

COMPARISON OF FIVE REAL-TIME PCR VALIDATED METHODS FOR SPECIFIC IDENTIFICATION AND QUANTIFICATION OF *ZEA MAYS*

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According to the European Regulations (Reg 1829/2003; Reg 1830/2003), to obtain the authorization to place on the market a Genetically Modified Organism (GMO) for feed and/or food use, the applicant must submit a dossier containing detailed information about several aspects such as description of the GM trait, risk analysis studies and other relevant issues. One of the most important tools to be provided by the applicant to the enforcement system is a suitable method for detection identification and quantification of the transformation event.

The more widespread techniques for GMO detection and quantification, are based on PCR and real time PCR, for qualitative and quantitative analysis respectively. The GM percentage for a single food/feed ingredient is calculated by the ratio of the GM vs. the food/feed ingredient DNA content. This ratio is obtained by comparing the quantitative results of two different real time PCR: GM event-specific one, and species-specific one. The CRL (Community Reference Laboratory for GM food and feed) has to evaluate and validate the method provided by the applicant on the bases of defined guidelines. Since different applicants generally submit different species-specific real time PCR methods for the same species, a number of *Zea mays* species-specific real time PCR has been validated by the CRL so far.

In order to harmonize methods among the enforcement laboratories involved in GMO detection, we compared five validated real time PCR methods for maize quantification whose PCR targets are *adh* (alcohol dehydrogenase) gene for three of them, and *hmg* (high mobility group) gene for the other two.

Template DNA extracted from seven GM maize Certified Reference Material (CRM) and a pool DNA template made by mixing an identical portion of each extracted DNA were tested under repeatability conditions (same operator using the same equipment within short intervals of time) , at several dilution levels, according to the five real time PCR methods. Slopes and R^2 coefficients obtained from the adopted regression model and other validation parameters were compared, within the same method and among all the five methods, by analysing their distribution at statistical level.