# CERTIFICATION

## AOAC® *Performance Tested*SM

Certificate No.

**041201**

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

## QuickTox™ Kit for QuickScan Aflatoxin

manufactured by

**EnviroLogix Inc.**
**500 Riverside Industrial Parkway**
**Portland, ME 04103**
**USA**

This method has been evaluated in the AOAC® *Performance Tested Methods*SM 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 Tested*SM 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 (January 1, 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

December 15, 2015
Date

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

**KIT NAME(S)**
QuickTox™ Kit for QuickScan Aflatoxin

**CATALOG NUMBERS**
AQ-109-BG (Single Kit), AQ-109-BGV (Bulk Packaged)

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

**AOAC EXPERTS AND PEER REVIEWERS**
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**APPLICABILITY OF METHOD**
**Target analyte – Aflatoxins**
**Matrices – Corn**
**Performance claims – Detection of Aflatoxin ranging from 2.5 – 100 ppb (ng/g)**

**REFERENCE METHOD**
**Compared to acceptable ranges specified by the AOAC *Performance Tested Methods℠* Program**

**PRINCIPLE OF THE METHOD**
The EnviroLogix QuickTox Kit for QuickScan Aflatoxin is a competitive, lateral flow immunoassay. Total aflatoxins are extracted from ground corn samples by shaking with 50% ethanol and the extracts are allowed to settle. Assay diluent buffer and extracts are added to reaction vessels followed by placement of assay strips. Test and control lines are allowed to develop as the sample and buffer move through the devices 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 encoded in the strips' barcodes. The system software reports and archives the total aflatoxin results. Archived results may be exported in spreadsheet form for further analyses as desired by the user.

**DISCUSSION OF THE VALIDATION STUDY**
Grain contaminated with aflatoxin commands a lower price compared to non-contaminated grain, and should be segregated in storage facilities. Grain with aflatoxin levels exceeding thresholds is deemed unfit for human food or for inclusion in livestock feed. A variety of technologies are commonly used for aflatoxin determination including TLC, HPLC, flourometry, EIA, LC/MS/MS and qualitative and quantitative lateral flow assays (4, 5). More sophisticated analytical techniques are used mainly in reference and academic laboratories. Visual and reader-based EIA and lateral flow assays are used in many grain operations specializing in the purchase, storage and resale of bulk commodities.

The availability of a rapid, robust, field-based, quantitative total aflatoxin assay can facilitate aflatoxin testing and grain movement. As of this writing a number of aflatoxin assays have received Performance Tested Method Certification although none use lateral flow devices (6).

The assay employs lot specific calibration curves which are encoded in each strip's barcode. Results do not depend on performing run-specific standard curves. The system software interprets signal intensities and uses the encoded calibration curve to determine the results. Inclusion of run-specific standards is not required and calculations and visual interpretation of assay results are eliminated.

Aflatoxin B1 is the type found in highest prevalence in corn (7). Moreover, naturally contaminated, certified reference corn samples used in this study had B1: B2 ratios of at least 10:1 and only one sample had detectable aflatoxin G2. The selectivity studies indicated that all four common types of aflatoxin: B1, B2, G1, and G2 are detected in the assay with B1 being the most reactive form. Thus the assay has highest sensitivity for the most prevalent aflatoxin type.

Other common mycotoxins, such as Fumonisin B1, Ochratoxin A, Vomitoxin, or Zearalenone did not react or interfere in the assay when tested at 100 ppm in corn extract. This approximates 200 ppm in a ground corn sample since the assay protocol specifies two volumes of extractant per weight of sample. The aflatoxin assay is not affected by other common mycotoxins even when present in extremely high levels.

**DISCUSSION OF THE VALIDATION STUDY Cont.**
The protocol for obtaining PTM certification for this assay was agreed upon by AOAC RI and the assay sponsor prior to initiation of these studies. The protocol included criteria for assay performance as outlined in Table 2. All assay results obtained in the sponsor's laboratory and in the third-party, independent laboratory were well within these acceptable ranges. The assay is robust, meeting performance requirements dictated by an external body in addition to meeting the sponsor's performance claims. The matrix studies performed at the sponsor's laboratory and an independent facility tested correlation of dose versus HPLC and the repeatability of six aflatoxin levels when multiple samples were extracted and run by two operators at each site across four reader systems. The data generated at the two sites showed good correlation with slopes of 1.26 and 1.11, respectively, for the internal and independent laboratory results, and corresponding $R^2$ values of 0.9897 and 0.9896 showing the assay response is linear between 0 – 100 ppb. Recoveries ranged from 85 – 126% across the two testing sites indicating assay efficacy over the entire assay range. $RSD_r$ % ranged from 4.65 – 17.67% across all contaminated samples with both testing sites exhibiting variances well within the 25 - 30% specified for the acceptable ranges (Table 2). The LOD for both the internal and independent laboratory studies were 1.36 and 2.13 ppb, respectively, which is below the LOD of 2.5 ppb specified by the assay sponsor. The combined data from both laboratories is presented in Figure 3C and Table 5C.

The lot-to-lot consistency and stability study was performed using a 10.9 ppb frozen extract. The mean dose ranged from 10 – 14 ppb, when tested at curve set (Day 0), a mid-life time point, and an end-life time point on five strip lots, and all individual points remained within the allowable range.

All results obtained with the 11.3 ppb corn in the robustness study fell within the acceptable range despite employing assay parameters that are beyond those stated in the assay product insert. Assay time (4 - 8 minutes, nominal 5 minutes) was deemed the most influential of the parameters tested. Assay temperature (18 - 30ºC) was also identified as a significant factor, whereas sample volume (80 - 120 µL, nominal 100 µL) did not exhibit a significant effect. Individual result means (n = 3 strips) for the nine combinations tested ranged from 9.20 – 14.33 ppb. Accuracy is further improved by adherence to the product insert.

**REFERENCES CITED**


1. Bailey, Cheryl and Davis, Alan., Evaluation of the QuickTox™ Kit for QuickScan Aflatoxin in corn, AOAC® *Performance Tested℠* certification number 041201.
2. AOAC Research Institute Validation Outline for QuickTox™ Kit for QuickScan Aflatoxin, Approved – April 2012.
3. Cody, RP, & Smith JK (1997) Applied Statistics and the SAS Programming Language, Chapter 7, Prentiss-Hall, Upper Saddle River, NJ
4. Rahmani, A.; Jinap, S.; & Soleimany, F. (2009) Qualitative and Quantitative Analysis of Mycotoxins Comprehensive Reviews in Food Science and Food Safety 8: 202-251.
5. Shephard, G.S.; Berthiller, F.; Burdaspal P.A.; Crews, C.; Jonker, M.A.; Krska, R.; MacDonald, S.; Malone, R.J.; Maragos, C.; Sabino M.; Solfrizzo, M.; Van Egmond, H.H.; & Whitaker T. B. Developments in mycotoxin analysis: an update for 2010-2011 World Mycotoxin Journal 5: 3 2012
6. http://aoac.org/testkits/testedmethods.html
7. Vargas EA, Preis RA, Castro L, & Silva CM (2001) Co-occurrence of aflatoxins B1, B2, G1, G2, zearalenone and fumonisin B1 in Brazilian corn Food Addit Contam. 2001 18:981-6.

**Table 5. Summary of Matrix Study for Corn**

## A. Run at Sponsor's Laboratory

| Cup | < 0.5 ppb | | 4.5 ppb | 11.3 ppb | 19.0 ppb | 50.8 ppb | 98.7 ppb |
|---|---|---|---|---|---|---|---|
| 1 | 0.35 | | 4.3 | 12 | 18 | 57 | 120 |
| 2 | 0.00 | | 3.7 | 11 | 17 | 55 | 110 |
| 3 | 0.59 | | 3.6 | 11 | 17 | 60 | 130 |
| 4 | 0.05 | | 3.9 | 13 | 17 | 60 | 120 |
| 5 | 0.66 | | 5.1 | 13 | 18 | 63 | 120 |
| 6 | 1.29 | | 5.8 | 12 | 17 | 68 | 120 |
| 7 | 0.00 | | 4.8 | 11 | 17 | 54 | 130 |
| 8 | 0.47 | | 5.8 | 13 | 19 | 60 | 140 |
| 9 | 1.04 | | 5.5 | 12 | 18 | 54 | 120 |
| 10 | 0.00 | | 5.1 | 12 | 19 | 61 | 130 |
| **Mean** | **0.45** | | **4.76** | **12.00** | **17.70** | **59.20** | **124.00** |
| $s_r$ | 0.46 | | 0.84 | 0.82 | 0.82 | 4.39 | 8.43 |
| LOD (mean + 2sd) | 1.36 | $RSD_r$ | 17.67% | 6.80% | 4.65% | 7.42% | 6.80% |
| LOQ = 3 x LOD | 4.09 | Recovery | 105.78% | 106.19% | 93.16% | 116.54% | 125.63% |
| | | Bias | 0.26 | 0.70 | -1.30 | 8.40 | 25.30 |

## B. Run at Independent Laboratory

| Cup | < 0.5 ppb | | 4.5 ppb | 11.3 ppb | 19.0 ppb | 50.8 ppb | 98.7 ppb |
|---|---|---|---|---|---|---|---|
| 1 | 1.64 | | 5.1 | 11 | 15 | 61 | 110 |
| 2 | 1.36 | | 4.7 | 11 | 15 | 54 | 95 |
| 3 | 1.61 | | 4.8 | 11 | 16 | 59 | 120 |
| 4 | 0.69 | | 5.1 | 11 | 16 | 54 | 120 |
| 5 | 2.01 | | 4.9 | 11 | 16 | 63 | 110 |
| 6 | 1.14 | | 4.4 | 11 | 15 | 52 | 110 |
| 7 | 1.64 | | 4.8 | 10 | 15 | 53 | 110 |
| 8 | 1.48 | | 4.4 | 10 | 18 | 57 | 100 |
| 9 | 1.16 | | 4.8 | 12 | 20 | 51 | 110 |
| 10 | 1.08 | | 4.5 | 12 | 15 | 53 | 110 |
| **Mean** | **1.38** | | **4.75** | **11.00** | **16.10** | **55.70** | **109.50** |
| $s_r$ | 0.38 | | 0.25 | 0.67 | 1.66 | 4.08 | 7.62 |
| LOD (mean + 2sd) | 2.13 | $RSD_r$ | 5.37% | 6.06% | 10.33% | 7.33% | 6.96% |
| LOQ = 3 x LOD | 6.39 | Recovery | 105.56% | 97.35% | 84.74% | 109.65% | 110.94% |
| | | Bias | 0.25 | -0.30 | -2.90 | 4.90 | 10.80 |

## C. Combined Data - Both Laboratories

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Mean** | **0.91** | | **4.76** | **11.50** | **16.90** | **57.45** | **116.75** |
| $s_r$ | 0.63 | | 0.60 | 0.89 | 1.52 | 4.50 | 10.79 |
| LOD (mean + 2sd) | 2.17 | RSDr | 12.72% | 7.73% | 8.98% | 7.83% | 9.25% |
| LOQ = 3 x LOD | 6.52 | Recovery | 105.67% | 101.77% | 88.95% | 113.09% | 118.29% |
| | | Bias | 0.26 | 0.20 | -2.10 | 6.65 | 18.05 |

**ORIGINAL CERTIFICATION DATE**
**April 12, 2012**

**CERTIFICATION RENEWAL RECORD**
**Renewed Annually through December 2016**

**METHOD MODIFICATION RECORD**
None

**SUMMARY OF MODIFICATION**

**Under this AOAC® *Performance Tested*℠ License Number, 041201 this method is distributed by:**

**Under this AOAC® *Performance Tested*℠ License Number, 041201 this method is distributed as:**