



# CERTIFICATION

**AOAC<sup>®</sup> Performance Tested<sup>SM</sup>**

Certificate No.

**012104**

The AOAC Research Institute hereby certifies the method known as:

**TotalTox<sup>™</sup> Aflatoxin**

manufactured by

**EnviroLogix**

**500 Riverside Industrial Parkway**

**Portland, ME 04103**

**USA**

This method has been evaluated in the AOAC<sup>®</sup> *Performance Tested Methods<sup>SM</sup>* Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC<sup>®</sup> Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested<sup>SM</sup>* 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 (November 15, 2021 – December 31, 2022). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads "Scott Coates".

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Scott Coates, Senior Director  
Signature for AOAC Research Institute

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November 15, 2021

Date

<b>METHOD AUTHORS</b> Cheryl Bailey, Russel Roberts, Suzanne McManus, Terry Goddard, Susan Tapley, Brendan Gow	<b>SUBMITTING COMPANY</b> EnviroLogix 500 Riverside Industrial Parkway Portland, ME 04103 USA
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<b>KIT NAME(S)</b> TotalTox™ Aflatoxin Formerly QuickTox™ Kit for Aflatoxin Flex	<b>CATALOG NUMBERS</b> AQ 309 BG
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<b>INDEPENDENT LABORATORY</b> Trilogy Analytical Laboratory 870 Vossbrink Dr. Washington, MO 63090	<b>AOAC EXPERTS AND PEER REVIEWERS</b> Tetsuhito Goto <sup>1</sup> , Joe Boison <sup>2</sup> , Wayne Ziermer <sup>3</sup> <sup>1</sup> Central Science, Saskatoon, CANADA <sup>2</sup> Retired Research Scientist, Saskatoon, CANADA <sup>3</sup> Independent Consultant, GA, USA
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#### APPLICABILITY OF METHOD

**Analytes – Total Aflatoxins**

**Matrixes – Corn.**

**Performance claims - Detection of total aflatoxins in corn with contamination levels ranging from 2.7 (Limit of Detection, LOD) – 300 ppb.**

<b>ORIGINAL CERTIFICATION DATE</b> January 29, 2021	<b>CERTIFICATION RENEWAL RECORD</b> Renewed annually through December 2022
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<b>METHOD MODIFICATION RECORD</b> 1. March 2021 Level 1 2. November 2021 Level 1	<b>SUMMARY OF MODIFICATION</b> 1. Rebranding to TotalTox™ Aflatoxin. 2. Non-method related changes to software.
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<b>Under this AOAC® Performance Tested<sup>SM</sup> License Number, 012104 this method is distributed by:</b> NONE	<b>Under this AOAC® Performance Tested<sup>SM</sup> License Number, 012104 this method is distributed as:</b> NONE
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#### PRINCIPLE OF THE METHOD (1)

The EnviroLogix TotalTox Kit for QuickScan Aflatoxin is a competitive, lateral flow immunoassay. Aflatoxin is extracted from ground corn by shaking. Water and a dissolvable packet containing EB17 extraction powder are used for corn extraction. Clarified extracts are produced by filtration or centrifugation. The test sample is created by 1:1 dilution with DB5 assay diluent and equilibrated to 22°C in the incubator unit and then tested with the assay strip. Development of the assay strip occurs as the test sample moves vertically through the strip by capillary action revealing test and control lines that are identified and quantified with the reader and associated system software. The reader systems use matrix-specific calibration curves, input by scanning the multi-matrix barcode card, to determine the quantitation level of the sample.

#### DISCUSSION OF THE VALIDATION STUDY (1)

The TotalTox Kit for QuickScan Aflatoxin Flex test method was developed to provide a rapid, easy to use, consistent, and highly accurate test for quantitation of aflatoxin levels in corn grain. The test method is a competitive lateral flow format with a test strip reader and associated software. This method was validated in these studies for use with the QuickScan System and QuickScan II Reader, each with the QuickScan software for quantitative results reporting.

Performance assessed by linearity and corn matrix studies in the sponsor's lab and the independent lab demonstrated a highly linear dose response of the method to levels of aflatoxin from 0–300 ppb, using both the QuickScan System and the QuickScan II Reader. The correlation coefficients ( $R^2$  value) of linear regression analysis for the combined data sets was 0.991, indicating a very good linear fit which demonstrates that both reader types are capable of accurately quantitating aflatoxin levels in comparison to HPLC-determined values. In all studies, the data produced RSD<sub>r</sub> values well below the acceptance criteria Max %RSD for each aflatoxin level, with the combined corn matrix studies exhibiting no more than 14% RSD<sub>r</sub> at the 5.9 ppb dose. Therefore, the test method, in conjunction with either reader type, displays suitable repeatability.

The selectivity study to determine the method's relative reactivity to different aflatoxins showed a similar response to B1, B2 and G1 (100%, 90%, 97% respectively), with a slightly reduced relative reactivity to G2 (77%). This reactivity balance will facilitate accurate aflatoxin analyses in the scenario where the ratio of the different aflatoxins varies within a sample type. The presence of other common mycotoxins in a sample will not interfere with aflatoxin determination.

The product consistency was demonstrated with multiple lots tested to the claimed shelf-life of 12 months. Data presented in the Product Consistency and Stability Study remain within the acceptable range for the 19.2 ppb aflatoxin level for 12 months post-manufacture, supporting the 12-month product stability claim.

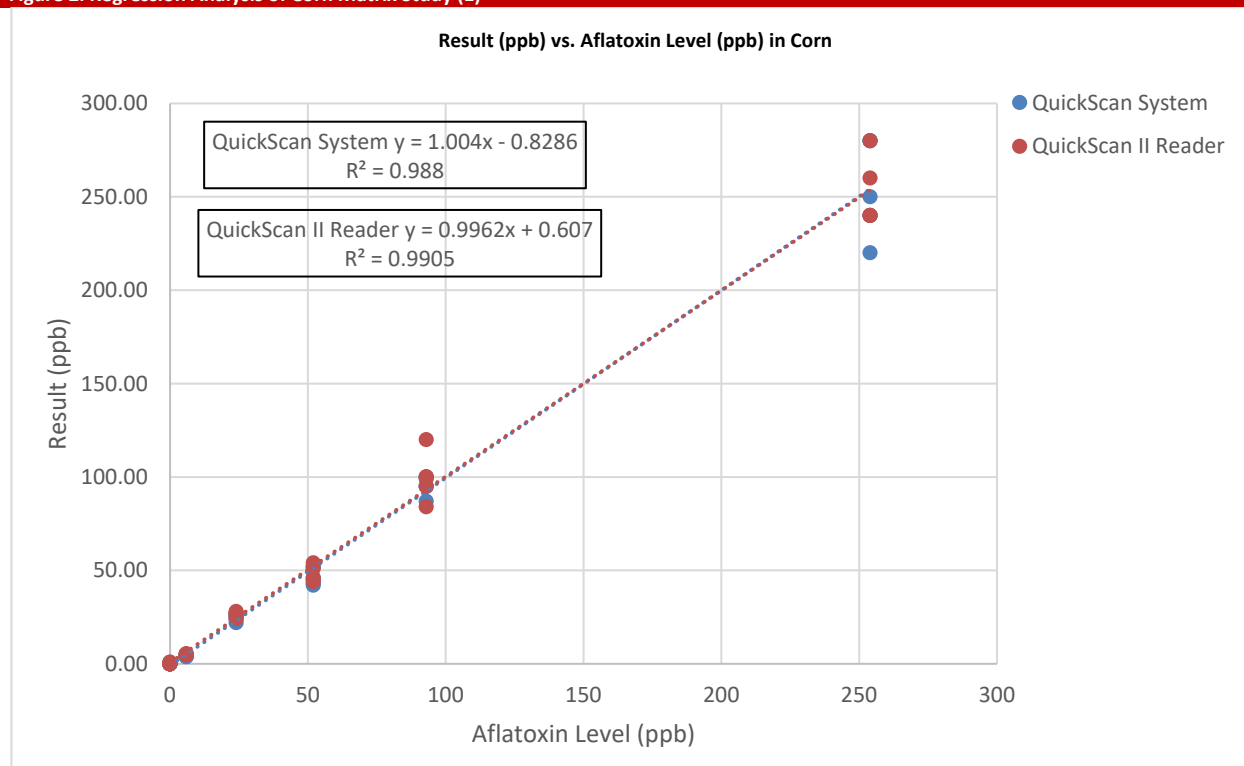
By using a combinatorial factor design to vary three user-effected variables, the robustness of the assay was explored in the Robustness Study. When co-varying sample test size, extraction shake time, and strip development time, all results fell within the acceptable ranges for the 19.2 ppb aflatoxin level using both readers. The generalized linear model analysis indicates that all three variables, test sample size, development time and shake time had a significant impact on the reported results. These data show that the sample should be accurately weighed, precisely extracted and the development time should be tightly controlled to ensure accurate test results are reported.

The lots produced using the Sartorius nitrocellulose showed comparable performance to the Millipore nitrocellulose lots used in the PTM method. In addition, four lots using the Sartorius nitrocellulose membrane showed acceptable performance in accelerated stability to support the assay's 1-year stability claim. Results presented in this study support the use of the nitrocellulose membrane sourced from Sartorius in the Aflatoxin Flex PTM test method for detection of aflatoxin in corn.

Table 7. Matrix study results for corn (1)

Table 7. Matrix study results for corn (2)													
Replicate	Aflatoxin concentration - Corn												Reader
	0		5.9 ppb		23.7 ppb		52.2 ppb		92.8 ppb		254 ppb		
	QSS	QSSII	QSS	QSSII	QSS	QSSII	QSS	QSSII	QSS	QSSII	QSS	QSSII	
1	0	0	4.5	5.4	25	28	46	46	99	100	280	260	
2	0	0	4.1	4.6	22	24	52	51	95	120	280	280	
3	0	0	3.7	4.9	26	26	52	54	95	100	250	240	
4	0	0	3.9	5.1	24	27	43	45	100	95	240	240	
5	0	0	3.8	5.1	26	27	42	44	87	84	220	240	
6	0	0											
7	0	0.88											
8	0	0.42											
9	0	0											
10	0	0.17											
Mean	0.00	0.15	4.0	5.0	24.6	26.4	47.0	48.0	95.2	99.8	254	252	
S <sub>r</sub>	0.00	0.29	0.32	0.29	1.67	1.52	4.8	4.3	5.1	13.0	26.1	17.9	
RSD <sub>r</sub> %			7.9	5.9	6.8	5.7	10.2	9.0	5.4	13.1	10.3	7.1	
Recovery %			68%	85%	104%	111%	90%	92%	103%	108%	100%	99%	
Bias			-1.90	-0.88	0.90	2.70	-5.20	-4.20	2.40	7.00	0.00	-2.00	
LOD	0.00	0.73											
LOQ	0.00	2.19											

Figure 2. Regression Analysis of Corn Matrix Study (1)



#### REFERENCE CITED

1. Bailey, C., Roberts, R., McManus, S., Goddard, T., Tapely, S. and Gow, B., Validation of the QuickTox™ Kit for QuickScan Aflatoxin Flex for Determination of Aflatoxin in Corn, AOAC® Performance Tested<sup>SM</sup> certification number 012104.