Food Res Technol, 2007. **226**: 7-18.

[6] Trapmann, S., et al., *Guidance document on measurement uncertainty for GMO testing laboratories*, JRC, 2007. 1-41.

[7] Žel, J., et al., *Calculation of measurement uncertainty in quantitative analysis of genetically modified organisms using intermediate precision--a practical approach.* J AOAC Int, 2007. **90**(2): 582-6.

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