APPLICATION NOTE

Food Safety - ELISA

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High Throughput Screening of Aflatoxin M_1 in Milk Samples by Automated ELISA Platform

Introduction

Aflatoxins are a group of naturally occurring mycotoxins produced by molds such as *Aspergillus flavus* and *Apsergillus parasiticus,* which have detrimental effects on humans, animals, and crops resulting in illness and economic losses. Aflatoxin M_1 is a metabolite of aflatoxin B_1 commonly found in dairy products. Presence of aflatoxin M_1 occurs when dairy cattle ingest feed contaminated with aflatoxin B_1. Aflatoxins are known to be carcinogenic and it is important to monitor the supply of dairy and dairy based products for contamination. As both aflatoxins B_1 and M_1 are carcinogenic in humans, the action level of 0.5 parts per billion of aflatoxin M_1 in milk is strictly enforced by the United States Food and Drug Administration (FDA). EU imposed an action limit of 0.05 parts per billions of aflatoxin M_1 in milk.

ELISA assays are widely used for the detection of aflatoxins M_1 in milk for regulatory conformance owing to the high sensitivity, selectivity, and ease of use of the method. In the following study we demonstrate the accuracy and precision of the MaxSignal® Aflatoxin M_1 ELISA kit (Cat# FOOD-1060-05) at the EU MRL of 0.05 ppb and the US MRL of 0.5 ppb. We also provide data showing the limit of detection (LOD) of the kit.

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Further, we show the variability between performance of manual ELISA and DS2 automated ELISA platform. We also show the correlation of MaxSignal method performance with the official AOAC reference method (AOAC 2000.08). Lastly, we show that the assay is effective at quantitating aflatoxin M_1 in both full fat and de-fatted milk samples well under the action limits imposed by US FDA and EU.

Experimental

Materials and Methods

Milk samples, shown to be free of Aflatoxin M_1 by the reference method (AOAC 2000.08)1, were used in this study. The experiments were performed with the MaxSignal Aflatoxin M_1 ELISA Kit. The spike solution provided in the kit was used to create all the contaminated samples mentioned in the study.

Results and Discussion

Accuracy and Precision

Milk samples were tested at ND (none detected), 0.05, 0.2, and 0.5 ppb. For each concentration, 2 milk samples were used with each milk sample being tested in 6 replicates. For 0.5 ppb samples, the spiked sample was diluted 1:2 in water for the quantitation to fall within the range of the standard curve. The concentrations for these samples were then corrected for the dilution factor to determine the actual concentration.

Results were evaluated for accuracy (recovery of 60-140%) as well as for precision (%CV <20%)

Table 1. Table 1 summarizes the accuracy and precision results.

	Blank	0.05 ppb	0.2 ppb	0.5 ppb
Mean reported concentration (ppb)	0.012	0.061	0.207	0.562
Mean recovery (%)	-	122%	104%	112%
%CV	17.60%	13.12%	4.22%	3.48%

Sensitivity (LOD)

LOD was established using blank sample matrices. A population of ten replicates of blank samples were used for the LOD measurement. The LOD of a method is defined as the lowest concentration that can be reliably measured. The LOD is defined as the mean+3*StDev reported for the negative/blank samples.

Table 2. Table 2 represents the results from LOD determinations.

	Mean Reported Concentration (ppb)	Standard Deviation	LOD (ppb)
Blank Milk	0.016	0.002	0.023

Method comparisons

The performance of the manual ELISA method and DS2 automation method was compared to establish the performance of DS2 analysis. The validation design outlined in accuracy and precision studies was adopted for this purpose. A variation of <20% was established as good correlation between the manual method and DS2 automation method. The variation was calculated as the difference between mean spike recovery between the methods compared to the manual method.

A correlation study for the ELISA method was carried out with the official reference method for M_1 testing (AOAC 2000.8). The results show a CV less than indicating a very good correlation between the methods.

Table 3. The results indicate a good correlation between the established manual method of ELISA and theDS2 automated method.

Spike Level (ppb)	Meal recovery (Manual)	Mean recovery (DS2)	Variation in methods
0.05	122%	129%	5.43%
0.2	103%	108%	4.63%
Mean blank (ppb)	0.012	0.008	

Table 4. Table 4 summarizes the correlation between ELISA method and reference method.

Spike Level (ppb)	MaxSignal method (n=10)	Reference Method (AOAC 2000.8) (n=3)	Variation in methods (%)
Blank	0.011 ppb	0.08 ppb	6.90%

Full fat vs. De-fatted milk

Milk from the same source was tested to compare performance of the assay in samples that contained their original fat content and samples that had been de-fatted. De-fatting was done by centrifugation at 4000 x g for 5 minutes. The upper, fat layer was then removed.

Table 5. Table 5 summarizes the results of sample variability testing.

	Mean recovery (0.2 ppb)	CV (0.2 ppb)	Mean (blank samples)	CV (blank) samples
Full Fat	111%	6.48%	0.017ppb	12.49%
De-fatted	106%	4.70%	0.016ppb	15.65%

Conclusion

MaxSignal Aflatoxin M_1 ELISA demonstrates effective detection of aflatoxin M_1 in milk samples at both the EU and US MRLs. Further, the kit shows good compatibility with the DS2 automated ELISA platform. The DS2 allows for higher throughput than traditional ELISA methods with a large reduction in

technician hands on time. The assay is able to detect aflatoxin M_1 in full fat milk equally as well as in de-fatted milk allowing technicians to chose to skip the defatting step, saving valuable time. Finally, the ELISA method shows good correlation with the official reference method and could be used in lieu of reference method for screening of contaminated milk samples.

References

1) Official Methods of Analysis of AOAC International. AOAC Official Method 2000.08. In Aflatoxin M_1 in Liquid Milk, Immunoaffinity Column by Liquid Chromatography, 18th ed.; AOAC International: Gaithersburg, MD, USA, 2005; pp. 45–47

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