CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

071402

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

QuickTox™ Kit for QuickScan DON3

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 03, 2018 – December 31, 2018). 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

January 03, 2018
Date

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

KIT NAME(S)
QuickTox™ Kit for QuickScan DON3

CATALOG NUMBERS
AQ-254-BG (Single Kit), AQ-254-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 – DON residues in commodities

Matrices – Wheat, maize, wheat bran, wheat flour, and barley

Performance claims – Detection of DON ranging from 0.3 – 5.0 ppm

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

ORIGINAL CERTIFICATION DATE
July 16, 2014

CERTIFICATION RENEWAL RECORD
Renewed Annually through December 2018

METHOD MODIFICATION RECORD
1. **January 2018 Level 1**

SUMMARY OF MODIFICATION
1. **Editorial changes**

Under this AOAC® *Performance Tested℠* License Number, 071402 this method is distributed by:
NONE

Under this AOAC® *Performance Tested℠* License Number, 071402 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
Water is used to extract DON from ground grain commodities. Extraction volumes are varied according to the type of sample being tested. Filtration or settling, depending on the matrix, may be used for clarification of extracts. The extract is diluted five-fold in assay buffer DB6; an aliquot of the dilution is placed in a reaction vial. The assay device is a competitive lateral flow immunoassay strip. A strip is placed into the reaction vial; test and control lines are developed for 3 min. Line intensities are read on the QuickScan system and automatically compared to the lot specific calibration curve encoded in the strip's barcode yielding quantitative DON results in the range of 0.3-12 ppm. Procedures used herein always employed filtration. The kit reports DON results up to 12 ppm; work reported herein was performed to obtain AOAC certification for a validated range up to 5 ppm.

DISCUSSION OF THE VALIDATION STUDY (1)
The QuickTox Kit for QuickScan DON, a rapid, competitive lateral flow immunoassay for detection of DON, was certified by the AOAC RI in 2012. Since then, several changes have been made to this assay, including shortening assay development time from 10 to 3 min, use of matrix specific extraction ratios and higher total sample dilutions to normalize matrix differences enabling use of a single calibration curve and provision for faster sample preparation time. The biologic reagents have not been altered in the updated assay. Rather, changes in assay strip architecture improved sample and gold conjugate flow through the assay device resulting in faster development of the test and control lines. Moreover, simplified device architecture facilitates manufacturing. Of note, faster sample preparation and shorter strip development decrease assay turnaround time in the hands of the user.
The data described in the present work indicate that the updated assay exhibits equivalent performance to the original product judged against acceptable ranges for non-detect and up to 5 ppm DON contaminated matrices. All data points in all internal and external studies were within the acceptable ranges. Linear regression analyses in the matrix and linearity studies showed high correlation values indicating that assay results exhibit predictable dose responses compared to HPLC determinations. Assay performance in the hands of multiple users, at two sites, across multiple lots, and after intentional variation in user interface parameters indicate that the assay procedure and manufacture of the assay are robust. The updated assay uses the same biologic reagents as used in the original kit; it is unsurprising that relative reactivity to DON and DON analogs was concordant with the previously reported data.
Use of lateral flow mycotoxin assays is becoming more widespread likely reflecting user demands for assay speed, ease of use, and accuracy of results judged against sophisticated reference techniques (3). The utility of these rapid assays may be best observed at grain elevators with multiple trucks awaiting delivery in longs queues. The availability of a 3 min DON assay with performance comparable to HPLC analyses serves the need for faster results facilitating grain handling.

Table 4. Summary of matrix studies for whole wheat (1)

Replicate	< 0.1 ppm	0.5 ppm	0.9 ppm	2.1 ppm	3.5 ppm	4.9 ppm
A. Run at sponsor's laboratory						
1	0.03	0.61	0.94	1.6	3.1	5.5
2	0	0.56	0.92	1.6	3.1	5.3
3	0.03	0.63	0.96	1.7	2.9	5.8
4	0	0.65	0.93	2	3.4	5.8
5	0	0.63	1	1.9	2.9	5.4
6	0	0.63	1	2	3.2	5.4
7	0	0.66	0.99	2	3.7	5.8
8	0	0.61	0.9	2	3.5	5.4
9	0	0.48	0.94	2.1	3.6	4.8
10	0	0.53	0.91	1.9	3.4	5.1
Mean	0.01	0.60	0.95	1.88	3.28	5.43
S_r	0.01	0.06	0.04	0.18	0.28	0.32
LOD mean + 2 S_r	0.03					
LOQ (3 x LOD)	0.09					
RSD_r, %		9.62	3.89	9.65	8.60	5.95
Recovery, %		120	105	90	94	111
Bias		0.10	0.05	-0.22	-0.22	0.53
B. Run at independent laboratory						
1	0	0.45	0.81	1.8	3.2	4.4
2	0	0.52	0.83	1.8	3.3	4.7
3	0	0.52	0.86	2	3.6	4.4
4	0	0.48	0.85	1.8	3.2	4.4
5	0	0.5	0.88	2.2	3.3	4.9
6	0					
7	0					
8	0					
9	0					
10	0					
Mean	0.00	0.49	0.85	1.92	3.32	4.56
S_r	0.00	0.03	0.03	0.18	0.16	0.23
LOD mean + 2 S_r	0.00					
LOQ (3 x LOD)	0.00					
RSD_r, %		6.01	3.19	9.32	4.95	5.05
Recovery, %		99	94	91	95	93
Bias		-0.01	-0.05	-0.18	-0.18	-0.34
C. Combined data-both laboratories						
Mean	0.00	0.56	0.91	1.89	3.29	5.14
S_r	0.00	0.07	0.06	0.18	0.24	0.51
LOD mean + 2 S_r	0.00					
LOQ (3 x LOD)	0.00					
RSD_r, %		12.55	6.57	9.25	7.39	9.97
Recovery, %		113	102	90	94	105
Bias		0.06	0.01	-0.21	-0.21	0.24

Table 5. Summary of matrix studies for maize (1)

Replicate	< 0.1 ppm	0.5 ppm	1.1 ppm	1.9 ppm	3.6 ppm	5.3 ppm
A. Run at sponsor's laboratory						
1	0	0.56	0.9	1.9	3.7	5.4
2	0.04	0.5	0.96	1.7	4	5.1
3	0.1	0.45	0.96	1.8	3.7	5.1
4	0	0.58	1	1.8	3.6	5.3
5	0.04	0.48	1.1	1.8	3.2	4.9
6	0.07	0.62	0.99	1.9	3.4	5.7
7	0.07	0.46	0.97	1.8	3.5	5.4
8	0.05	0.53	1	2	2.9	5.6
9	0	0.5	1	1.7	3.4	5.1
10	0	0.49	0.99	1.7	3.7	4.9
Mean	0.037	0.52	0.99	1.81	3.51	5.25
S_r	0.036	0.05	0.05	0.10	0.31	0.28
LOD mean + 2 S_r	0.11					
LOQ (3 x LOD)	0.33					
RSD_r, %		10.60	5.08	5.49	8.75	5.25
Recovery, %		103	90	95	98	99
Bias		0.02	-0.11	-0.09	-0.09	-0.05
B. Run at independent laboratory						
1	0.05	0.47	0.93	1.9	3.5	5.7
2	0.05	0.39	0.91	1.8	3.6	5.9
3	0.05	0.49	0.98	2	3.4	6
4	0.09	0.53	0.93	1.8	3.5	5.4
5	0.06	0.49	1.2	2	3.6	5.6
6	0.05					
7	0.06					
8	0.07					
9	0.01					
10	0.08					
Mean	0.06	0.47	0.99	1.90	3.52	5.72
S_r	0.02	0.05	0.12	0.10	0.08	0.24
LOD mean + 2 S_r	0.10					
LOQ (3 x LOD)	0.30					
RSD_r, %		10.92	12.14	5.26	2.38	4.17
Recovery, %		95	90	100	98	108
Bias		-0.03	-0.11	0.00	-0.08	0.42
C. Combined data-both laboratories						
Mean	0.05	0.5	0.99	1.84	3.51	5.41
S_r	0.03	0.06	0.08	0.11	0.25	0.34
LOD mean + 2 S_r	0					
LOQ (3 x LOD)	0					
RSD_r, %		11.1	7.67	5.74	7.13	6.35
Recovery, %		101	90	97	98	102
Bias		0	-0.11	-0.06	-0.09	0.11

Table 6. Summary of matrix study for wheat bran (1)

	Run at sponsor's laboratory					
Replicate	< 0.1 ppm	0.5 ppm	1.0 ppm	2.0 ppm	3.5 ppm	5.0 ppm
1	0	0.45	0.81	1.8	3	4.8
2	0.03	0.58	0.9	1.8	3.4	5
3	0	0.45	0.95	1.7	3.7	5
4	0.05	0.49	1.1	1.7	3.4	4.5
5	0.05	0.45	1	1.8	3.3	4.2
6	0.02					
7	0.08					
8	0.1					
9	0					
10	0					
Mean	0.03	0.48	0.95	1.76	3.36	4.70
S_r	0.04	0.06	0.11	0.05	0.25	0.35
LOD mean + 2 S_r	0.11					
LOQ (3 x LOD)	0.33					
RSD_r, %		11.65	11.40	3.11	7.47	7.37
Recovery, %		97	95	88	96	94
Bias		-0.02	-0.05	-0.24	-0.14	-0.30

Table 7 . Summary of matrix study for white wheat flour (1)

	Run at sponsor's laboratory					
Replicate	< 0.1 ppm	0.5 ppm	1.0 ppm	2.0 ppm	3.5 ppm	5.0 ppm
1	0.02	0.58	0.96	1.8	3.5	5.1
2	0.12	0.7	1	1.8	3.4	4.8
3	0.03	0.62	1	2	3.5	5.1
4	0.08	0.53	0.93	1.8	3.5	5.1
5	0.06	0.52	0.96	1.9	3.2	4.4
6	0.06					
7	0.03					
8	0.04					
9	0.02					
10	0					
Mean	0.05	0.59	0.97	1.86	3.42	4.90
S_r	0.04	0.07	0.03	0.09	0.13	0.31
LOD mean + 2 S_r	0.13					
LOQ (3 x LOD)	0.39					
RSD_r, %		12.46	3.09	4.81	3.81	6.29
Recovery, %		118	97	93	98	98
Bias		0.09	-0.03	-0.14	-0.08	-0.10

Table 8 . Summary of matrix study for barley (1)

	Run at sponsor's laboratory					
Replicate	< 0.1 ppm	0.5 ppm	1.0 ppm	2.0 ppm	3.5 ppm	5.0 ppm
1	0	0.49	0.97	1.9	3.3	4.1
2	0	0.55	0.97	1.9	3.2	4.6
3	0	0.57	0.94	1.9	3.1	4.6
4	0	0.48	0.98	2	3.1	5
5	0	0.43	1	1.9	3.4	4.7
6	0					
7	0					
8	0					
9	0					
10	0					
Mean	0.00	0.50	0.97	1.92	3.22	4.60
S_r	0.00	0.06	0.02	0.04	0.13	0.32
LOD mean + 2 S_r	0.00					
LOQ (3 x LOD)	0.00					
RSD_r, %		11.19	2.23	2.33	4.05	7.04
Recovery, %		101	97	96	92	92
Bias		0.00	-0.03	-0.08	-0.28	-0.40

REFERENCES CITED

1. Polakowski, S., Davis, A., Albert, A, and Gow, B.., Evaluation of the QuickTox™ Kit for QuickScan DON3, AOAC® *Performance Tested*℠ certification number 071402.
2. AOAC Research Institute Validation Outline for QuickTox™ kit for QuickScan DON3, Approved July 2014
3. Albert, A.L., Champoux, P.D., & Davis, A.H. (2013) JAOAC Int. 96, 1006-16