

Development and Validation of a Quantitative Lateral Flow Device for the Detection and Quantification of Fumonisin in Corn

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ABSTRACT

Neogen's Reveal Q+ for Fumonisin is a rapid lateral flow device used to quantitate levels of fumonisin in corn. This rapid (6 minute) and accurate device utilizes a simple extraction process and detects fumonisin from 0.3 to 6.0 ppm. Accurate fumonisin measurements up to 12 ppm can be obtained by performing a supplemental analysis involving a diluted extract.

Purpose: The purpose of this study was to develop and validate the Reveal Q+ for Fumonisin device and demonstrate the accuracy and robustness in testing contaminated corn samples.

Methods: Ten gram (10g) naturally incurred samples were extracted using a denatured ethanol solution (10g in 50 mL of 65% Ethanol/ 35% water). After a 3 minute shake, the extracted samples were filtered through a glass fiber filter and then diluted with the provided diluent in a sample cup. The device was introduced into the sample and allowed to run for 6 minutes. The device was then inserted into the AccuScan III reader and the results from the device were determined from a lot specific standard curve that had been previously entered into the reader.

Results: The Reveal Q+ for Fumonisin device detected fumonisin in naturally contaminated samples from 0.3 to 6.0 ppm. The device was demonstrated to be highly accurate with a low level of variability.

Significance: These data demonstrate that Neogen's Reveal Q+ for Fumonisin device is a highly accurate, reliable and rapid assay for the determination of fumonisin levels in corn.

INTRODUCTION

The Reveal Q+ for Fumonisin kit (Neogen item 8885) is intended for the quantitative analysis of fumonisin in corn. Quantitation ranges from 0.3 to 6 parts per million (ppm) and can be extended by dilution of positive extracts. This poster details the finding of the experimental evaluations to establish product claims for the Reveal Q+ for Fumonisin test kit.

LIMIT OF DETECTION

The limit of detection is 0.2 ppm. The limit of detection is calculated by the mean of 48 NDA samples plus 2 standard deviations. Data presented in Table 1.

TABLE 1. Results of Limit of Detection (LOD)

Sample	Result	Sample	Result	Sample	Result	Sample	Result
1	0.1	13	0.0	25	0.1	37	0.1
2	0.1	14	0.1	26	0.0	38	0.1
3	0.1	15	0.1	27	0.0	39	0.1
4	0.1	16	0.1	28	0.1	40	0.1
5	0.1	17	0.1	29	0.1	41	0.1
6	0.2	18	0.0	30	0.1	42	0.1
7	0.1	19	0.1	31	0.1	43	0.1
8	0.1	20	0.1	32	0.0	44	0.1
9	0.2	21	0.1	33	0.0	45	0.1
10	0.1	22	0.1	34	0.1	46	0.1
11	0.1	23	0.1	35	0.1	47	0.0
12	0.1	24	0.1	36	0.0	48	0.1

Mean: 0.09

Standard deviation: 0.037

LOD: 0.163

CROSS-REACTIVITY

TABLE 2.

Type	Cross-reactivity
Fumonisin B ₁	100%
Fumonisin B ₂	80%
Fumonisin B ₃	80%

Reveal Q+ for Fumonisin Simple and Fast Detection of Fumonisin

- EXTRACT:** 1:5 in 65% ethanol; Shake 3 minutes.
- DILUTE:** 200 µL diluent and 100 µL extract in the dilution cup.
- TRANSFER:** 100 µL diluted extract into the sample cup.
- TEST:** Insert test device and set timer for 6 minutes.
- INTERPRET:** Read device using the AccuScan® III reader.



TEST METHODOLOGY ROBUSTNESS

Corn samples naturally contaminated at 0.6, 2.0 and 5.5 ppm of fumonisin were tested using multiple lots of product, operators, readers and sample preparations across different days. The technicians had limited Reveal Q+ test experience before performing two extractions on each sample, testing three Reveal Q+ for Fumonisin strips on each extraction and reading each strip on three different Reveal AccuScan III readers. The process was repeated on day 2 for a total 972 data points. Results are summarized in Table 3.

TABLE 3.

		Operator 1		Operator 2		Operator 3		AVG	%CV
		Day 1	Day 2	Day 1	Day 2	Day 1	Day 2		
0.6 ppm	AVG	0.7	0.6	0.7	0.7	0.7	0.7	0.7	13.1
	% CV	8.0	15.3	12.7	13.7	8.7	20.0		
	AVG	2.4	2.2	2.6	2.3	2.3	2.2		
2.0 ppm	% CV	9.7	8.3	9.7	13.0	8.0	11.7	2.3	10.1
	AVG	6.1	5.5	5.9	6.0	5.7	5.9		
5.5 ppm	% CV	7.0	5.3	7.0	6.7	8.0	7.3	5.9	6.9
	AVG	6.1	5.5	5.9	6.0	5.7	5.9		



BETA SITE RESULTS

Reveal Q+ for Fumonisin was tested by 8 different industry professionals in corn. Blind samples were submitted for analysis, and independent testing was performed at each site. The mean recovery was 93% and correlates well with reference material. Results presented in Table 4.

TABLE 4.

	Sample expected value (ppm)	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Average Value (ppm)	Mean Recovery	Standard Deviation
Replicate 1	0.6	*	0.6	0.5	0.6	0.6	0.4	0.5	0.6	0.5	91%	0.10
		0.6	0.6	0.5	0.5	0.4	0.5	0.7				
		0.6	0.7	0.5	0.5	0.5	0.4	0.5				
	2	2.1	2.0	1.6	1.5	2.1	1.7	1.8	2.5	1.8	89%	0.39
		1.9	1.9	1.7	1.5	1.8	1.7	1.7	2.5			
		1.1	2.2	1.6	1.5	1.6	1.8	1.6	2.2			
4.4	4.7	4.7	3.2	3.5	5.0	3.2	3.8	4.5	4.2	94%	0.58	
	4.5	4.5	4.0	3.7	4.3	3.3	3.9	5.0				
	4.5	4.5	4.2	3.5	4.8	3.7	3.8	4.9				
Replicate 2	0.6	0.6	0.6	0.5	0.6	0.6	0.5	0.5	0.6	0.6	94%	0.06
		0.6	0.6	0.5	0.5	0.6	0.5	0.6	0.6			
		0.6	0.7	0.5	0.5	0.5	0.5	0.6	0.7			
	2	1.9	2.0	1.9	1.6	2.0	1.4	1.6	2.5	1.9	95%	0.28
		2.1	2.1	1.8	1.7	2.0	1.6	1.8	2.5			
		2.0	2.0	1.7	1.8	2.1	1.7	1.6	2.3			
4.4	*	4.7	3.7	3.7	4.5	2.9	3.6	5.1	4.2	94%	0.68	
	4.2	5.5	3.6	3.8	4.8	3.5	3.6	4.5				
	4.3	4.9	3.8	4.5	5.1	3.2	3.6	4.5				

*Device not inserted properly in the AccuScan III reader resulting in an invalid read.

GIPSA TESTING

The Reveal Q+ for Fumonisin validation study was performed in accordance with the USDA's Grain Inspection, Packers and Stockyards Administration (GIPSA) Design Criteria and Test Performance Specification for Quantitative Fumonisin Test Kits (dated Sept. 7, 2010). Commodity validation data was submitted for corn. The Reveal Q+ for Fumonisin validation study was submitted to GIPSA in June 2012 and is awaiting the administration's laboratory evaluation. Submission data is summarized in Table 5.

TABLE 5.

	Accuracy on corn samples naturally contaminated with fumonisin			
Reference material	0.6 ppm	1.1 ppm	2.0 ppm	5.3 ppm
Overall mean	0.61 ppm	1.04 ppm	2.2 ppm	5.25 ppm
Overall st. dev.	0.09 ppm	0.187 ppm	0.49	0.49 ppm
N=	63	63	63	63

CONCLUSIONS

The results of the Reveal Q+ for Fumonisin robustness study demonstrate that the assay is robust and provides accurate and reliable values even when tested between multiple operators, multiple readers and multiple lots of devices.

Beta site testing also support the accuracy, robustness and ease of use of this device.

This data demonstrates that Neogen's Reveal Q+ for Fumonisin device is a highly accurate, reliable and rapid assay for the determination of fumonisin levels in corn.

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