

Validation of a multiplex PCR system based on international guidelines

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Abstract

With the upcoming of genetically modified food products, PCR technology has become an important tool in food analysis. Many protocols have been developed and published. However, no data about validation of such a system has been published yet. To fulfill the requirements of quality control a multiplex PCR system detecting maize, soya and the 35S-promoter was validated. This validation makes a reliable GMO (genetically modified organism) screening possible and guarantees a highly reproducible sensitivity of 0,05% (>95%) for the 35S-promoter. The validation data comply with international guidelines and can serve as an example how to validate a PCR system for a diagnostic food laboratory.

Keywords: GMO-screening, PCR, multiplex, validation, international guidelines

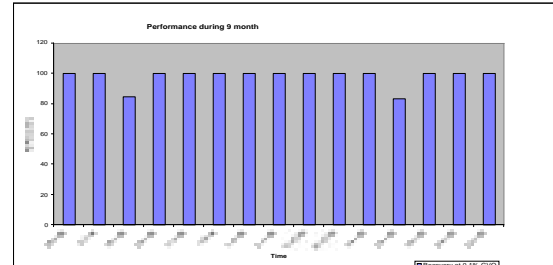


Figure 2

Guidelines for Validation

DIN 58967-60

DIN 58967-61

EMEA CPMP ICH 381/95 Topic Q2A

EMEA CPMP ICH 281/95 Topic Q2B

Paul-Ehrlich-Institut Richtlinie

OMCL

Polymerase-Kettenreaktion Begriffe, allgemeine methodenspezifische Anforderungen

Polymerase-Kettenreaktion Spezielle Anforderungen für den Nachweis von Nukleinsäuresequenzen der HIV-1 und HIV-2

Validation of analytical methods: Definitions and terminology

Validation of analytical procedures: Methodology

Anforderungen an Validierung bzw. Routinebetrieb der HCV-NAT im Blutspendewesen (1.7.98)

Guidelines for validation of NAT for the detection of HCV RNA in plasma pools (29.1.99)

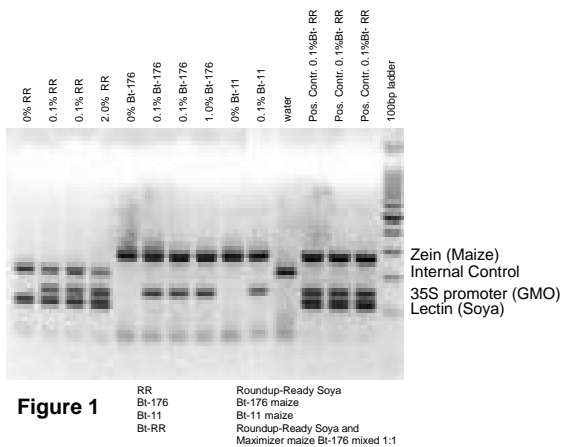


Figure 1

RR Bt-176 Roundup-Ready Soya
Bt-11 Bt-176 maize
Bt-RR Bt-11 maize
Roundup-Ready Soya and
Maximizer maize Bt-176 mixed 1:1

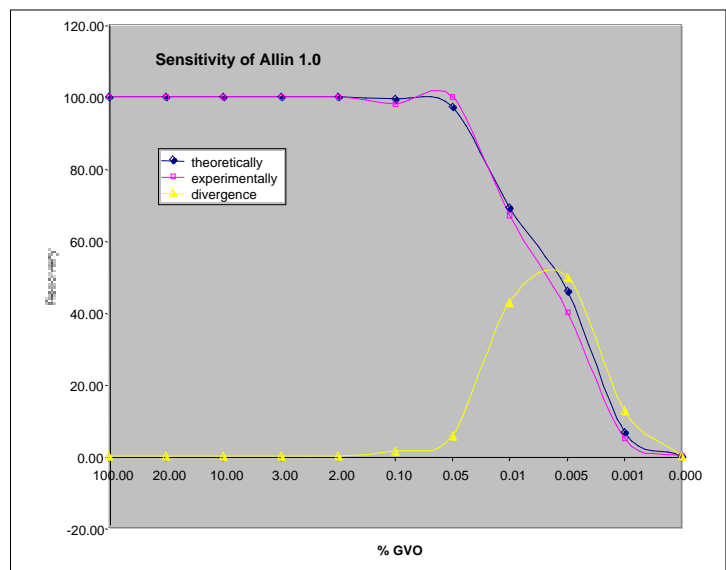


Figure 3

Materials and Methods

DNA-isolation

DNA-isolation was done applying the Wizard protocol published in "Schweiz. Lebensmittelbuch" chapter 52B (Wizard protocol, Promega). After RNase digestion, the content of DNA was measured photometrically (Genequant II, Pharmacia).

Amplification and Detection

400ng of DNA-template was added to the mastermix of the first PCR of Allin 1.0 multiplex screeningsystem, according to the manufacturers protocol (full protocol see www.biosmart.ch).

The PCR was run on a Hybaid thermocycler (OMN-E).

The nested PCR was performed equally using 1µl of the first reaction as template for the second PCR-reaction. The amplicons were detected on a 2% agarose gel after 35 minutes of electrophoresis at 200V (Biosmart Gelelectrophoresis Maxi-system). The result was photographed using a digital camera (Mavica, Sony).

Interpretation of the nested PCR-system was clear (Fig. 1).

Standards

For the validation and as a run control certified standards (Fluka) were used. Total DNA content was kept to 400ng per reaction. High amounts of GMO do not disturb the results of the analysis.

Discussion

The screening system Allin 1.0 presented here exhibit a detection limit (95%) for GMO of 0.05% which is sensitive enough to fulfill the requirements of the swiss law (Fig. 3).

A negative result indicates clearly that the product has not to be labeled as GMO. Due to the internal control of Allin 1.0 false negative results are detected clearly. Therefore no second analysis with spiked samples has to be performed. Additionally the detection of soya and maize in the same assay is very helpful because some samples show contaminations of maize in soya and vice versa.

The reproducibility which was assayed over a prolonged time was satisfactory (Fig. 2). This is a basic necessity of a high and constant quality of an analysis-system.

The use of different Taq DNA polymerases had no significant influence on the sensitivity of the analyses show a good robustness like the fact that 3 different operators received the same sensitivity.

The drawback of this method is its time consuming nested assay and the possible risk of contaminations. However we found no GMO positive signal in a negative control, indicating that contamination can be eliminated by good laboratory practice and a strict room-concept. The

here presented data are an example how to apply the international guidelines for the validation of a PCR-system in practice.