



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

071502

The AOAC Research Institute hereby certifies that the performance of the test kit known as:

QuickToxTM Kit for QuickScan Aflatoxin FREE

manufactured by

EnviroLogix, Inc.

500 Riverside Industrial Parkway

Portland, ME 04103

USA

This method has been evaluated in the AOAC[®] *Performance Tested MethodsSM* Program, and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC[®] Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance TestedSM* certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (September 24, 2016 – December 31, 2016). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Deborah McKenzie

Deborah McKenzie, Senior Director
Signature for AOAC Research Institute

September 24, 2016

Date

METHOD AUTHORS

Original Validation: Sergiusz Polakowski, Russell Roberts, Keith Tanguay, Cheryl Bailey, Alan H. Davis and Brendan Gow

August 2016 Modification: Anna Rice and Brendan Gow

September 2016 Modification: Sergiusz Polakowski, Russell Roberts, Keith Tanguay, Joseph Morrill, Cheryl Bailey, Alan Davis, Brendan Gow, and Anna Rice

SUBMITTING COMPANY

EnviroLogix Inc.
500 Riverside Industrial Parkway
Portland, ME 04103

KIT NAME(S)

QuickTox™ Kit for QuickScan Aflatoxin FREE

CATALOG NUMBERS

AQ 209 BG

INDEPENDENT LABORATORY

Trilogy Analytical Laboratory
870 Vossbrink Dr.
Washington, MO 63090
USA

AOAC EXPERTS AND PEER REVIEWERS

Gordon Shephard^{1,4,5}, Gary Lonbaert², Wayne Ziemer³
¹ Institute of Biomedical and Microbial Biotechnology, Bellville, South Africa
² Retired Health Canada, Winnipeg, Manitoba, Canada
³ Consultant, Loganville, GA, USA
⁴ Modification August 2016
⁵ Modification September 2016

APPLICABILITY OF METHOD

Target analyte – Total Aflatoxins

Matrices – corn, barley, oats, sorghum, wheat, whole peanut, peanut seed, peanut hull

Performance claims - Detection of total aflatoxins with contamination levels ranging from 2.7 (LOD) – 100 ppb in corn, barley, oats, sorghum, and wheat and 7.5 (LOD) – 99 ppb in whole peanut, peanut seed, and peanut hull.

September 2016 Modification: Detection of aflatoxins in peanut seed in the range of 2.5-30 ppb.

Results compared to acceptable ranges specified by the AOAC *Performance Tested Methods*SM program.

PRINCIPLE OF THE METHOD (1)

The EnviroLogix QuickTox Kit for QuickScan Aflatoxin FREE is a competitive, lateral flow immunoassay. Aflatoxin is extracted from ground samples by shaking. Water and EB17 extraction buffer (in powdered form) supplied in the kit are used for corn and wheat samples; 50% ethanol is used to extract barley, oats, and sorghum samples; 80% ethanol is used to extract whole peanut, peanut seed and peanut hull samples. Clarified extracts are produced by filtration (corn, whole peanut, peanut seed, and peanut hull), or centrifugation (barley, oats, sorghum, and wheat). Matrix specific volumes of DB5 assay diluent buffer and clarified extracts are added to reaction vessels followed by placement of assay strip. Test and control lines are allowed to develop for matrix specific defined times as the sample and buffer move through the device by capillary action. Line intensities developed on the strips are quantitated using a reader and associated system software and are compared to lot specific calibration curves previously entered into the reader system by scanning lot specific barcodes. The system software reports and archives the aflatoxin results. Archived results may be exported in spreadsheet form for further analyses as desired by the user.

DISCUSSION OF THE ORIGINAL VALIDATION STUDY (1)

We previously reported on a quantitative, lateral flow based, aflatoxin assay (3). AOAC Research Institute PTM certification was achieved for corn (No. 041201). The aim of the present work was to receive PTM certification for a second aflatoxin assay that was developed to enable use on additional matrices and has a more balanced recognition of the different aflatoxin forms (B1, B2 G1, and G2). The new kit provides for aqueous extraction of some matrices including corn and wheat. Corn is commonly analyzed for the presence of aflatoxins early in the grain supply chain at grain elevators. Aqueous extraction reduces the need for storage and disposal of hazardous solvents which is of benefit to facilities that may lack sophisticated storage facilities. Analytical mycotoxin analyses using sophisticated technologies such as HPLC or LC MS/MS involve solvent extraction and cleanup using immunoaffinity columns (IAC) or solid phase extractions designed, in part, to minimize matrix effects. Despite cleanup efforts, correction for matrix effects is often necessary and usually involves spiking with mycotoxin standards for use as calibrator(s) during analysis (4–8). Similarly, matrix specific calibration curves are incorporated in the Aflatoxin FREE kit to compensate for matrix effects.

DISCUSSION OF THE ORIGINAL VALIDATION STUDY (1)

Moreover, the antibody employed in the original kit exhibited considerable bias towards detection of Aflatoxin B1 (3). The present assay employs a new antibody which exhibits a more balanced recognition of aflatoxin forms. This should facilitate aflatoxin analyses in matrices with different aflatoxin B2, G1, and G2 ratios relative to B1 as has been reported for some peanut samples (9, 10). As was the case for the original assay, the presence of other common mycotoxins in a sample will not interfere with aflatoxin determination. The Linearity and Matrix Studies demonstrated linear dose responses from 0 (non-detect) - 100 ppb aflatoxin. As a whole, RSD, % calculations and within range results, indicate the kit provides accurate and precise aflatoxin quantitation for multiple matrices confirming that sample preparation, assay procedures and matrix specific calibration curves produce acceptable results. Data produced in the Lot-to-Lot Consistency and Stability study show within range results for multiple lots of kit components with varied ages including those used post expiration. This speaks to the ruggedness of manufacturing and quality assurance processes. Kit quality and performance over time are assured. The robustness study showed that two combinations produce unacceptable results upon co-variation of sample extract volume, assay temperature and assay development time. Further analyses indicated that high assay temperature was not a root cause suggesting that unacceptable results would require two simultaneous user errors (sample volume and assay development time). The package insert defines the correct protocols; attention to the procedures will reduce the likelihood of making two mistakes at the same time. Moreover, the package insert emphasizes the importance of following the stated protocols. In summary, the QuickTox Kit for QuickScan Aflatoxin FREE has a number of features directed towards ease of use. The assay development time has been reduced to 4 min for corn and aqueous extraction reduces the need for use of solvents. Dedicated assay procedures and calibration curves minimize matrix effects and the assay has a more balanced recognition of the different aflatoxin types (B1, B2, G1, and G2) enhancing analyses in varied matrices.

DISCUSSION OF THE MODIFICATION STUDY Approve August 2016 (11)

Based on results from two representative matrices across three kit lots tested, the proposed software modification generates test results that are either equivalent to the original software or with improved accuracy across the diluted sample range. These data also confirm that the performance for samples run in the base range (2.7 to 30ppb), without additional dilution, is not impacted by the proposed software modification. This study supports the proposed software modification to the QuickTox™ Kit for QuickScan Aflatoxin FREE test method (AOAC PTM certification 071502).

DISCUSSION OF THE MODIFICATION STUDY Approve September 2016 (12)

The QuickTox™ Aflatoxin FREE test currently holds AOAC Research Institute PTM certification for a number of matrices including peanut seed from 7.5 ppb to 99 ppb. An additional protocol for peanut seed matrix was developed in order to reach a higher sensitivity of detection. In the European Union, the maximum total aflatoxins level is 4 ppb for direct consumption. The high sensitivity peanut protocol was assessed for additional AOAC certification with a range of 2.5 ppb to 30 ppb. For any result reported over 30 ppb by this protocol, the customer may use the lower sensitivity peanut protocol.

The Linearity and Matrix Studies, herein, demonstrated a linear dose response from 0 (non-detect) - 30 ppb aflatoxin. As a whole, RSD, % calculations and within range results, indicated the kit provides accurate and precise total aflatoxin quantitation for peanut seed within the range of 2.5 ppb to 30 ppb, confirming that sample preparation, assay procedure, and a matrix-specific calibration curve produce acceptable results.

The new high sensitivity method provides an additional detection range for the peanut seed matrix and allows for accurate aflatoxin detection encompassing the European maximum levels for the matrix. Addition of this protocol to the PTM certified method # 071502 expands the sensitivity offerings of the EnviroLogix QuickTox™ Kit for QuickScan Aflatoxin FREE.

REFERENCES CITED

1. Polakowski, S., Roberts, R., Tanguay, K., Bailey, C., Davis, A.H., and Gow, B., Evaluation of the QuickTox™ Kit for QuickScan Aflatoxin FREE, AOAC® *Performance TestedSM* certification number 071502.
2. AOAC Research Institute Validation Outline for QuickTox™ Kit for QuickScan Aflatoxin FREE, Approved – July 2015.
3. Bailey, C., & Davis, A. H. (2012). QuickTox™ Kit for QuickScan Aflatoxin. *Journal of AOAC International*, 95(5), 1460-1468
4. Berthiller, F. P. A., Burdaspal, C., Crews, M. H., Iha, R., Krska, V. M. T., Lattanzio, S., MacDonald, S., Malone, R.J., Maragos, C., Solfrizzo, M., Stroka, J., & Whitaker, T.B. (2014): *World Mycotoxin Journal* 7, no. 1 3-33
5. Lattanzio, V. M. T., Solfrizzo, M., Powers, S., & Visconti, A. (2007) *Rapid Communications in Mass Spectrometry*, 21(20), 3253-3261
6. Sulyok, M., Krska, R., & Schuhmacher, R. (2007) *Food additives and contaminants*, 24(10), 1184-1195
7. Vaclavik, L., Vaclavikova, M., Begley, T. H., Krynetsky, A. J., & Rader, J. I. (2013) *Journal of agricultural and food chemistry*, 61(20), 4822-4830
8. Yogendrarajah, P., Van Poucke, C., De Meulenaer, B., & De Saeger, S. (2013) *Journal of Chromatography A*, 1297, 1-11
9. Oliveira, C. A., Gonçalves, N. B., Rosim, R. E., & Fernandes, A. M. (2009) *International journal of molecular sciences*, 10(1), 174-183
10. Yentür, G., Er, B., Özkan, M. G., & Öktem, A. B. (2006) *European Food Research and Technology*, 224(2), 167-170
11. Rice, A. and Gow, B., QuickTox™ Kit for QuickScan Aflatoxin FREE, Method Modification for PTM Certification 071502, AOAC® *Performance TestedSM* certification number 071502. Approved August 2016.
12. Polakowski, S., Roberts, R., Tanguay, K., Morrill, J., Bailey, C., Davis, A., Gow, B., and Rice, A., Evaluation of QuickTox™ for QuickScan Aflatoxin FREE AOAC® *Performance TestedSM* 071502, Method Modification for Peanut Seed Matrix. Approved September 2016.

Original Validation Data (1)

Table 5. Summary of matrix studies for corn

A. Run at sponsor's laboratory kit lot 045-13							
Replicate	< 1 ppb		5.3 ppb	11.3 ppb	19 ppb	50.8 ppb	98.7 ppb
1	1.9		6.1	12	18	59	100
2	0.0		5.6	12	18	56	110
3	0.1		5.9	13	18	59	110
4	1.4		5.6	13	18	57	110
5	0.0		5.6	12	17	62	100
6	0.6		5.1	12	19	59	94
7	0.6		4.7	12	20	60	120
8	0.0		4.6	13	19	55	120
9	0.5		6.0	14	17	67	100
10	0.0		5.4	12	18	59	110
11			3.9	13	21		100
12			5.1	11	19		120
12			5.6	12	21		110
14			5.8	14	16		110
Mean	0.50		5.36	12.50	18.50	59.30	108.14
S _r	0.65		0.62	0.85	1.45	3.37	8.36
LOD (mean ^a + 2 S _r)	1.80	RSD _r , %	11.57	6.80	7.84	5.68	7.73
LOQ = 3 x LOD	5.40	Recovery, %	101.13	110.62	97.37	116.73	109.56
		Bias	0.06	1.20	-0.50	8.50	9.44
B. Run at independent laboratory kit lot 216-14							
1	0		3.5	11	19	59	100
2	0		4.4	9.1	18	57	94
3	0		2.8	9.7	16	58	97
4	0		4.1	11	17	61	96
5	0		3.6	9.8	15	60	96
6	0						
7	0						
8	0						
9	0						
10	0						
Mean	0.0		3.68	10.12	17.00	59.00	96.60
S _r	0.00		0.61	0.85	1.58	1.58	2.19
LOD (mean ^a + 2 S _r)	0.00	RSD _r , %	16.58	8.40	9.29	2.68	2.27
LOQ = 3 x LOD	0.00	Recovery, %	69.43	89.56	89.47	116.14	97.87
		Bias	-1.62	-1.18	-2.00	8.20	-2.10

^a mean and S_r of results for aflatoxin non-detect sample

Table 6. Summary of matrix studies for barley

A. Run at sponsor's laboratory							
Replicate	<1 ppb		5 ppb	10 ppb	20 ppb	50 ppb	100 ppb
1	0.0		2.8	9.2	16	44	94
2	0.0		3.7	8.6	17	42	110
3	0.0		3.4	8.4	17	43	98
4	0.0		4.1	10	18	33	92
5	0.0		3.6	8.3	20	37	93
6	0.0						
7	0.0						
8	0.0						
9	0.0						
10	0.0						
Mean	0.0		3.52	8.90	17.60	39.80	97.40
S _r	0.0		0.48	0.71	1.52	4.66	7.40
LOD (mean ^a + 2 S _r)	0.0	RSD _r , %	13.64	7.98	8.64	11.71	7.60
LOQ = 3 x LOD	0.0	Recovery, %	70.40	89.00	88.00	79.60	97.40
		Bias	-1.48	-1.10	-2.40	-10.20	-2.60
B. Run at independent laboratory							
1	0.0		3.7	10	20	48	120
2	0.0		5.7	9.9	22	50	110
3	0.0		4.9	11	23	51	110
4	0.0		4.2	11	22	58	110
5	0.0		4.4	11	19	56	110
6	0.0						
7	0.0						
8	0.0						
9	0.0						
10	0.0						
Mean	0.0		4.58	10.58	21.20	52.60	112.00
S _r	0.00		0.76	0.58	1.64	4.22	4.47
LOD (mean ^a + 2 S _r)	0.00	RSD _r , %	16.59	5.48	7.74	8.02	3.99
LOQ = 3 x LOD	0.00	Recovery, %	91.60	105.80	106.00	105.20	112.00
		Bias	-0.42	0.58	1.20	2.60	12.00
C. Combined data-both laboratories							
Mean	0.0		4.05	9.74	19.40	46.20	104.70
S _r	0		0.82	1.07	2.41	7.94	9.62
LOD (mean ^a + 2 S _r)	0	RSD _r , %	20.25	10.99	12.42	17.19	9.19
LOQ = 3 x LOD	0	Recovery, %	81.00	97.40	97.00	92.40	104.70
		Bias	-0.95	-0.26	-0.60	-3.80	4.70

^a mean and S_r of results for aflatoxin non-detect sample

Table 7. Summary of matrix studies for oats, sorghum, and wheat- sponsor's lab

A. Oats						
Replicate	< 1 ppb	5 ppb	10 ppb	20 ppb	50 ppb	100 ppb
1	0.0	4.8	11	18	39	82
2	0.0	5.3	9.3	22	45	98
3	0.0	4.3	9.1	17	39	99
4	0.0	4.4	10	20	47	97
5	0.0	5.2	10	22	46	98
6	0.0					
7	0.0					
8	0.0					
9	0.0					
10	0.0					
Mean	0.00	4.80	9.88	19.80	43.20	94.80
S _r	0.00	0.45	0.75	2.28	3.90	7.19
LOD (mean ^a + 2 S _r)	0.00	RSDr, % 9.38	7.59	11.52	9.03	7.58
LOQ = 3 x LOD	0.00	Recovery, % 96.00	98.80	99.00	86.40	94.80
		Bias -0.20	-0.12	-0.20	-6.80	-5.20
B. Sorghum						
Replicate	< 1 ppb	5 ppb	10 ppb	20 ppb	50 ppb	100 ppb
1	0.0	3.8	9.5	18	38	87
2	0.0	3.7	8.2	16	45	82
3	0.0	3.5	7.8	16	36	100
4	0.0	3.2	8.1	16	40	88
5	0.0	2.7	7.6	16	38	85
6	0.0					
7	0.0					
8	0.0					
9	0.0					
10	0.0					
Mean	0.00	3.38	8.24	16.40	39.40	88.40
S _r	0.00	0.44	0.74	0.89	3.44	6.88
LOD (mean ^a + 2 S _r)	0.00	RSDr, % 13.02	8.98	5.43	8.73	7.78
LOQ = 3 x LOD	0.00	Recovery, % 67.60	82.40	82.00	78.80	88.40
		Bias -1.62	-1.76	-3.60	-10.60	-11.60
C. Wheat						
Replicate	< 1 ppb	5 ppb	10 ppb	20 ppb	50 ppb	100 ppb
1	0.21	4.3	9.4	24	65	100
2	0	5.4	8.9	20	65	110
3	0	4.0	9.5	20	63	90
4	0	3.9	7.8	22	68	100
5	0	3.9	9.8	23	62	110
6	0					
7	0					
8	0					
9	0					
10	0					
Mean	0.02	4.30	9.08	21.80	64.60	102.00
S _r	0.07	0.64	0.79	1.79	2.30	8.37
LOD (mean ^a + 2 S _r)	0.16	RSDr, % 14.88	8.70	8.21	3.56	8.21
LOQ = 3 x LOD	0.48	Recovery, % 86.00	90.80	109.00	129.20	102.00
		Bias -0.70	-0.92	1.80	14.60	2.00

^a mean and S_r of results for aflatoxin negative sample

Table 8. Summary of matrix studies for peanuts

A. Whole peanut							
Replicate	<1 ppb		15 ppb	25 ppb	50 ppb	70 ppb	100 ppb
1	0		14	25	44	67	120
2	0		17	23	48	58	110
3	0		16	24	46	62	130
4	0		16	24	47	64	110
5	0		15	24	43	71	100
6	0						
7	0						
8	0						
9	0						
10	0						
Mean	0.00		15.60	24.00	45.60	64.40	114.00
Sr	0.00		1.14	0.71	2.07	4.93	11.40
LOD (mean ^a + 2 S _r)	0.00	RSD _r , %	7.31	2.96	4.54	7.66	10.00
LOQ = 3 x LOD	0.00	Recovery, %	104.00	96.00	91.20	92.00	114.00
		Bias	0.60	-1.00	-4.40	-5.60	14.00
B. Peanut seed							
1	0		23	27	44	69	120
2	0		18	27	51	56	110
3	0		18	24	48	62	99
4	0		21	29	52	62	99
5	0		17	25	46	63	80
6	0						
7	0						
8	0						
9	0						
10	0						
Mean	0.00		19.40	26.40	48.20	62.40	101.60
Sr	0.00		2.51	1.95	3.35	4.62	14.91
LOD (mean ^a + 2 S _r)	0.00	RSD _r , %	12.94	7.39	6.95	7.40	14.68
LOQ = 3 x LOD	0.00	Recovery, %	129.33	105.60	96.40	89.14	101.60
		Bias	4.40	1.40	-1.80	-7.60	1.60
C. Peanut hull							
1	0		12	17	53	69	110
2	0		18	17	52	77	75
3	0		18	16	40	76	130
4	0		18	21	51	70	95
5	0		14	23	41	76	140
6	0						
7	0						
8	0						
9	0						
10	0						
Mean	0.00		16.00	18.80	47.40	73.60	110.00
Sr	0.00		2.83	3.03	6.35	3.78	26.22
LOD (mean ^a + 2 S _r)	0.00	RSD _r , %	17.69	16.12	13.40	5.14	23.84
LOQ = 3 x LOD	0.00	Recovery, %	106.67	75.20	94.80	105.14	110.00
		Bias	1.00	-6.20	-2.60	3.60	10.00

^a mean and S_r of results for aflatoxin negative sample

Modification Data Approved August 2016 (11)

Table 2. Results for Corn Matrix - Aqueous EB17 Sample Extraction

AQ209BG Kit Lot	748-16		750-16		751-16	
Sample (ppb)	Current Software	Modified Software	Current Software	Modified Software	Current Software	Modified Software
Negative	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Mean	NA	NA	NA	NA	NA	NA
<i>SD</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>
<i>%CV</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>
5.3	4.8	4.8	5.4	5.4	6.3	6.3
	5.6	5.6	5.5	5.5	6.2	6.2
	5.1	5.1	4.1	4.1	5.4	5.4
	4.5	4.5	5.2	5.2	5.3	5.3
	4.6	4.6	4.4	4.4	5.5	5.5
	4.9	4.9	4.2	4.2	5.6	5.6
	4.6	4.6	5.8	5.8	5.5	5.5
	5.8	5.8	5.4	5.4	5.3	5.3
Mean	5.0	5.0	5.0	5.0	5.6	5.6
<i>SD</i>	<i>0.5</i>	<i>0.5</i>	<i>0.7</i>	<i>0.7</i>	<i>0.4</i>	<i>0.4</i>
<i>%CV</i>	<i>9.8</i>	<i>9.8</i>	<i>13.1</i>	<i>13.1</i>	<i>6.8</i>	<i>6.8</i>
11.3	13	13	11	11	12	12
	12	12	12	12	11	11
	11	11	13	13	11	11
	12	12	11	11	11	11
	10	10	10	10	11	11
	11	11	11	11	12	12
	748-16		750-16		751-16	
	Current Software	Modified Software	Current Software	Modified Software	Current Software	Modified Software
	11	11	12	12	11	11
	12	12	10	10	12	12
Mean	12	12	11	11	11	
<i>SD</i>	<i>0.8</i>	<i>0.8</i>	<i>1.0</i>	<i>1.0</i>	<i>0.4</i>	<i>0.4</i>
<i>%CV</i>	<i>7.2</i>	<i>7.2</i>	<i>8.8</i>	<i>8.8</i>	<i>3.5</i>	<i>3.5</i>
18.3	19	19	21	21	17	17
	18	18	20	20	20	20
	18	18	18	18	17	17
	18	18	19	19	19	19
	20	20	18	18	19	19

	18	18	19	19	18	18
	18	18	21	21	18	18
	20	20	17	17	17	17
Mean	19	19	19	19	18	18
<i>SD</i>	1.1	1.1	1.4	1.4	1.0	1.0
<i>%CV</i>	6.0	6.0	7.4	7.4	5.5	5.5
31.2	>30	>30	30	30	30	30
	>30	>30	>30	>30	>30	>30
	30	30	>30	>30	29	29
	>30	>30	>30	>30	>30	>30
	>30	>30	29	29	>30	>30
	29	29	30	30	28	28
	>30	>30	>30	>30	30	30
	>30	>30	29	29	30	30
Mean	NA	NA	NA	NA	NA	NA
<i>SD</i>	NA	NA	NA	NA	NA	NA
<i>%CV</i>	NA	NA	NA	NA	NA	NA
31.2 diluted	34	30	37	32	36	31
	36	31	35	31	36	32
	37	32	34	30	34	30
	37	32	37	32	34	30
	32	28	33	29	35	31
	33	29	36	31	37	32
	31	27	37	32	36	31
	35	31	37	33	35	31
Mean	34	30	36	31	35	31
<i>SD</i>	2.1	1.8	1.7	1.5	0.8	0.7
<i>%CV</i>	6.1	6.1	4.7	4.7	2.3	2.3
	748-16		750-16		751-16	
	Current Software	Modified Software	Current Software	Modified Software	Current Software	Modified Software
50.9 diluted	47	53	51	58	48	54
	49	55	52	59	50	57
	51	57	48	54	49	55
	52	59	49	55	49	55
	48	54	45	50	40	45
	46	52	49	56	45	51
	46	51	49	55	51	58
	51	58	50	56	47	53
Mean	49	55	49	55	47	53
<i>SD</i>	2.5	2.8	2.3	2.6	3.5	3.9
<i>%CV</i>	5.0	5.0	4.6	4.6	7.3	7.3
103.4 diluted	91	110	83	98	77	91
	89	110	82	97	84	100
	82	97	83	98	83	98
	88	110	84	100	86	100
	85	100	84	100	83	99

	84	100	78	93	75	89
	86	100	85	100	78	92
	86	100	84	100	85	100
Mean	86	103	83	98	81	96
<i>SD</i>	3.0	5.5	2.2	2.5	4.1	4.5
<i>%CV</i>	3.5	5.3	2.7	2.5	5.0	4.7

Table 4. Results for Sorghum Matrix- 50% Ethanol Extracted

AQ209BG Kit Lot	748-16		750-16		751-16	
	Current Software	Modified Software	Current Software	Modified Software	Current Software	Modified Software
Negative	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Mean	NA	NA	NA	NA	NA	NA
<i>SD</i>	NA	NA	NA	NA	NA	NA
<i>%CV</i>	NA	NA	NA	NA	NA	NA
5	4.1	4.1	4.3	4.3	4.0	4.0
	4.7	4.7	4.3	4.3	4.2	4.2
	4.2	4.2	4.2	4.2	4.0	4.0
	4.2	4.2	4.0	4.0	3.8	3.8
	4.5	4.5	3.6	3.6	3.5	3.5
Mean	4.3	4.3	4.1	4.1	3.9	3.9
<i>SD</i>	0.3	0.3	0.3	0.3	0.3	0.3
<i>%CV</i>	6.3	6.3	6.9	6.9	7.0	7.0
20	19	19	21	21	20	20
	23	23	19	19	19	19
	21	21	19	19	20	20
	22	22	20	20	22	22
	21	21	19	19	22	22
Mean	21	21	20	20	20	20
<i>SD</i>	1.5	1.5	0.9	0.9	1.1	1.1
<i>%CV</i>	7.2	7.2	4.7	4.7	5.4	5.4
30	>30	>30	>30	>30	30	30
	>30	>30	30	30	29	29
	>30	>30	29	29	>30	>30
	>30	>30	28	28	29	29
	>30	>30	29	29	>30	>30
Mean	NA	NA	NA	NA	NA	NA
<i>SD</i>	NA	NA	NA	NA	NA	NA
<i>%CV</i>	NA	NA	NA	NA	NA	NA
	748-16		750-16		751-16	
	Current	Modified	Current Software	Modified	Current Software	Modified

	Software	Software		Software		Software
30 diluted	29	29	29	29	27	27
	30	30	28	28	23	23
	26	26	26	26	27	27
	29	29	24	24	24	24
	27	27	26	26	25	25
Mean	28	28	26	26	25	25
<i>SD</i>	1.6	1.6	1.8	1.8	1.8	1.8
<i>%CV</i>	5.6	5.6	7.0	7.0	7.0	7.0
50 diluted	58	58	48	48	48	48
	46	46	49	49	46	46
	48	48	48	48	48	48
	49	49	49	49	42	42
	48	48	47	47	47	47
Mean	50	50	48	48	46	46
<i>SD</i>	4.6	4.6	0.7	0.7	2.3	2.3
<i>%CV</i>	9.3	9.3	1.5	1.5	5.1	5.1
100 diluted	93	93	100	100	120	120
	91	91	100	100	99	99
	95	95	110	110	110	110
	100	100	120	120	98	98
	86	86	97	97	98	98
Mean	93	93	105.6	105.6	105.1	105.1
<i>SD</i>	5.2	5.2	9.5	9.5	9.8	9.8
<i>%CV</i>	5.6	5.6	9.0	9.0	9.3	9.3

Method Modification Data Approved September 2016 (12)

Table 2. Summary of Matrix studies for peanut seed

Replicate	<1 ppb		3.8 ppb	5 ppb	7 ppb	12 ppb	20 ppb
1	0		3.0	4.9	6.2	10	17
2	0		3.4	4.9	6.4	10	17
3	0		4.0	6.6	6.4	10	17
4	0		<LOD (1.6 ^b)	4.8	6.2	11	16
5	0		2.9	4.4	5.9	11	19
6	0.64						
7	0						
8	0						
9	0						
10	0						
Mean	0.064		3.3	5.1	6.2	10	17
Sr	0.20		0.53	0.86	0.22	0.35	0.88
LOD (mean ^a + 2S _r)	0.47	RSD _r %	15.93	16.87	3.49	3.35	5.08
LOQ = 3 X LOD	1.40	Recovery, %	87.20	102.50	88.85	87.34	86.88
		Bias	-0.49	0.12	-0.78	-1.52	-2.62

^aMean and S_r of results for aflatoxin negative sample.

^bExtrapolation of the reported <LOD result off the calibration curve. LOD for peanut seed protocol =2.5 ppb.

ORIGINAL CERTIFICATION DATE

July 18, 2015

CERTIFICATION RENEWAL RECORD

Renewed through December 2016

METHOD MODIFICATION RECORD

1. August 2016
2. September 2016

SUMMARY OF MODIFICATION

1. Software update to modify the standard curve to enable curve-specific dilution adjustments across the dilution range (30-100ppb)
2. Detection range of Peanut Seed changed to 2.5-30 ppb.

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