CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

121701

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

QuickTox™ Kit for QuickScan DON Flex

manufactured by

EnviroLogix
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 (August 01, 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

August 01, 2018
Date

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

KIT NAME(S)
QuickTox™ Kit for QuickScan DON Flex

CATALOG NUMBERS
AQ 304 BG

INDEPENDENT LABORATORY
Trilogy
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Washington, MO 63090
USA

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APPLICABILITY OF METHOD
Target organisms – deoxynivalenol (DON)

Matrices – (20 g) - corn and wheat

Performance claims - Detection of DON ranging from 0.1 – 30 ppm.

ORIGINAL CERTIFICATION DATE	**CERTIFICATION RENEWAL RECORD**
December 19, 2017	**New Approval 2018**

METHOD MODIFICATION RECORD	**SUMMARY OF MODIFICATION**
1. August 2018 Level 2	**1. Certification of QuickScan System II**

Under this AOAC® *Performance Tested*℠ License Number, 121701 this method is distributed by:	**Under this AOAC® *Performance Tested*℠ License Number, 121701 this method is distributed as:**
NONE	**NONE**

PRINCIPLE OF THE METHOD (1)
The QuickTox™ Kit for QuickScan DON Flex test method utilizes competitive, lateral flow immunoassay to detect DON contamination in various matrices. The mycotoxin is extracted from ground wheat or corn samples with water and clarified through filtration or centrifugation. The test sample is created by 1:1 dilution with DB6 assay diluent and equilibration 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 system uses matrix-specific calibration curves, input by scanning the multi-matrix barcode card, in order to determine the quantitation level of the sample.

DISCUSSION OF THE VALIDATION STUDY (1)
The QuickTox™ Kit for QuickScan DON Flex test method was developed to provide a rapid, easy to use, consistent, and highly accurate test for quantitation of deoxynivalenol levels in grain commodities. 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 two types of readers (500+, 550+), each with the QuickScan software for quantitative results reporting.
Performance assessed by linearity and matrix studies in the sponsor's lab and the independent lab demonstrated a highly linear dose response of the method to levels of deoxynivalenol from 0 – 30 ppm, using both the 500+ and 550+ scanners. The correlation coefficients (R^2 value) of linear regression analysis reached 1.00, indicating a perfect linear fit which demonstrates that both scanner types are capable of accurately quantitating DON 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 DON level, with the combined wheat matrix studies exhibiting no more than 17% RSD_r at the 0.5ppm dose. Therefore, the test method, in conjunction with either scanner type, displays suitable repeatability.
To challenge the product claim of dilution accuracy down to 2 ppm *(3),* the 5 ppm sample was tested using the dilution protocol for the 8 – 30 ppm quantitation range as part of the matrix studies. Results from both scanners were within the acceptable ranges regardless of the test protocol used (Tables 5, 6, and 9), indicating the QuickScan software accurately reports results in the lower range of the dilution protocol.
The selectivity study to determine the method's relative reactivity to DON analogs showed a strong response to both DON and the 3-acetyl-form. The most recent evaluation of DON and its derivatives by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) issued a group tolerable daily intake for DON and its acetylated forms and in so doing, assessed their toxicities to be equal, as the acetylated forms are hydrolyzed to DON in mammals *(4)*. A more recent study has shown that their toxicities vary according to the model being used *(5)*. Nevertheless, regulations for DON remain set only for DON itself due to challenges in assessing the acetylated forms separately. In regards to the occurrence of 3-acetyl-DON, the same JECFA noted that the acetylated derivative, if it occurs at all, is generally present at levels less than 10% of DON. Thus the cross reactivity noted in the test kit does not detract from its ability to appropriately identify DON contamination in wheat.
The product consistency was demonstrated with multiple lots tested during and beyond the claimed shelf-life of 12 months. Data presented in the Product Consistency and Stability Study remain within the acceptable ranges for the 2.1 ppm DON level for as long as 15 months post-manufacture, supporting the 12 month product stability claim.

DISCUSSION OF THE VALIDATION STUDY CONTINUED (1)

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 test portion size, extraction shake time, and strip development time, the majority of results fell within the acceptable ranges for the 2.3 ppm DON level using both scanners, whereas 15% of the results were out of range low in combinations containing the low test size. The generalized linear model analysis (Figure 6) indicates that only test portion size had a significant impact on the reported results. These data show that the assay is robust to small user discrepancies in extraction shake time and test strip development time, however, the sample should be sufficiently weighed to ensure accurate test results are reported.

As a whole, the studies herein which employed multiple users across multiple sites, multiple kit lots, and multiple instruments, indicate the assay procedure is robust, the matrix-specific calibration curves are accurate, and the manufacture of both assay and instruments is highly consistent. The QuickTox™ Kit for QuickScan DON Flex method provides end users with a test kit that is not only easy to use, but produces rapid, accurate quantitative results allowing grain handlers to make decisions at the point of need with high confidence.

Table 5. Matrix study results for wheat (1)

	DON concentration - Wheat												
	0		**0.5 ppm**		**0.9 ppm**		**2.3 ppm**		**5.0 ppm***		**28.6 ppm**		
Replicate	*500+*	*550+*	*500+*	*550+*	*500+*	*550+*	*500+*	*550+*	*500+*	*550+*	*500+*	*550+*	*Scanner*
1	0.00	0.01	0.53	0.46	0.79	1.0	2.0	2.1	5.8	6.1	29	26	
2	0.00	0.00	0.52	0.63	0.86	0.83	2.2	2.3	5.0	4.5	27	28	
3	0.00	0.00	0.55	0.53	0.77	0.94	2.2	2.2	5.4	4.7	27	29	
4	0.02	0.00	0.60	0.63	0.87	0.86	2.2	2.4	5.2	4.6	28	29	
5	0.00	0.00	0.59	0.61	0.80	0.83	1.8	1.9	5.1	5.3	30	27	
6	0.00	0.00							4.3*	4.5*			
7	0.00	0.00							4.8*	5.0*			
8	0.00	0.00							4.8*	3.7*			
9	0.00	0.00							5.0*	5.0*			
10	0.01	0.00							4.8*	4.2*			
Mean	0.00	0.00	0.56	0.57	0.82	0.89	2.1	2.2	5.3	5.0	28	28	
S_r	0.01	0.00	0.04	0.07	0.04	0.08	0.17	0.17	0.34	0.65	1.07	1.27	
RSD_r %			6.39	13.11	5.43	8.44	8.07	7.67	6.32	12.81	3.79	4.54	
Recovery %			112%	114%	91%	99%	91%	94%	106%	101%	99%	98%	
Bias			0.06	0.07	-0.08	-0.01	-0.21	-0.14	0.32	0.04	-0.30	-0.61	
LOD	0.02	0.01											
LOQ	0.05	0.02											

*Results from dilution protocol, not included in mean analysis or additional analyses

Table 6. Matrix study results for corn (1)

	DON concentration - Corn											
	0		0.5 ppm		0.9 ppm		1.9 ppm		5.3 ppm		30.4 ppm	
Replicate	*500+*	*550+*	*500+*	*550+*	*500+*	*550+*	*500+*	*550+*	*500+*	*550+*	*500+*	*550+*
1	0.00	0.00	0.43	0.49	0.80	0.9	1.8	1.9	5.0	4.5	33	30
2	0.00	0.00	0.46	0.41	0.94	0.93	1.9	1.8	5.1	4.6	31	34
3	0.00	0.00	0.44	0.54	0.86	0.84	1.6	1.7	5.1	4.9	30	32
4	0.00	0.00	0.41	0.38	0.93	0.94	2.0	2.1	5.1	5.0	35	35
5	0.00	0.00	0.45	0.39	0.86	0.95	1.8	1.8	5.2	5.1	35	36
6	0.00	0.00							4.6*	5.2*		
7	0.00	0.00							4.4*	4.2*		
8	0.00	0.00							5.0*	5.0*		
9	0.00	0.00							4.7*	5.0*		
10	0.00	0.00							4.8*	4.4*		
Mean	0.00	0.00	0.44	0.44	0.88	0.92	1.8	1.9	5.1	4.8	33	33
S_r	0.00	0.00	0.02	0.07	0.06	0.04	0.15	0.14	0.08	0.27	2.24	2.35
RSD_r %			4.39	15.79	6.56	4.80	8.28	7.34	1.64	5.57	6.83	7.12
Recovery %			88%	88%	98%	102%	96%	97%	96%	91%	108%	109%
Bias			-0.06	-0.06	-0.02	0.02	-0.08	-0.05	-0.22	-0.48	2.42	2.64
LOD	0.00	0.00										
LOQ	0.00	0.00										

*Results from dilution protocol, not included in mean analysis or additional analyses

DISCUSSION OF THE AUGUST 2018 MODIFICATION (7)

The Linearity study was performed using non-detect wheat and wheat naturally contaminated at 0, 0.5, 0.9, 2.3, 5.0, and 28.6 ppm levels of DON. Replicate test strips were run by a single operator and read on the 550+ scanner and two QSSII instruments. Results from each scanner type were graphed against the reference value. All test results were within the acceptable range of the reference samples (Table 1). Linear regression analysis produced R^2 values of 1.0 for both scanner types indicating equivalent alignment of scanners with the reference values.

Matrix studies were conducted according to the Validation Outline for the QuickTox Kit for QuickScan Don Flex and test strips read by one operator on the 550+ scanner and two QSSII instruments. Individual test strip results, as well as precision, recovery, bias, LOD and LOQ values for wheat are shown in Table 2. The mean values for each level were similar across all readers with the highest RSD_r of 9.4% being exhibited on one of the QSSII units evaluated. LOD and LOQ values were equivalent between the two QSSII units, whereas the 550+ reader resulted in slightly higher LOD and LOQ values but were well below the acceptance criteria (≤0.1ppm) established in the Validation Outline.

Results from the corn matrix study on the two reader types are shown in Table 3. All results were within acceptable ranges for each level indicated in Table 1. The LOD and LOQ values were determined to be 0.00 for all readers, equivalent to those values obtained in the original PTM validation corn matrix study using the 550+ scanner. The mean result for each level was comparable among the instruments tested and the RSD_r remained below 10% for all levels on all instruments.

Table 2. Matrix Study Results for Wheat Samples using QuickScan 550+ and QSSII Readers. (7)

DON concentration - Wheat																		
	0			0.5 ppm			0.9 ppm			2.3 ppm			5.0 ppm			28.6 ppm			
Replicate	*550+*	*QSSII-1*	*QSSII-2*	*550+*	*QSSII-1*	*QSSII-2*	*550+*	*QSSII-1*	*QSSII-2*	*550+*	*QSSII-1*	*QSSII-2*	*550+*	*QSSII-1*	*QSSII-2*	*550+*	*QSSII-1*	*QSSII-2*	*Reader*
1	0.00	0.00	0.00	0.52	0.50	0.57	0.78	0.78	0.8	1.7	1.8	1.7	4.1	4.1	4.0	28	31	29	
2	0.00	0.00	0.00	0.54	0.57	0.57	0.78	0.79	0.81	1.7	1.7	1.7	4.6	4.8	4.5	28	27	26	
3	0.00	0.00	0.00	0.51	0.53	0.58	0.82	0.85	0.85	2.1	2.0	2.1	4.2	4.3	4.4	26	28	28	
4	0.02	0.00	0.00	0.51	0.53	0.52	0.73	0.76	0.73	1.9	2.0	1.9	4.3	4.6	4.2	26	28	29	
5	0.00	0.00	0.00	0.51	0.52	0.58	0.88	0.82	0.79	1.9	1.8	1.9	4.0	4.2	5.1	27	25	27	
6	0.00	0.00	0.00										5.2*	5.3*	5.0*				
7	0.00	0.00	0.00										5.1*	5.4*	5.5*				
8	0.00	0.00	0.00										5.2*	5.6*	5.2*				
9	0.00	0.00	0.00										5.2*	5.1*	5.2*				
10	0.00	0.00	0.00										5.2*	5.2*	5.4*				
Mean	0.00	0.00	0.00	0.52	0.53	0.56	0.80	0.80	0.79	1.9	1.9	1.9	4.2	4.4	4.4	27	28	28	
S_r	0.01	0.00	0.00	0.01	0.03	0.03	0.06	0.04	0.04	0.17	0.13	0.17	0.23	0.29	0.42	1.00	2.17	1.30	
RSD_r %				2.52	4.81	4.45	7.00	4.42	5.46	9.00	7.21	9.00	5.43	6.63	9.37	3.70	7.80	4.69	
Recovery %				104%	106%	113%	89%	89%	88%	81%	81%	81%	85%	88%	89%	94%	97%	97%	
Bias				0.02	0.03	0.06	-0.10	-0.10	-0.11	-0.44	-0.44	-0.44	-0.76	-0.60	-0.56	-1.60	-0.80	-0.80	
LOD	0.01	0.00	0.00																
LOQ	0.04	0.00	0.00																

*Results from dilution protocol, not included in mean analysis or additional analyses

Table 3. Matrix Study Results for Corn Samples using QuickScan 550+ and QSSII Readers (7)

	DON concentration - Corn																	
	0			0.5 ppm			0.9 ppm			1.9 ppm			5.3 ppm			30.4 ppm		
Replicate	*550+*	*QSSII-1*	*QSSII-2*	*550+*	*QSSII-1*	*QSSII-2*	*550+*	*QSSII-1*	*QSSII-2*	*550+*	*QSSII-1*	*QSSII-2*	*550+*	*QSSII-1*	*QSSII-2*	*550+*	*QSSII-1*	*QSSII-2*	*Reader*
1	0.00	0.00	0.00	0.47	0.48	0.46	0.92	0.92	0.9	1.8	1.8	1.8	4.3	4.2	4.8	28	31	28	
2	0.00	0.00	0.00	0.42	0.47	0.42	0.83	0.83	0.88	1.6	1.6	1.6	4.5	5.0	4.4	26	26	27	
3	0.00	0.00	0.00	0.46	0.48	0.46	0.86	0.84	0.89	1.5	1.6	1.4	4.5	4.2	4.4	26	26	30	
4	0.00	0.00	0.00	0.43	0.40	0.47	0.81	0.89	0.83	1.7	1.6	1.7	4.4	4.7	4.5	26	25	24	
5	0.00	0.00	0.00	0.45	0.44	0.51	0.83	0.86	0.83	1.6	1.7	1.5	4.7	4.1	4.4	27	30	27	
6	0.00	0.00	0.00										4.9*	5.4*	5.1*				
7	0.00	0.00	0.00										4.9*	5.0*	5.1*				
8	0.00	0.00	0.00										4.9*	5.3*	5.0*				
9	0.00	0.00	0.00										5.3*	5.3*	5.6*				
10	0.00	0.00	0.00										5.0*	4.9*	5.0*				
Mean	0.00	0.00	0.00	0.45	0.45	0.46	0.85	0.87	0.86	1.6	1.7	1.6	4.5	4.4	4.5	27	28	27	
S_r	0.00	0.00	0.00	0.02	0.03	0.03	0.04	0.04	0.03	0.11	0.09	0.16	0.15	0.39	0.17	0.89	2.70	2.17	
RSD_r %				4.65	7.57	6.92	5.06	4.26	3.62	6.95	5.39	9.88	3.31	8.81	3.85	3.36	9.79	7.97	
Recovery %				89%	91%	93%	94%	96%	96%	86%	87%	84%	85%	84%	85%	88%	91%	89%	
Bias				-0.05	-0.05	-0.04	-0.05	-0.03	-0.04	-0.26	-0.24	-0.30	-0.82	-0.86	-0.80	-3.80	-2.80	-3.20	
LOD	0.00	0.00	0.00																
LOQ	0.00	0.00	0.00																

*Results from dilution protocol, not included in mean analysis or additional analyses

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