Validation Report

Aflatoxin M1 Rapid

(Cat. # GEB24005)

Contents

1. Scope

This validation study was conducted to evaluate the performance of the Aflatoxin M1 Rapid Kit (Cat# GEB24005) in accordance with the kit's instructions for use.

2. Calibration Study

Standards (0, 50, 100, 250, 500 and 1000 ng/L) were ran five times by one operator and read with two separate microplate readers. Measured OD values were converted to %B/B0 values to plot a standard curve using a four-parameter curve fit. In each run, regression equation generated was used to calculate aflatoxin M1 concentration and recorded (Table 1). All runs showed excellent goodness of fit with R^2 value of 0.99 or higher. Residual analysis was conducted by plotting residuals (observed - predicted %B/Bo value) versus aflatoxin M1 concentration. Data points are randomly distributed around zero residual (graph not shown).

Table 1. Results of calibration study

Standard (ng/L)	Calculated value (ng/L)					Mean	Sr[a]	RSDr[b] (%)	Bias (ng/mL)
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5				
0	15	3	0	0	8	5	6.38	N/A[c]	5
50	57	54	53	49	55	54	2.97	5.53	4
100	122	118	116	123	120	120	2.86	2.39	20
250	251	266	256	268	255	259	7.40	2.85	9
500	486	532	506	487	525	507	21.16	4.17	7
1000	893	941	903	934	967	928	29.90	3.22	-72

[a] Sr = Standard deviation
[b] RSDr = Relative standard deviation
[c] N/A = Not applicable

3. Cross reactivity

Cross-reactivity was determined by comparing the IC_{50} values of other aflatoxins (B1, B2, G1 and G2) to that of aflatoxin M1. The IC_{50} is defined as the concentration of analyte that produces 50% inhibition of the maximum signal. Standard curves were established for aflatoxin M1 and for other aflatoxins under identical assay conditions. Cross-reactivity was calculated using the formula: Cross-reactivity (%) = (IC_{50} of aflatoxin M1 / IC_{50} of the other aflatoxin) × 100.

Table 2. Results of cross reactivity

Aflatoxin	Cross reactivity
M1	100%
B1	< 0.01%
B2	< 0.01%
G1	< 0.01%
G2	< 0.01%

4. Matrix Study

Liquid milk was accurately measured out and spiked with aflatoxin M1 solution to make 100 and 500 ng/L (ppt) of contamination levels. Powdered milk was weighed out and spiked with 10 times higher concentrations of aflatoxin M1 (1000 and 5000 ng/L) due to reconstitution with DI water for test (1:10). In order to prevent variation due to different spike volumes, same spike volume (20 µL) for different contamination levels was applied by using different concentrations of total aflatoxins solution. After spike, tube was gently shaken to homogenize. Results are shown in Table 3. Recoveries from all matrixes ranged from 81 and 91%. Repeatability precision (RSDr, %) from all matrixes ranged from 2.28 and 6.64%.

Table 3. Results of matrix studies: spiked with 100 and 500 ppt aflatoxin M1

	Spike level (ppt)	n[a]	Mean (ppt)	Sr[b]	RSDr[c]	Recovery (%)[d]	Bias (ppt)[e]
Raw milk	0	5	22	N/A	N/A	N/A	N/A
	100	5	104	6.45	6.20	85	-18.0
	500	5	455	19.33	4.25	87	-67.0
			Mean	18.39	5.23	86	N/A
Homogenized milk	0	5	14	N/A	N/A	N/A	N/A
	100	5	92	6.11	6.64	81	-22.0
	500	5	426	24.41	5.73	83	-88.0
			Mean	22.76	6.19	82	N/A
Skim milk	0	5	5	N/A	N/A	N/A	N/A
	100	5	94	3.67	3.90	90	-11.0
	500	5	434	12.33	2.84	86	-71.0
			Mean	24.92	3.37	88	N/A
Non-fat dry milk	0	5	18	N/A	N/A	N/A	N/A
	100	5	107	4.52	4.22	91	-11.0
	500	5	451	10.27	2.28	87	-67.0
			Mean	19.35	3.25	89	N/A

[a] n = Number of determinations
[b] Sr = Standard deviation
[c] RSDr = Relative standard deviation
[d] Recovery (%) = (Mean $_{calculated}$/ known concentration)*100
[e] Bias = Mean $_{calculated}$ - known concentration

5. Robustness

Minor, reasonable changes such as assay temperature, incubation time, and wash were introduced to evaluate the ability of method to remain unaffected. The factorial design of the experiment is described in Table 4. Ten replicates of skim milk were tested per condition. Condition 1 through 8 represent deviations from the assay procedure. Condition 9 represents the baseline (assay method). Robustness testing of the kit revealed that the method remained robust throughout the changes with recoveries in the 86 – 104 % range.

Table 4. Results of robustness studies: skim milk spiked with 250 ppt aflatoxin M1

Condition	Temperature	Time	Washes	Mean aflatoxin M1 spike level (ppt, n=5)		Recovery (%)
				0	250	
1	18°C	8/4/4 min	4	5	263	103
2	18°C	8/4/4 min	6	11	241	92
3	18°C	12/6/6 min	4	7	221	86
4	18°C	12/6/6 min	6	8	247	96
5	27°C	8/4/4 min	4	14	233	88
6	27°C	8/4/4 min	6	3	217	86
7	27°C	12/6/6 min	4	7	255	99
8	27°C	12/6/6 min	6	11	225	86
9 (Base line)	20–25°C	10/5/5 min	5	7	266	104

6. Consistency and stability

Lot-to-lot consistency was established by testing three lots of the kits each at three time points, using corn samples spiked at 100 and 500 ppt aflatoxin M1. Results remained consistent between all three lots at all time points. The product remained stable for the full 12-month expiration period.

Table 5. Results of product consistency and stability studies: skim milk spiked with 100 and 500 ppt aflatoxin M1

	Months after manufacturing								
	0 (Lot A)			6 (Lot B)			11 (Lot C)		
Spike (ppt)	0	100	500	0	100	500	0	100	500
1	5	108	417	9	91	485	4	92	427
2	13	88	483	5	94	481	11	82	434
3	17	87	464	16	85	466	18	87	418
Mean	12	94	455	10	90	477	11	87	426
Rec.(%)	N/A	106	93	N/A	100	97	N/A	98	87