

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

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ABSTRACT

Neogen's **Reveal[®] Q+ for Zearalenone** is a rapid lateral flow device used to quantitate levels of zearalenone in grains. This rapid (6 minute) and accurate device utilizes a simple extraction process and detects zearalenone from 50 ppb to 1200 ppb. Accurate zearalenone 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 Zearalenone device and demonstrate the accuracy and robustness in testing grain based commodities.

Methods: Ten gram (10 g) naturally incurred corn or spiked wheat samples were extracted using a denatured ethanol solution (10g into 300 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 Zearalenone device detected zearalenone in naturally contaminated samples from 50 to 1200 ppb. The device was demonstrated to be highly accurate with a coefficient of variation of 15% across the levels tested.

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

INTRODUCTION

The Reveal Q+ for Zearalenone test kit is intended for the quantitative analysis of commodities including wheat and corn.

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 25–1200 parts per billion (ppb) zearalenone in wheat and between 50–1200 ppb zearalenone in corn.

Here we will report assay robustness and validation results.

LIMIT OF DETECTION

The limit of detection (LOD) was found to be 25 ppb for wheat and 50 ppb for corn. LOD was calculated by analyzing 48 non-detect corn samples and 48 non-detect wheat samples. See Table 1a corn and Table 1b wheat.

TABLE 1a. Results of Limit of Detection (LOD): Corn

Sample	Result	Sample	Result	Sample	Result	Sample	Result
1	1.6	13	6.7	25	6.7	37	16.4
2	2.1	14	11.0	26	14.4	38	32.2
3	6.5	15	16.6	27	31.0	39	28.0
4	15.9	16	6.6	28	19.6	40	30.2
5	17.3	17	1.5	29	41.0	41	5.9
6	23.5	18	18.3	30	18.9	42	15.6
7	22.6	19	22.3	31	12.2	43	29.4
8	19.0	20	14.8	32	17.3	44	26.3
9	23.8	21	27.8	33	22.3	45	2.1
10	13.3	22	27.5	34	11.7	46	22.4
11	27.3	23	17.6	35	18.5	47	20.9
12	14.2	24	13.9	36	16.6	48	44.3

Mean: 18.2 ppb • Standard Deviation: 9.7 • LOD: 37.5 ppb

TABLE 1b. Results of Limit of Detection (LOD): Wheat

Sample	Result	Sample	Result	Sample	Result	Sample	Result
1	15.2	13	16.6	25	14.5	37	19.2
2	25.0	14	8	26	14.5	38	17.5
3	18.4	15	18.4	27	16.6	39	19.3
4	17.0	16	20.7	28	13.5	40	10.5
5	17.3	17	14.3	29	11.3	41	7.6
6	14.8	18	22.6	30	13.5	42	4.7
7	17.2	19	16.1	31	13.6	43	7.9
8	17.5	20	20.5	32	14.5	44	11.7
9	12.5	21	14.3	33	17.5	45	7.5
10	19.5	22	13.9	34	20.6	46	4.8
11	17.3	23	8.7	35	19.7	47	9.2
12	20.6	24	14.4	36	10.3	48	7.8

Mean: 14.8 ppb • Standard Deviation: 4.7 • LOD: 24.3 ppb

Reveal[®] Q+ for Zearalenone Simple and Fast Zearalenone Detection

- EXTRACT:** Corn - 1:3 in 65% ethanol; Shake 3 minutes.
- FILTER:** Through filter paper or syringe filter.
- DILUTE:** 200 μ L diluent and 100 μ L extract in dilution cup.
- TRANSFER:** 100 μ L 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 3 spiked wheat samples (50 ppb, 250 ppb and 1000 ppb). Independent extractions were made of each sample and tested on 3 different device lots. Data are presented in Tables 2 and 3.

TABLE 2

Operator	50 ppb			250 ppb			1000 ppb		
	1	2	3	1	2	3	1	2	3
Average	43.8	41.5	42.6	225.6	231.7	246	826.1	808.2	1007.1
% CV	17.5	21.7	16.9	11.3	13.4	10.5	11.8	8.2	13.4

TABLE 3

Lot	1			2			3		
	50	250	1000	50	250	1000	50	250	1000
Average	40.2	219.6	861.8	46.5	243.6	910.0	42.8	240.1	869.5
% CV	20.2	13.7	14.3	17.6	10.6	15.7	16	10.2	16.1

These data represent the highest level of variability since results represent data generated and averaged from multiple extractions, multiple reader, multiple day, multiple lot and multiple operators.



GIPSA TESTING

The Reveal Q+ for Zearalenone validation study was performed in accordance with the USDA-Grain Inspection, Packers and Stockyards Administration (GIPSA) Design Criteria and Test Performance Specifications for Quantitative Zearalenone Test Kits. Commodity validation was submitted for naturally incurred corn.

TABLE 4 GIPSA Validation Accuracy on Corn Samples

Reference Material	108 ppb	266.7 ppb	1020.7 ppb	104.3 ppb
Overall mean	114.3	239.1	1075.8	102.2 ppb
Overall st. dev.	15.8	8.1	4.7	18.3
N=	63	63	63	63

CONCLUSIONS

- The results of the Reveal Q+ for Zearalenone 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 (data not shown).
- These data demonstrate that Neogen's Reveal Q+ for Zearalenone device is a highly accurate, reliable and rapid assay for the determination of zearalenone levels in corn and wheat.

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