CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

021301

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

QuickTox™ Kit for QuickScan Ochratoxin-A

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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KIT NAME(S)
QuickTox™ Kit for QuickScan Ochratoxin-A

CATALOG NUMBERS
AQ-113-BG

INDEPENDENT LABORATORY
Trilogy Analytical Laboratory
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Washington, MO 63090
USA

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APPLICABILITY OF METHOD
Target analyte – Ochratoxin
Matrices – Wheat
Performance claims – Detection of ochratoxin ranging from 1.5 – 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 Ochratoxin-A uses competitive, lateral flow immunoassay technology and a reflectance reader system. Ground wheat is extracted with a proprietary buffer (diluted from concentrate supplied in the assay kit.) The extract is centrifuged to prepare a supernatant. Assay buffer and supernatant are then added to a reaction vessel followed by an assay strip. Capillary flow moves the extract-buffer solution through the device allowing development of test and control lines. The reader system and software process the strip's line intensities and provides results to the user by comparison to lot specific calibration curves encoded on the barcode affixed to the strip. Archived results may be exported in both report or spread sheets format for trend analyses as desired by the user.

DISCUSSION OF THE VALIDATION STUDY

Ochratoxins are thought to be nephrotoxic and carcinogenic (3, 6, 7). The US Food and Drug Administration has not defined acceptable guidance or regulatory levels for acceptance of ochratoxin contaminated wheat. However, several countries and the EU have set regulatory levels for ochratoxin contamination (8, 9). The importance of ochratoxin testing is evidenced by the number of techniques available including immunoaffinity or solid phase clean up and HPLC, enzyme immunoassay (EIA), and lateral flow membrane assay (10). A rapid and simple to use quantitative ochratoxin assay should add to this armamentarium and facilitate testing enabling ochratoxin determination early in the grain production and processing chain.

In addition to OTA and OTB other ochratoxin forms including Ochratoxin, C, α and β have been described (11, 12) but little information is available regarding levels produced during a natural Aspergillus infection. Hesseltine reported that a majority of Aspergillus isolates produced OTA in greater amounts than OTB when cultivated *in vitro* on wheat (13). The selectivity study in the present work indicated that the QuickTox assay exhibits a bias towards detection of OTA compared to OTB. Other ochratoxin forms are not readily available precluding determination of relative reactivity beyond OTA and OTB. The AOAC Official Method of Analyses no. **2000.03** for Ochratoxin (5) was used to determine ochratoxin levels in naturally contaminated reference samples used in the present work. This method quantitates OTA but does not allow determination of other ochratoxin forms (12). Thus, it is difficult to asses the importance of preferential detection of OTA by either HPLC or the QuickTox assay.

Aflatoxin B1, Fumonisin B1, Vomitoxin, and Zearalenone were not interferents nor did they cause false positive results in the assay despite being added to extremely high levels of 100 ppm in extracts. Moreover, the assay procedure uses 5 mL of extractant per g of sample; addition of 100 ppm spiked into the extract approximates 500 ppm in a wheat sample. The absence of interference adds confidence to the validity of assay results by the end user.

Variability in any assay's results reflect sampling and inherent device and operator variation. Moreover, the distribution of ochratoxin in contaminated wheat has been reported to be less uniform than is the case for vomitoxin (13).

DISCCUSION OF THE VALIDATION STUDY Continued

Despite these sources of variability, the R^2 values were 0.9719 and 0.9849 for the matrix studies run in the sponsor's and independent laboratories, respectively. These data indicate a linear dose response for the assay relative to HPLC determinations.

Two equal considerations were paramount in development of the QuickTox Kit for QuickScan Ochratoxin-A: reliable assay results and operator ease of use. All assay results obtained in both internal and external studies reported in this work were within the acceptable ranges established before performance tested method certification was attempted speaking to the validity of assay performance. The assay employs minimal steps and precludes user calculations and interpretations with use of lot specific calibration curves on assay devices and use of a reader and software system. Overall, these data speak to the assay's reliability and utility for the end user.

REFERENCES CITED

1. Davis, Alan and Roberts, Russell., Evaluation of the QuickTox™ Kit for QuickScan Ochratoxin-A in wheat, AOAC® *Performance Tested℠* certification number 021301.
2. AOAC Research Institute Validation Outline for QuickTox™ Kit for QuickScan Ochratoxin-A , Approved – February 2013.
3. Bayman, P & Baker, J.L. (2006) *Mycopathologia* (2006) 162: 215–223
4. Magan N. & Aldred, A. (2005) *Food Addit Contam, Supplement* 1: 10–16
5. *Official Methods of Analysis* (2005) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Method 2000.03
6. el Khoury, A., & Atoui, A. (2010) *Toxins* 2010, *2*, 461-493
7. Peraica,M., RadicÂ, B., Lucic, A., & Pavlovic, M. (1999) *B WORLD HEALTH ORGAN* 77, 754-759
8. ftp://ftp.fao.org/docrep/fao/007/y5499e/y5499e00.pdf
9. http://www.grainscanada.gc.ca/storage-entrepose/ota/ota-eng.htm
10. 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. (2012) *World Mycotoxin J.* **5**, 3–30
11. Stander, M.A., Steyn., P.S., Lubben, A., Miljkovic, A., Mantle, P.G., & Marais, G. *J. Agric. Food Chem.* (2000**)** *48,* 1865-1871
12. Harris, J.P. &, Mantle, P.G. (2001) *Phytochemistry* 58 709–716
13. Hesseltine, C.W., Vandegraft, E.E., , DOROTHY 1. Fenell, D.I., Smith, M.L., & ODETTE L. Shotwell, O.L. (1972) L *Mycologia* 64, 539-550
14. Entwisle, A. C.; Williams, A. C.; Mann, P. J.; Slack, P. T. & Gilbert, J. (2000) *JAOAC Int.*.83 1377- 1383
15. Biselli, S., C. Persin, C., & M. Syben, M. (2008) *Mycotoxin Research* 24, 98-104

Table 5 Summary of matrix study for wheat run at sponsor's laboratory

Replicate	< 1ppb		4.9 ppb	21.5 ppb	54.2[a]	101.8[a]
1	0.1		3.6	16	65	91
2	0.0		4.0	24	56	110
3	0.0		3.4	19	51	120
4	0.2		3.1	17	50	130
5	0.2		2.8	16	57	110
6	0.3		3.9	20	60	110
7	0.1		3.5	21	42	110
8	0.2		3.8	20	60	110
9	0.0		3.4	16	70	130
10	0.0		2.7	21	77	96
Mean	0.11		3.40	19.11	58.88	111.64
S_r	0.11		0.45	2.72	10.07	12.66
LOD (mean + 2 * S_r)	0.33	RSD_r, %	13.10	14.22	17.10	11.34
LOQ= 3 X LOD	0.99	Recovery, %	69.36	88.87	108.64	109.66
		Bias	-1.50	-2.39	4.68	9.84

[a]Data reflect six-fold dilution and correction for the dilution factor.

Table 9 Summary of matrix study for wheat run at independent laboratory

Replicate	< 1ppb		7.0 ppb	21.5 ppb	54.2[a]	101.8[a]
1	0.0		6.7	19	36	72
2	0.0		6.4	22	43	79
3	0.0		8.3	20	43	67
4	0.0		6.6	20	38	69
5	0.0		7.4	16	43	80
6	0.0		5.6	20	33	66
7	0.0		5.1	17	46	68
8	0.1		5.2	21	36	68
9	0.0		5.0	18	42	74
10	0.0		5.6	19	39	74
Mean	0.01		6.19	19.20	39.90	71.70
S_r	0.03		1.03	1.72	3.91	4.71
LOD (mean + 2 * Sr)	0.07	RSD_r, %	16.69	8.96	9.80	6.57
LOQ= 3 X LOD	0.20	Recovery, %	88.43	89.3	73.6	70.4
		Bias	-0.8	-2.3	-14.3	-30.1

[a]Data reflect six-fold dilution and correction for the dilution factor.

ORIGINAL CERTIFICATION DATE
February 12, 2013

CERTIFICATION RENEWAL RECORD
Renewed Annually through December 2016

METHOD MODIFICATION RECORD
None

SUMMARY OF MODIFICATION

Under this AOAC® *Performance Tested*SM License Number, 021301 this method is distributed by:

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