

Development and Validation of a Quantitative Lateral Flow Device for the Detection and Quantification of Deoxynivalenol in Grains

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ABSTRACT

Neogen's **Reveal Q+ for DON** is a rapid lateral flow device used to quantitate levels of deoxynivalenol (DON) in grains. This rapid (3 minute) and accurate device utilizes a simple extraction process and detects DON from 0.3 to 6.0 ppm. Accurate DON measurements from 0.6 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 DON device and demonstrate the accuracy and robustness in testing a range of commodities.

Methods: Ten gram (10 g) naturally incurred samples were extracted using 100 mL of deionized water. After a 3 minute hand shake, the extracted samples were filtered through a glass fiber filter and then diluted with the provided diluents in a sample cup. The device was introduced into the sample and allowed to run for 3 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 DON device detected DON in naturally contaminated samples from 0.3 to 6 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 DON device is a highly accurate, reliable and rapid assay for the determination of DON levels in various commodities.

INTRODUCTION

The Reveal Q+ for DON test kit is intended for the quantitative analysis of commodities including corn, DDGS, wheat, oats, malted barley and barley for DON.

The total assay run time is 6 minutes which includes a simple 3 minute extraction and a 3 minute device run time.

The device is quantitative between 0.3 to 6 parts per million (ppm) and can be extended by dilution of positive extracts.

This poster will report assay robustness, validation and beta site test results.

LIMIT OF DETECTION

The limit of detection (LOD) was found to be 0.3 ppm. LOD was calculated by analyzing non-detect wheat samples.

TABLE 1. Results of Limit of Detection (LOD)

Sample	Result	Sample	Result	Sample	Result	Sample	Result
1	0.2	13	0.1	25	0.2	37	0.2
2	0.2	14	0.1	26	0.2	38	0.2
3	0.2	15	0.1	27	0.2	39	0.2
4	0.1	16	0.1	28	0.2	40	0.2
5	0.2	17	0.2	29	0.2	41	0.2
6	0.1	18	0.1	30	0.2	42	0.2
7	0.2	19	0.1	31	0.1	43	0.2
8	0.1	20	0.1	32	0.2	44	0.3
9	0.2	21	0.1	33	0.2	45	0.3
10	0.1	22	0.2	34	0.2	46	0.2
11	0.1	23	0.1	35	0.1	47	0.3
12	0.1	24	0.3	36	0.2	48	0.2

Mean: 0.17
Standard deviation: 0.061
LOD: 0.3 ppm

Reveal Q+ for DON Simple and Fast Detection of DON

- EXTRACT:** 1:10 in water; Shake 3 minutes.
- DILUTE:** 1000 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 3 minutes.
- INTERPRET:** Read device using the AccuScan III reader.



TEST METHODOLOGY ROBUSTNESS

The assay was tested for robustness in a multi-operator, multi-day, multi-reader, multi-device randomized experiment. Three operators (previously unfamiliar with the assay) evaluated 3 different wheat samples (0.6 ppm, 2.0 ppm and 5.8 ppm). Independent extractions were made of each sample and tested on 3 different device lots. Data are presented in Table 2.

TABLE 2.

		Operator 1		Operator 2		Operator 3		AVG	%CV
		Day 1	Day 2	Day 1	Day 2	Day 1	Day 2		
0.6 ppm	AVG	0.6	0.6	0.7	0.7	0.6	0.6	0.6	18.4
	% CV	19.6	17.0	21.5	14.3	16.3	14.5		
	2.0 ppm	AVG	1.9	1.8	2.1	2.1	2.1		
% CV	9.5	7.7	9.2	8.8	11.9	8.0			
5.8 ppm	AVG	5.8	5.5	6.0	6.2	6.2	6.1	6.0	9.3
% CV	10.4	8.1	7.2	8.1	8.6	7.4			



BETA SITE RESULTS

Reveal Q+ for DON was tested by 9 different industry professionals in corn. Blind samples were submitted for analysis, and independent testing was performed at each site. The mean recovery was 92% and correlates well with reference material. Results presented in Table 3.

TABLE 3.

Sample expected value (ppm)	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Average value (ppm)	Mean recovery	Standard deviation
	0.2	0.1	0.1	0.2	*	0.2	0.1	0.1	0.2			
	0.2	0.1	*	0.1	0.1	0.2	0.2	0.1	0.1			
NDA	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.13	n/a	0.06
	0.3	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.1			
	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2			
0.9	1.3	0.8	0.7	0.8	0.7	0.8	0.9	0.8	0.8	0.84	94%	0.16
	1.3	0.7	0.7	0.8	1.0	0.9	0.8	0.8	0.7			
	1.1	0.7	0.7	0.9	0.9	0.8	0.9	0.7	0.8			
0.9	0.7	0.6	0.8	0.7	0.8	0.8	1.0	0.7	0.8	0.76	84%	0.16
	0.8	0.7	0.7	0.7	0.6	0.7	0.9	0.6	0.9			
	0.7	0.7	0.6	0.7	0.7	0.8	0.8	1.0	0.9			
4.9	4.0	4.8	4.6	3.9	3.9	5.4	5.7	4.4	5.1	4.66	95%	0.62
	4.5	4.4	5.3	3.5	4.0	5.8	4.9	4.1	4.8			
	5.1	4.4	5.3	4.0	4.1	5.5	5.1	4.4	4.9			
4.9	4.8	4.6	4.5	4.0	4.6	5.3	5.0	5.0	5.8	4.73	97%	0.55
	4.6	5.1	5.2	4.0	3.9	4.7	4.8	4.3	5.5			
	4.4	4.6	5.4	4.1	3.7	4.8	5.5	4.3	5.3			

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

GIPSA TESTING

The Reveal Q+ for DON validation study was performed in accordance with the USDA-Grain Inspection, Packers and Stockyards Administration (GIPSA) Design Criteria and Test Performance Specifications for Quantitative DON test Kits. Commodity validation was submitted for corn, wheat, barley, oats, dried distillers grains solubles (DDGS), corn gluten meal, rice and malted barley. Data are presented in Table 4.

TABLE 4.

Accuracy on corn samples naturally-contaminated with DON				
Reference material	0.5 ppm	1.1 ppm	1.9 ppm	4.8 ppm
Overall mean	0.53 ppm	0.98 ppm	1.92 ppm	4.72 ppm
Overall st. dev.	0.06	0.11	0.14	0.21
N=	63	63	63	63
Accuracy on wheat samples naturally-contaminated with DON				
Reference material	0.5 ppm	1.0 ppm	2.0 ppm	4.9 ppm
Overall mean	0.60 ppm	1.0 ppm	2.15 ppm	5.18 ppm
Overall st. dev.	0.07	0.10	0.17	0.28
N=	63	63	63	63

CONCLUSIONS

The results of the Reveal Q+ for DON 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.

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

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