

Validation of a quantitative duplex real-time PCR system to determine the amount of GMO in food

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Abstract

With the upcoming of genetically modified food products, PCR technology has become an important tool in food analysis. Recently quantitative PCR systems became available (real-time and competitive systems). However, no validation data of such a system has been published yet. Here we present the validation data of a duplex quantitative system. We used the LightCycler (Roche Diagnostics) for real-time quantification (FRET). The data show the capability to quantify GMO in food and the limits. The validation data comply with EN 45001 and were approved by the Swiss Accreditation Service (SAS).

Keywords: GMO-quantification, real-time PCR, validation, international guidelines

Materials and Methods

DNA-isolation

DNA-isolation was done applying the Wizard protocol according to the protocol SLMB, chapter 52B (Wizard protocol, Promega). After RNase digestion, the content of DNA was measured photometrically (Genequant II, Pharmacia).

Amplification and Detection

The hybridization probes for the Zein-gen was labeled with RED705 and for the 35S promoter with RED640. 45 PCR cycles were run on a LightCycler (Roche). The standardization curve was set manually using the proportional mode.

Standards

For the validation we used certified standards (maize Bt-176) from Fluka.

generation of the data

Each datapoint was generated by at least 24 individual experiments done by 3 persons at more than 3 different days over 2 months. The right choice and stability of standards are crucial points. Maize DNA-standard was diluted in DEPC treated water.

35S system (GMO-marker)

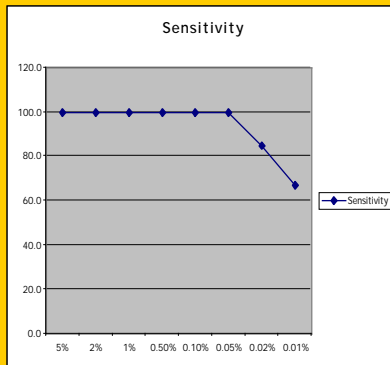


Figure 1

A clear peak at the correct melting temperature was validated as a positive signal. This shows only qualitative and not quantitative data.

Linearity of the duplex system (zein/35S)

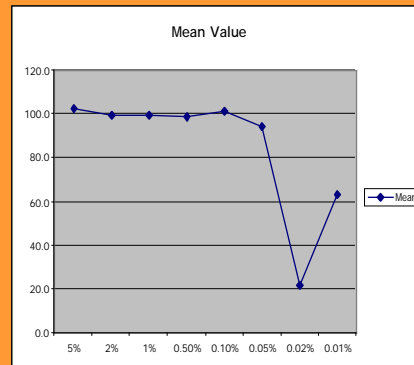


Figure 2

If the measured value meets the theoretical value the expectation was 100% fulfilled

Precision of the duplex system (zein/35S)

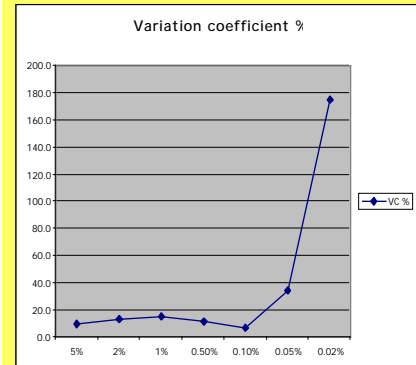


Figure 3

The variation coefficient indicates the interval where 63% of the realizations of a single measurement have its value.

sensitivity limit

The sensitivity limit (95%) for the 35S system was between 0.05% (100%) and 0.02% (84%) of GMO standard (Bt-176) (Fig 1). The sensitivity limit for the zein system for maize (95%) was below 1.6ng (100%) of GMO standard (Bt-176) (data not shown).

repeatability and intermediate precision

The repeatability is the lower criteria than the intermediate precision. Therefore if the intermediate precision is determined, the criteria for the repeatability is enclosed. During two months the sensitivity remained at least at 0.05% GMO (100%), done by at least 3 different persons.

linear range

The linearity (Fig 2) in the range of 0.1% to 5% is between 102.3% and 99.6% of the theoretical expectation.

precision and working range

The variation coefficient was used to measure the precision. When decreasing the amount of template below 0.1% the variation coefficient increased. In the range of 0.1% to 5% the maximal variation coefficient was 16% for the duplex system (Fig 3).

uncertainty

The maximal difference of the mean values to the theoretical value in the range of 0.1% to 5% was taken to measure the uncertainty. This gives +2.3/-0.4% (Fig 2).

Discussion

Although validation of qualitative PCR-systems are difficult, quantitative PCR-systems are even more difficult to validate. The here shown data concern the following criteria for both systems (zein for maize and 35S for GMO): sensitivity, repeatability, intermediate precision, linear range, the working range, precision and uncertainty. The data of the other validation criteria are not presented here but were also investigated during the validation procedure (specificity, inhibition, robustness). The final precision for the PCR system was characterized by the variation coefficient maximal 16% and the uncertainty of +2.3/-0.4%. Pipetting errors for the samples can be excluded when using a duplex system.

Using a biological system these results are surprisingly precise considering the fact that two markers have to be determined for the final result. Additionally sampling contributes also to the variation of results especially in the lower range, and was not investigated during this validation. The data presented here can serve as basic to set limits and to adjust the expectations from measurements using PCR-methods to a more objective level.