CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

012104

The AOAC Research Institute hereby certifies the method known as:

TotalTox™ Aflatoxin

manufactured by

EnviroLogix
500 Riverside Industrial Parkway
Portland, ME 04103
USA

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*[SM] Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*[SM] certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date December 1, 2

Expiration Date December 31, 2024

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SUBMITTING COMPANY
EnviroLogix
500 Riverside Industrial Parkway
Portland, ME 04103 USA

METHOD NAME
TotalTox™ Aflatoxin
Formerly QuickTox™ Kit for Aflatoxin Flex

CATALOG NUMBER
AQ 309 BG

INDEPENDENT LABORATORY
Trilogy Analytical Labor
870 Vossbrink Dr.
Washington, MO 63090

APPLICABILITY OF METHOD
Analytes – Total Aflatoxins.

Matrixes – Co

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

ORIGINAL CERTIFICATION DATE	**CERTIFICATION RENEWAL RECORD**
January 29, 2021	**Renewed annually through December 2024.**

METHOD MODIFICATION RECORD	**SUMMARY OF MODIFICATION**
1. March 2021 Lev	**1. Rebranding to TotalTox™ Aflatoxin.**
2. November 2021 Level 1	**2. Non-method related changes to software.**
3. November 2023 Level 1	**3. Non-method related changes to software and editorial/clerical.**

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NONE	**NONE**

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_r values well below the acceptance criteria Max %RSD for each aflatoxin level, with the combined corn matrix studies exhibiting no more than 14% RSD_r 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 (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 determinatio
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 tes 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.