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Food safety: screening tests used to detect and quantify GMO proteins

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Abstract GMO protein content in maize flours or fresh crops can be easily assessed by the enzyme-linked immunosorbent assays (ELISA) and immunochromatographic (lateral flow) strip tests commercially available. Therefore, ELISA can be seen as a useful tool for screening, for control purposes and for traceability implementation. In order to highlight the importance of monitoring GMO protein presence in food products and to investigate the performance of representative ELISA commercial kits, we evaluated three commercial kits by measuring the amount of Cry1Ab/Cry1Ac in IRMM certified reference materials.

Keywords GMO · Immunoassay · ELISA · Cry 1Ab

Introduction

A large number of qualitative and quantitative methods are currently available for GMO detection. At the moment the most commonly applied approach for GMO quantification is based on the real-time PCR technique (RT-PCR). The RT-PCR is an expensive and sophisticated technique requiring well-equipped laboratories and trained operators. Another approach, based on protein detection, could be eventually used for screening purposes when a huge number of samples have to be analysed. The protein-based methods have the potential of serving as cost-efficient, practical and quick tools for screening, for multiplex analysis and for traceability. For example, Cry 1Ab GMO protein content in maize flours or fresh crops can be easily assessed by the enzyme-linked immunosorbent assays (ELISA) tests commercially available. In the present communication, we describe the results obtained in our laboratory using three commercial kits. Three operators tested the ability of the kits to quantify the target and to provide repeatable and reproducible results. The same mi-

croplate reader (Bio-Rad model 550, Bio-Rad Laboratories Inc., Hercules, California) was used by measuring the amount of Cry1Ab/Cry1Ac in IRMM certified reference materials over a 3-day period (short-interval time). Prior to application of this method for control purposes, the method should be tested through a complete validation study.

Material and methods

Selected ELISA kits tested

Three different commercial ELISA kits for recognizing MON810 in raw materials were randomly selected after a search on the web. The three kits selected were:

- K1: S.D.I. GMOCheck Bt1 Maize Test Kit cat.n. 7110000.
- K2: EnviroLogix Inc. Cry1Ab/Cry1Ac Plate Kit cat. n. AP 003.

Table 1 Data represent the repeatability, reproducibility and accuracy of results for each percentage level of IRMM standard

Kit	Level	Theoretical value (in % of GMO content)	Measured value (in % of GMO content)	Accuracy (%)	Repeatability variance	Reproducibility variance
1	1	0	0.0001	—	0.0001	0.0002
	2	0.1	0.2664	266.4	0.0041	0.0120
	3	0.5	1.0324	206.5	0.2039	0.1315
	4	1	1.8460	184.6	0.1103	0.5227
	5	2	2.8642	143.2	0.2710	1.2009
	6	5	3.7894	75.8	0.1136	1.6877
2	1	0	0.0316	—	0.0008	0.0009
	2	0.1	0.2453	245.3	0.0690	0.0962
	3	0.5	0.6426	128.5	0.0226	0.0292
	4	1	1.2779	127.8	0.0433	0.0445
	5	2	2.5260	126.3	0.2448	0.2915
	6	5	5.7559	115.1	0.6412	0.8600
3	1	0	0.0102	—	0.0001	0.0002
	2	0.1	0.1203	120.3	0.0006	0.0011
	3	0.5	0.5783	115.7	0.0032	0.0076
	4	1	0.9986	99.9	0.0093	0.0149
	5	2	1.6412	82.1	0.0200	0.0256
	6	5	2.6745	52.9	0.0039	0.0105

K3: Abraxis LLC ELISA kit called BT CryAC/Cry1Ab code 510001.

Samples

Certified reference material of genetically modified MON810 dried maize was provided by IRMM. In addition, the internal standards were provided in all kits and were used as indicated in the user's instructions. Certified reference material from IRMM (IRMM-413) is available in six different mass fractions of GMO maize: <0.02%, 0.1%, 0.5%, 1%, 2% and 5%.

Experimental design

Tests were repeated three times for each kit. Each kit contained a standard plate of 96 wells. The testing involved three different operators and was conducted on different days. The standard samples were defined according to the user's instructions to mimic the normal operational procedure.

The unknown samples, derived from the IRMM-413 MON810 certified reference material, were quantified according to the manufacturer's standard curve supplied with the kits. Unknown samples of each concentration level were tested, each in triplicate.

Results

The relative repeatability standard deviation (RSD_r) expressed by the formula $RSD_r = SD_r / \bar{x}$ (in %) was used to characterize the kits performance under repeatability con-

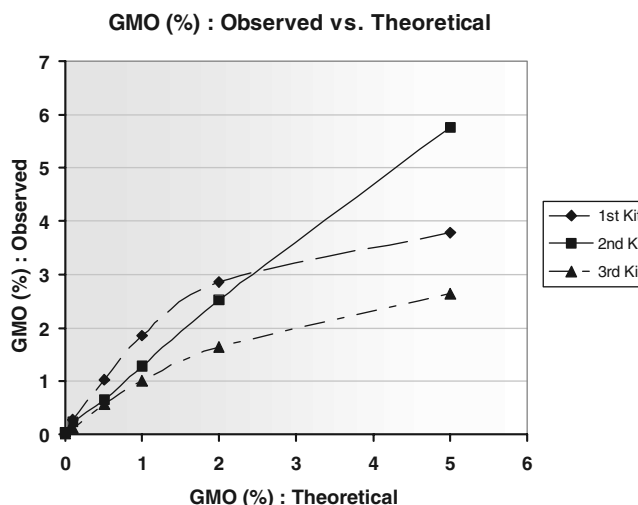


Fig. 1 Scatter plot. Relationship between observed vs. theoretical values for three kits expressed in % of the GMO content

ditions. It was calculated as the mean of the RSD_r s across all levels; where RSD_r at the level was acquired as the mean of all partial RSD_r s obtained for all operators—K1, $RSD_r=12.4\%$; K2, $RSD_r=16.1\%$ and K3, $RSD_r=7.6\%$.

The relative reproducibility repeatability standard deviation (RSD_R) expressed by the formula $RSD_R = SD_R / \bar{x}$ (in %) was used to characterize the performance of the kits under reproducibility conditions. It was calculated as the mean of the RSD_R s across all levels; where RSD_R at the level was calculated among all operators—K1, $RSD_R=37.2\%$; K2, $RSD_R=18.0\%$ and K3, $RSD_R=8.6\%$.

The limit of detection (LOD) is the lowest amount or concentration of analyte in a sample which can be reliably detected. It was calculated using the formula $LOD = \bar{x}(\text{Blank})$

$+3 \times SD$ (in % of GMO content) – K1, LOD=0.034%; K2, LOD=0.107% and K3, LOD=0.037%.

The limit of quantitation (LOQ) is the lowest amount or concentration of analyte in a sample that can be reliably quantified with an acceptable level of precision and accuracy. It was calculated using the formula $LOQ = \bar{x}(\text{Blank}) + 9 \times SD$ (in % of GMO content)—K1, LOQ=0.082%; K2, LOQ=0.259% and K3, LOQ=0.089%.

Accuracy is the closeness of agreement between a test result and the accepted reference value. It is calculated using the formula $(\text{observed value}/\text{theoretical value}) \times 100$ and is expressed in % (Table 1).

Conclusion

We have considered parameters such as accuracy, repeatability and reproducibility, RSD, LOD and LOQ to evaluate the performance of the kits according to the level of GMO content. The results were collected in Table 1 and visualized

in Fig. 1. They indicate that kits differ from each other, especially at the highest levels, e.g. in the difference between the measured and theoretical values. The acceptable accuracy (within $\pm 25\%$ of the accepted theoretical value) with the lowest repeatability and reproducibility variance (fifth and sixth column in Table 1) in each level was achieved by the K3. As reported in Table 1 (second and third columns) and Fig. 1, only the performance of the K2 kit reflects a linear relationship between the theoretical (labelled on the certified material) and measured values in the range of 0 to 5% of GMO content; however, we have to consider that these kit results are not applicable for quantification of the GMO content below 0.26% because the LOD value is 0.259%. In conclusion, concerning the results obtained, K3 seems to be the most reliable, followed by K2 and the K1. Provided that immunoassays are potentially powerful tools in food analysis, further optimization and formal validation are necessary for a complete evaluation of their performance before they are used routinely for control purposes.

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