

# Development And Validation of a Quantitative Lateral Flow Device For the Detection and Quantification of Ochratoxin in Grains

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## ABSTRACT

Neogen's Reveal<sup>®</sup> Q+ for Ochratoxin is a rapid lateral flow device used to quantitate levels of ochratoxin in grains. This rapid (9 minute) and accurate device utilizes a simple 70% methanol extraction process and detects ochratoxin from 2 ppb–20 ppb. Accurate ochratoxin measurements beyond these detection limits 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 Ochratoxin device and demonstrate the accuracy and robustness in testing grain-based commodities.

**Methods:** 10 g naturally-incurred wheat or spiked grain samples were extracted using 70% methanol (10 g into 40 mL of 70% methanol). 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 9 minutes. The device was then inserted into the AccuScan<sup>®</sup> III or AccuScan Pro readers and the results from the device were determined from a lot-specific standard curve that had been previously entered into the readers.

**Results:** The Reveal Q+ for Ochratoxin device detected ochratoxin in naturally-contaminated samples from 2–20 ppb. The device was demonstrated to be highly accurate with a low coefficient of variation across all levels tested.

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

## INTRODUCTION

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

The assay run time is 12 minutes, which includes a simple 3 minute extraction and a 9 minute device run time.

The device is quantitative between 2–20 parts per billion (ppb) and can be extended by dilution of positive extracts.

Here we will report assay robustness, validation and beta site test results.

## LIMIT OF DETECTION

The limit of detection (LOD) was found to be 2.3 ppb. The LOD was calculated by analyzing 58 non-detect wheat samples. Data presented in Table 1.

**TABLE 1. Results of limit of detection (LOD)**

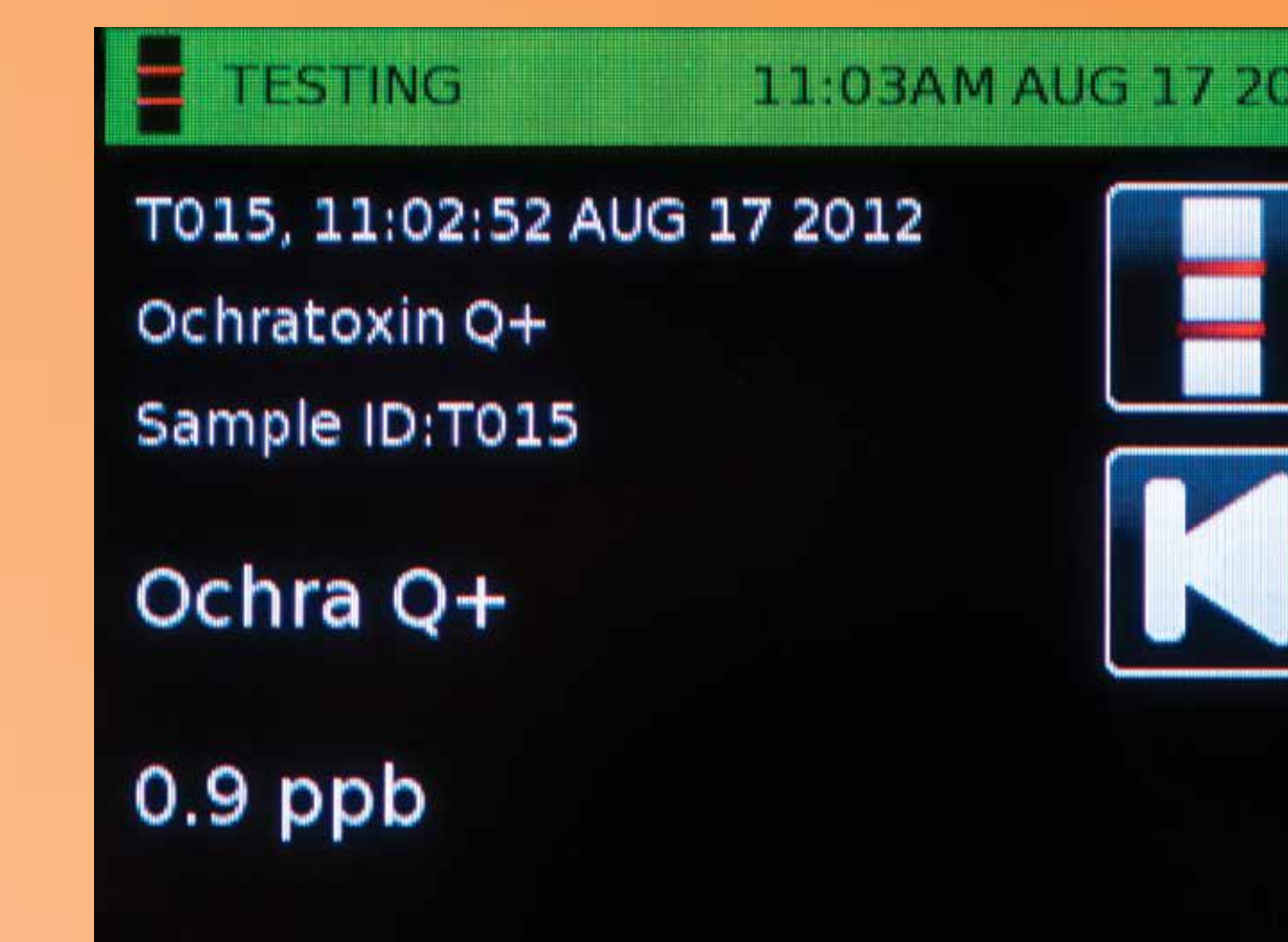
Sample	Result	Sample	Result	Sample	Result	Sample	Result	Sample	Result
1	0.0	11	1.6	21	1.4	31	1.8	41	1.8
2	1.1	12	0.2	22	1.3	32	1.7	42	1.2
3	1.0	13	0.2	23	1.1	33	1.6	43	1.8
4	2.3	14	0.6	24	1.4	34	2.6	44	1.8
5	0.8	15	1.0	25	1.3	35	2.0	45	1.1
6	0.8	16	1.1	26	1.4	36	1.2	46	1.5
7	0.8	17	1.1	27	1.7	37	1.1	47	1.0
8	1.4	18	0.8	28	1.8	38	1.5	48	2.4
9	0.9	19	1.0	29	0.8	39	1.4	49	1.4
10	0.7	20	1.7	30	1.7	40	1.4	50	1.3

Mean: 1.3  
Standard deviation: 0.527  
LOD: 2.3 ppm



## Reveal Q+ for Ochratoxin Simple and Fast Detection of Ochratoxin

- EXTRACT:** 1:4 in 70% methanol, shake 3 minutes.
- DILUTE:** 200 µL diluent and 100 µL extract in dilution cup.
- TRANSFER:** 100 µL diluted extract into sample cup.
- TEST:** Insert test device and set timer for 9 minutes.
- INTERPRET:** Read device using an AccuScan III or AccuScan Pro 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 three different wheat samples (5.2 ppb, 9.9 ppb and 19.7 ppb). Independent extractions were made of each sample and tested on three different device lots. Data 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		
		5.2 ppb	AVG	4.4	4.0	3.7	4.1		
	% CV	13.2	16.3	16.3	22.2	18.5	14.7		
9.9 ppb	AVG	8.3	10.0	9.7	10.0	11.3	11.9	10.2	14.7
	% CV	9.7	9.3	10.9	10.0	8.6	9.1		
19.7 ppb	AVG	16.9	17.0	18.1	17.3	17.7	18.3	17.5	8.4
	% CV	9.0	8.6	7.9	6.8	6.5	8.4		

## BETA SITE RESULTS

Reveal Q+ for Ochratoxin was tested by three different industry professionals in wheat. Blind samples were submitted for analysis, and independent testing was performed at each site. The mean recovery was 99% and correlates well with reference material. Data presented in Table 3.

**TABLE 3**

	Site 1	Site 2	Site 3	AVG (ppb)	Mean recovery (%)	Standard deviation
Sample 1 – NDA	0.0	0.0	*	0.1	NA	0.1
	0.0	0.0	*			
	0.0	0.4	0.1			
Sample 2 – 6.6 ppb corn	8.7	7.0	6.5	7.6	114.7	1.1
	8.5	7.7	*			
	9.1	6.8	6.2			
Sample 3 – NDA	0.0	0.0	*	0.1	NA	0.1
	0.0	0.0	0.3			
	0.0	0.0	0.2			
Sample 4 – 6.6 ppb corn	8.4	5.8	6.1	6.8	103.0	1.1
	7.6	6.1	6.0			
	7.8	6.9	6.5			
Sample 5 – 15.0 ppb wheat	13.4	13.6	12.1	13.4	89.6	0.6
	13.9	13.3	13.7			
	13.3	13.3	14.4			
Sample 6 – 15.0 ppb wheat	14.3	12.0	14.3	13.5	90.1	1.1
	14.6	11.3	13.5			
	14.2	13.3	14.1			

\*Indicates strip not placed in reader properly.

## STABILITY TESTING

The data in Table 4 is provided to demonstrate a minimum of six-month shelf life of all test kit reagents when stored at ambient temperature. Test kits in the beginning, middle, and end of their shelf life were tested using spiked corn samples at various levels.

**TABLE 4. Reveal Q+ OTA**

Day 0		1 month		3 month		6 month	
HPLC ppb	Device ppb	HPLC ppb	Device ppb	HPLC ppb	Device ppb	HPLC ppb	Device ppb
0	0.2	0	0.5	0	0.3	0	0.1
5.2	4.5	5.2	5.8	5.2	4.9	5.2	4.2
19.7	21.8	19.7	19.1	19.7	20.7	19.7	18.7
102	102.5	102	101.4	102	96.4	102	92.4

## CONCLUSIONS

- The results of the Reveal Q+ for Ochratoxin 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 Ochratoxin device is a highly accurate, stable, reliable and rapid assay for the determination of ochratoxin levels in wheat and corn.

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