

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

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ABSTRACT

Neogen's **Reveal Q+ for Aflatoxin** is a rapid lateral flow device used to quantitate levels of aflatoxin in grains. This rapid (6 minute) and accurate device utilizes a simple extraction process and detects aflatoxin from 2 to 150 ppb. Accurate aflatoxin measurements from 151 to 400 ppb can be obtained by performing a supplemental analysis involving a diluted extract.

Purpose: The purpose of this study was to develop and validate the Q+ device and demonstrate the accuracy and robustness in testing corn-based commodities.

Methods: Fifty gram (50 g) naturally-incurred corn samples were extracted using a denatured ethanol solution (65% ethanol/35% water). After a 3 minute hand 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 Neogen's **Reveal 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 Q+ device detected aflatoxin in naturally contaminated corn samples from 2 to 100 ppb. The device was demonstrated to be highly accurate with a low level of variability.

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

INTRODUCTION

The **Reveal Q+ for Aflatoxin** test kit is intended for the quantitative analysis of commodities including corn, corn products, rice, peanuts and sorghum for aflatoxin.

The assay run time is 9 minutes, which includes a simple 3 minute extraction and a 6 minute device run time.

The device is quantitative between 2–150 parts per billion (ppb) and can be extended by dilution of positive extracts.

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

CROSS-REACTIVITY

- Aflatoxin B₁ = 100%
- Aflatoxin G₁ = 74.9%
- Aflatoxin B₂ = 69.7%
- Aflatoxin G₂ = 20.4%

LIMIT OF DETECTION

The limit of detection (LOD) was found to be 2 ppb. LOD was calculated by analyzing 48 non-detect corn samples.

TABLE 1. Results of Limit of Detection (LOD)

Sample	Result	Sample	Result	Sample	Result	Sample	Result
1	0.5	13	0.8	25	0.3	37	0.1
2	1.5	14	0.2	26	0.3	38	0.1
3	0.4	15	3.4	27	0.9	39	0.9
4	1.4	16	1.4	28	1.5	40	0.0
5	1.4	17	0.9	29	0.6	41	1.5
6	0.3	18	0.6	30	0.1	42	0.9
7	1	19	1.0	31	1.3	43	0.8
8	1.1	20	1.7	32	1.4	44	0.4
9	0.6	21	0.6	33	0.1	45	1.0
10	1.1	22	0.2	34	1.0	46	0.7
11	1.1	23	1.2	35	0.5	47	1.0
12	0.4	24	0.5	36	0.6	48	1.4

Mean: 0.828

Standard deviation: 0.610

LOD: 2.0 ppb

Reveal Q+ Simple and Fast Aflatoxin Detection for Aflatoxin

- EXTRACT:** 1:5 in 65% ethanol; Shake 3 minutes.
- DILUTE:** 500 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

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 2 different corn samples (4.5 ppb and 24.7 ppb). Independent extractions were made of each sample and tested on 3 different device lots. Data is presented in Table 2.

TABLE 2.

		Operator 1				Operator 2				Operator 3				AVG	%CV
		Day 1 Extract 1	Day 1 Extract 2	Day 2 Extract 1	Day 2 Extract 2	Day 1 Extract 1	Day 1 Extract 2	Day 2 Extract 1	Day 2 Extract 2	Day 1 Extract 1	Day 1 Extract 2	Day 2 Extract 1	Day 2 Extract 2		
4.5 ppb	AVG	3.6	3.8	3.6	3.9	3.8	3.3	3.7	3.8	3.6	4	3.4	3.4	3.7	17.7
	%CV	14.4	13.4	18	21	1.6	26.5	16.7	16.9	13.7	15.9	16.2	16		
24.7 ppb	AVG	23.7	22.4	25.8	22.9	24.1	22.5	25.8	22.3	24.7	22.3	23.9	28.9	24.1	16.7
	%CV	14.1	13.5	15.7	20	12.3	11.7	13.2	17.5	17.3	15.7	12.3	14.3		

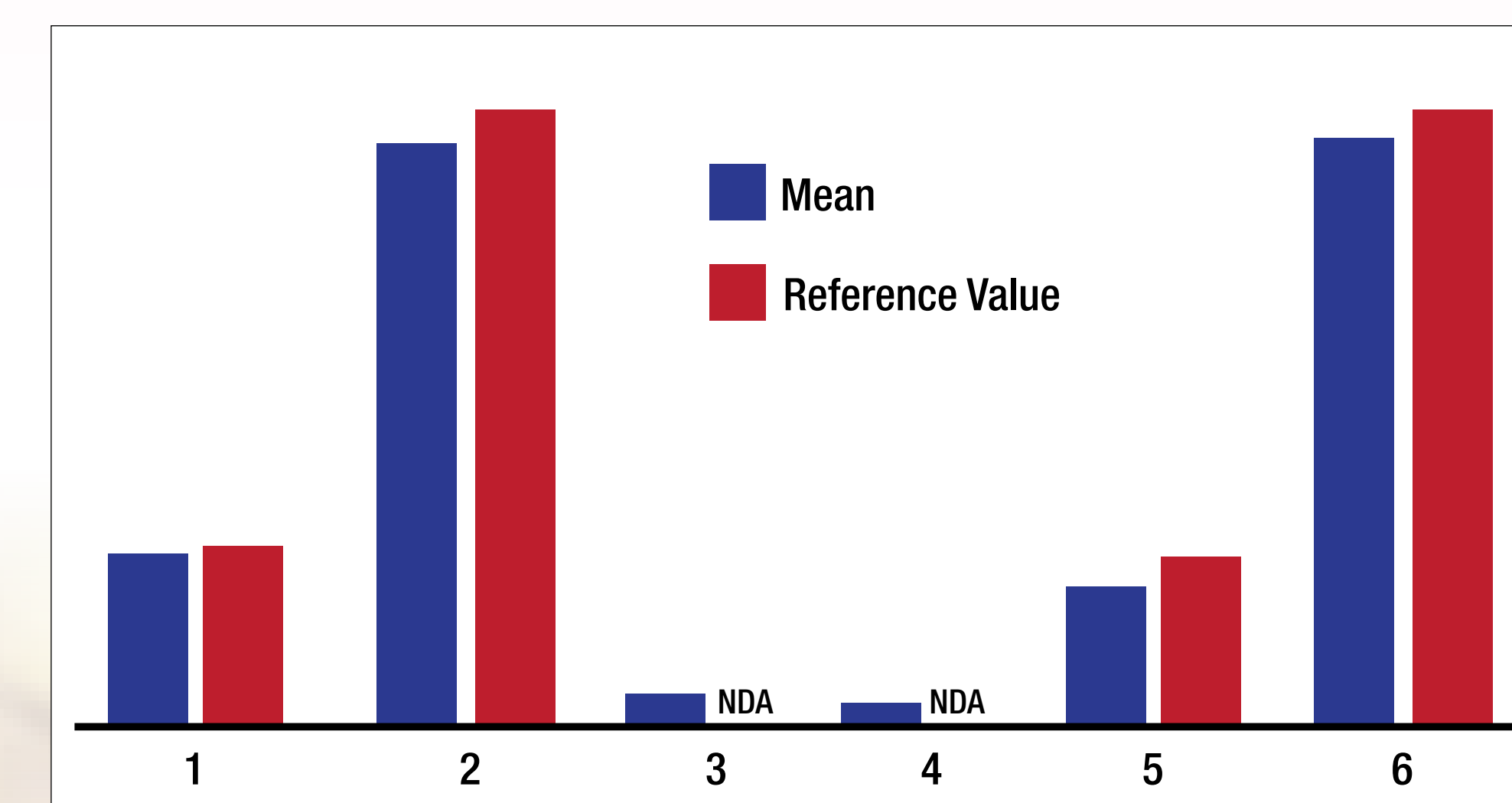
BETA SITE RESULTS

Reveal Q+ for Aflatoxin was tested by 11 different industry professionals in corn. The mean recovery was 92% and correlates well with reference material.

TABLE 3.

Site	Sample					
	4.5 ppb	24.7 ppb	Non detect	4.5 ppb	24.7 ppb	Non detect
1	3.9	19.8	3.3	3.9	20	2.5
	5.1	18.7	2.4	5.8	17.4	2.6
	4.5	20.3	2.1	5	20.4	2.2
2	6.3	7.7	0.4	5.1	28.1	0.9
	8.1	24.3	1.2	4	30.8	0.8
	5.8	24.6	1.1	3.6	31.8	1.2
3	5.2	28.3	0.5	4.1	21.3	0.1
	3.8	29.4	1.1	6.1	25.5	0.1
	4.5	23.3	0.4	5.8	34.8	0.5
4	3.3	22.9	0.9	4	25	3
	5.3	28.5	1.1	3.5	21.8	1
	3.9	21	1.5	4.6	21.1	2.1
5	6.1	25.2	na*	4.3	20.7	1.2
	3.8	28	2.2	4.2	28.8	1.4
	3.8	30.2	2	4	23.7	1.1
6	3.5	19.8	1.9	3.9	22.9	1.1
	4.1	26.8	1.7	3.8	25	0.6
	23.8*	26.7	1.5	3.4	21	1.4
7	4.7	29	0.5	3.2	30.2	0.4
	3.3	22.7	1.7	2.9	26	1.3
	3.8	26.4	0.9	3.2	28.7	0.6
8	3.9	20.5	1.1	2.4	22.4	0.6
	3.1	23.6	1	3.7	19.8	1.3
	2.6	24.3	0.6	3.3	24.5	1.6
9	2.7	23.2	0.5	3.9	25.2	1.6
	2.6	22.8	0.9	2.8	25	0.2
	4.4	25.3	1.1	3.5	28.3	1.1
10	na*	20.5	1.6	3.1	20.5	1.2
	5.3	13.9	2	3.4	24.8	1.5
	5.1	21.9	2.4	2.7	20.9	1.5
11	2.6	20.6	1.1	3	18.7	1.6
	4.5	19.8	1	3.1	17	0.6
	2.5	25.8	2	3	20.3	0.9

FIGURE 1. Reveal Q+ for Aflatoxin Beta Site Evaluation



CONCLUSIONS

- The results of the Reveal Q+ for Aflatoxin 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 supports the accuracy, robustness and ease of use of this device.
- These data demonstrate that Neogen's **Reveal Q+ for Aflatoxin** device is a highly accurate, reliable and rapid assay for the determination of aflatoxin levels in corn.

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