

# Development And Validation of a Quantitative Lateral Flow Device For the Detection and Quantification of T-2 and HT-2 in Grains

Paul Lewis, Josh Kuipers, Tim Goldy, Collin Walsh, Frank Klein, Jennifer Rice

## ABSTRACT

Neogen's Reveal<sup>®</sup> Q+ for T-2/HT-2 is a rapid lateral flow device used to quantitate levels of T-2/HT-2 in grains. This rapid (6 minute) and accurate device utilizes a simple water extraction process and detects T-2/HT-2 from 50 ppb to 600 ppb. Accurate T-2/HT-2 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 T-2/HT-2 device and demonstrate the accuracy and robustness in testing grain-based commodities.

**Methods:** 10 gram naturally-incurred corn or spiked samples were extracted using distilled water (10 g into 100 mL). After a 3 minute shake, the extracted samples were filtered through a glass fiber filter and 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<sup>®</sup> III or AccuScan Pro readers and results were determined from a lot-specific standard curve that had been previously entered into the readers.

**Results:** The Reveal Q+ for T-2/HT-2 device detected T-2/HT-2 in naturally-contaminated samples from 50 to 600 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 T-2/HT-2 device is a highly accurate, reliable and rapid assay for the determination of T-2/HT-2 levels in grain.

## INTRODUCTION

The Reveal Q+ for T-2 / HT-2 test kit is intended for the quantitative analysis of commodities including corn, DDGS, wheat, oats, malted barley and barley for T-2/HT-2.

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 50–600 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 39 ppb. The LOD was calculated by analyzing 50 non-detect corn 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	32.9	11	27.7	21	20.3	31	33.4	41	24.7
2	33.8	12	30.4	22	16.0	32	25.9	42	18.4
3	27.7	13	23.8	23	28.5	33	21.3	43	27.8
4	39.9	14	31.8	24	31.1	34	19.3	44	40.2
5	29.0	15	37.2	25	26.4	35	23.3	45	25.6
6	30.7	16	31.7	26	18.9	36	20.0	46	29.6
7	32.4	17	19.3	27	17.9	37	23.4	47	25.5
8	26.6	18	32.0	28	16.4	38	23.8	48	29.7
9	23.6	19	33.9	29	14.7	39	13.0	49	28.2
10	23.9	20	24.5	30	20.5	40	27.8	50	24.1

Mean: 26.2  
Standard deviation: 6.3  
LOD: 39 ppb

## Reveal Q+ for T-2/HT-2 Simple and Fast Detection of T-2/HT-2

- EXTRACT:** 1:10 in water, shake 3 minutes.
- DILUTE:** 500 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 6 minutes.
- INTERPRET:** Read device using the AccuScan<sup>®</sup> 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 corn samples (50 ppb, 400 ppb and 600 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		
50 ppb	AVG	52.7	55.4	52.2	59.3	58.6	54.0	55.4	20.5
	% CV	24.2	18.3	17.8	16.1	23.1	19.7		
400 ppb	AVG	413.5	413.4	371.0	414.1	403.3	412.5	404.6	15.2
	% CV	23.6	8.5	24.4	5.3	10.0	5.6		
600 ppb	AVG	561.1	512.1	503.1	543.9	548.1	534.4	533.8	8.7
	% CV	6.3	16.1	4.7	5.2	4.8	4.7		

## BETA SITE RESULTS

Reveal Q+ for T-2/HT-2 was tested by four 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. Data presented in Table 3.

**TABLE 3**

	Site 1	Site 2	Site 3	Site 4	AVG (ppb)	Mean recovery (%)	Standard deviation
Sample 1 – NDA	6.4	19.0	23.0	0.0	7.7	NA	8.1
	4.6	0.0	13.5	6.0			
	0.0	15.0	16.5	0.0			
	3.5	0.0	16.5	0.0			
Sample 2 – 100 ppb corn	79.0	78.1	74.7	109.6	98.2	98.2	19
	95.1	126.0	85.6	124.6			
	85.6	91.8	72.4	105.9			
	102.7	124.4	92.6	123.7			
Sample 3 – NDA	12.3	13.0	0.0	33.3	14.1	NA	14.2
	16.9	0.0	0.0	31.2			
	8.9	12.3	0.0	36.6			
	20.0	0.0	1.7	38.9			
Sample 4 – 100 ppb corn	85.7	84.7	81.5	77.0	84.9	84.9	11.8
	63.1	101.8	85.9	76.8			
	86.6	96.8	87.9	86.5			
	68.5	112.0	81.2	83.2			
Sample 5 – 400 ppb corn	405.7	264.7	405.7	264.7	346.7	86.7	51.3
	347.2	392.8	347.2	392.8			
	390.8	274.0	390.8	274.0			
	339.9	358.2	339.9	358.2			
Sample 6 – 400 ppb corn	384.8	453.3	384.8	453.3	387	96.8	35.1
	366.5	394.9	366.5	394.9			
	359.8	422.2	359.8	422.2			
	345.7	366.5	345.7	366.5			

## ADDITIONAL TESTING

Additional validation testing for Reveal Q+ for T-2/HT-2 was performed using three spiked corn levels with three operators and seven extractions each. All extracts were tested by each operator. Results are shown in Table 4.

**TABLE 4**

Reference Spike Level	50 ppb	250 ppb	500 ppb
Overall mean	54.6 ppb	282.5 ppb	525.1 ppb
Overall standard deviation	11.4	29.2	29.6
%CV	20.8	10.3	5.6
N=	63	63	63

## CONCLUSIONS

- The results of the T-2/HT-2 Q+ 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 T-2/HT-2 device is a highly accurate, reliable and rapid assay for the determination of T-2/HT-2 levels in corn.

## ACKNOWLEDGEMENTS

The authors thank Robert Gallavan for study design and statistical analysis, and Rod Poland for assistance in preparing the poster.

