

# Veratox<sup>®</sup> HS MAX for Total Aflatoxin

## Validation Report for Veratox HS MAX for Total Aflatoxin (Neogen item 8032)

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## Veratox HS MAX for Total Aflatoxin

### SUMMARY

Veratox HS MAX for Total Aflatoxin (Neogen item 8032) is a competitive direct enzyme-linked immunosorbent assay (CD-ELISA) that provides quantitative analysis of aflatoxin. The kit is designed to detect the four principle types of aflatoxin: B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. Quantitation ranges from 1–10 parts per billion (ppb) and can be extended by dilution of positive extracts. The validation report details the findings of the experimental evaluation to establish product claims for Veratox HS MAX for Total Aflatoxin.

**Limit of Detection:** 0.8 parts per billion (ppb)

**Range:** Quantitative range of the kit was demonstrated from 1–10 parts per billion (ppb)

**Specificity:** B<sub>1</sub> = 100 , B<sub>2</sub> = 112 , G<sub>1</sub> = 89, G<sub>2</sub> = 71

**Validated commodities:** Corn

**Robustness:** Veratox HS MAX for Total Aflatoxin demonstrated excellent recovery and reproductibility .

**Time period:** All assays were conducted in 2016. No alterations or updates to the method have taken place since that time.

### MATERIALS AND METHODS

All tests were conducted on standard quality control (QC) approved lots of Veratox HS MAX for Total Aflatoxin test kits. All assays were performed in accordance with the test kit insert.

All high performance liquid chromatography (HPLC) analyses were performed in an ISO:17025 accredited laboratory using AOAC Official Method 2005.08 and immunoaffinity cleanup.

### LIMIT OF DETECTION

The limit of detection was calculated using the method cited in the following:

Shrivastava A, Gupta VB. *Methods for the determination of limit of detection and limit of quantitation of the analytical methods.* Chron Young Sci 2011; 2:21-5.

LoB = mean blank + 1.645 (SD blank)

LoD = LoB + 1.645 (SD low concentration sample)

Replicate	Result
1	0.396
2	0.323
3	0.501
4	0.406
5	0.126
6	0.000
7	0.507
8	0.375
9	0.108
10	0.000
11	0.000
12	0.000
Avg	0.229
SD	0.208
LOB	0.571
LOD	0.768

### SPECIFICITY

Aflatoxin is a toxic and carcinogenic substance produced by certain strains of the molds *Aspergillus flavus* and *A. parasiticus*. There are four principal types of aflatoxin: B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. Aflatoxin B<sub>1</sub> is the most common and most toxic and, therefore, of the greatest concern.

Cross Reactivity Analysis	
Analyte	Cross Reactivity %
AFB <sub>1</sub>	100
AFB <sub>2</sub>	112
AFG <sub>1</sub>	89
AFG <sub>2</sub>	71

### ROBUSTNESS/RUGGEDNESS TESTING

#### Lot to lot variability was tested using the following protocol:

10 gram corn samples were prepared following the kit instructions. Commercially available reference materials at 4 levels were used.

Two extracts were prepared for each MRM on two days.

Three analysts performed testing following the kit instructions.

Two lots of kits were used for the study.

All assays were read on a Statfax 4700 reader.

Level (ppb)	Extract	Day 1						Day 2					
		Analyst 1		Analyst 2		Analyst 3		Analyst 1		Analyst 2		Analyst 3	
		Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
NDA	1	0.3	0.2	0.2	0.0	0.3	0.1	0.3	0.2	0.3	0.4	0.0	0.0
		0.0	0.1	0.2	0.0	0.3	0.1	0.2	0.2	0.2	0.3	0.1	0.1
	2	0.4	0.1	0.1	0.0	0.2	0.0	0.1	0.1	0.0	0.1	0.0	0.0
		0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.1	0.3	0.1	0.1
1.7 (1.4–2.0)	1	1.5	2.1	2.1	1.8	2.2	1.7	2.0	1.9	1.8	1.6	2.0	1.7
		1.9	2.0	1.8	1.7	2.0	1.6	2.2	1.8	2.0	1.9	2.1	1.7
	2	2.1	1.7	2.0	2.0	2.2	1.7	1.4	1.8	1.6	1.9	1.9	1.8
		1.8	1.8	2.1	1.8	1.5	1.8	1.4	1.8	2.2	1.9	2.0	1.8
5.2 (4.4–6.0)	1	5.7	5.1	7.0	5.5	5.6	6.0	6.0	5.4	5.9	5.8	6.1	5.9
		5.7	5.3	5.2	5.4	5.7	6.0	5.4	6.1	6.0	5.8	5.9	5.7
	2	5.5	5.0	6.0	5.9	5.8	4.7	5.1	4.5	5.4	5.5	6.0	6.0
		5.5	5.8	5.7	6.0	5.9	5.2	5.2	4.6	5.4	5.4	6.0	5.7
8.5 (7.4–9.6)	1	7.7	7.5	7.8	7.7	7.8	8.3	7.5	7.7	8.0	8.7	8.2	8.4
		7.1	7.8	8.0	7.4	7.9	6.9	7.6	8.5	8.6	9.1	7.6	8.6
	2	7.8	7.8	7.9	7.5	8.0	7.7	7.8	7.4	8.3	8.6	8.7	9.3
		9.4	8.6	8.5	8.4	8.1	8.0	7.6	6.7	7.8	8.8	8.6	9.6

One kit lot was used to challenge the variation in three test parameters: shake time, reagent volume and incubation time.

**Study Protocol:** one analyst tested four levels of reference material. Two separate extractions from each level of reference material were prepared. Each extraction was tested in duplicate.

The recommended shake time is 30 seconds. Testing was performed using 15, 30, and 45 seconds.

The recommended reagent volume is 100 µL. Testing was performed using 90 µL and 110 µL.

The recommended incubation time is 15 minutes continuous shake on a mechanical shaker at 900 rpm. Testing was performed using 13 and 17 minutes.

Level ppb	Extract	Shake Time			Reagent Volume		Incubation Time	
		15 sec	30 sec	45 sec	90 µL	110 µL	13 min	17 min
0.0	1	0.5	0.0	0.2	0.0	0.0	0.0	0.2
		0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	0.0	0.1	0.0	0.0	0.0	0.0	0.0
		0.3	0.0	0.0	0.3	0.1	0.0	0.0
1.7	1	2.9	2.7	2.5	2.6	2.9	2.7	2.4
		2.7	2.7	3.0	2.6	2.8	2.5	2.5
	2	2.4	2.1	2.0	1.9	2.0	2.2	2.0
		2.8	2.3	2.3	1.9	2.0	2.6	2.3
5.0	1	4.3	5.2	5.6	5.4	5.5	5.4	5.6
		4.9	5.1	4.6	4.9	5.3	5.0	5.1
	2	4.9	4.7	4.8	5.8	5.6	5.2	5.9
		5.2	4.9	5.2	4.8	5.6	5.4	5.7
8.5	1	8.4	8.5	8.2	8.5	7.8	7.9	8.8
		8.6	8.3	7.9	9.0	7.8	7.7	9.3
	2	8.6	8.8	9.3	8.8	8.2	7.5	9.4
		9.6	8.7	9.3	8.8	7.8	8.0	9.2

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### NEOGEN COLLABORATIVE STUDY

**Beta site testing:** External beta site evaluations were conducted to evaluate the performance of the Veratox HS MAX for Total Aflatoxin by ten independent industry professionals. Each laboratory was directed to analyze the samples following the kits written instructions. Each laboratory was provided seven ground corn samples (10 g size) to analyze. Samples were tested using two microwell strips at each laboratory. Data generated is represented below.

Lab		NDA - 0 ppb		1.7 ±0.3 ppb		5.2 ± 0.8 ppb		8.5 ±1.1 ppb
1	Strip 1	0.6	0.5	2.0	1.8	4.5	4.5	9.7
	Strip 2	0.5	0.3	2.0	2.0	4.4	4.1	9.2
2	Strip 1	0.0	0.0	2.6	2.0	6.3	4.8	7.9
	Strip 2	0.1	0.3	2.5	2.1	6.2	4.6	8.2
3	Strip 1	0.4	0.4	2.2	2.3	5.1	4.3	6.8
	Strip 2	0.4	0.6	2.6	2.2	5.1	4.5	7.3
4	Strip 1	0.0	0.2	2.0	3.0	4.8	5.2	9.9
	Strip 2	0.0	0.2	1.5	2.5	4.0	5.2	8.9
5	Strip 1	0.0	0.0	1.7	2.6	5.3	5.8	8.7
	Strip 2	0.0	0.0	1.7	2.2	4.7	5.2	9.3
6	Strip 1	0.1	0.0	1.0	1.6	5.5	4.9	9.1
	Strip 2	0.0	0.0	1.5	2.1	5.2	4.3	9.1
7	Strip 1	0.0	0.0	1.9	1.9	4.6	4.9	7.6
	Strip 2	0.3	0.0	1.8	1.7	4.5	4.9	7.9
8	Strip 1	0.6	0.9	3.0	1.6	4.8	4.0	9.6
	Strip 2	0.1	0.2	2.9	2.1	4.7	4.6	9.5
9	Strip 1	0.6	1.0	2.6	3.0	8.0	6.2	9.3
	Strip 2	1.0	1.2	2.7	3.2	7.0	5.9	8.7
10	Strip 1	0.0	0.0	1.3	2.7	4.2	4.4	8.5
	Strip 2	0.0	0.0	1.2	1.6	4.6	3.9	7.3
<b>Mean</b>		<b>0.2</b>	<b>0.3</b>	<b>2.0</b>	<b>2.2</b>	<b>5.2</b>	<b>4.8</b>	<b>8.6</b>
<b>% Recovery</b>				<b>120</b>	<b>130</b>	<b>100</b>	<b>120</b>	<b>101</b>
<b>St Dev</b>				<b>0.5</b>		<b>0.8</b>		<b>1.0</b>

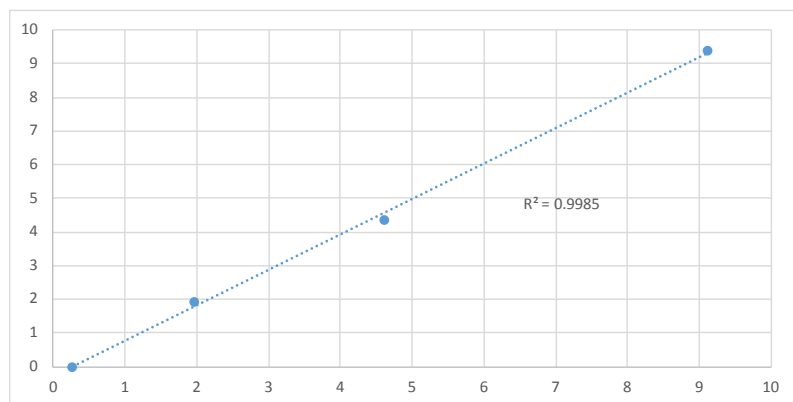
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### CORRELATION WITH HPLC REFERENCE METHOD

HPLC determinations on commercially obtained naturally contaminated corn samples were performed by an outside commercial laboratory and by Neogen internal HPLC laboratory. Results shown below:

Level	HPLC Points						HPLC Average
2 ppb	1.96	1.93	1.84	1.93	1.93	2.03	1.936
5 ppb	4.87	4.64	4.50	4.16	3.91	4.09	4.361
9.4 ppb	9.40	9.57	9.43	9.14	9.61	9.18	9.388

Level	Multi-User Average	HPLC Average
2.0 ppb	1.971	1.937
5.0 ppb	4.621	4.362
9.4 ppb	9.124	9.388



The Veratox HS MAX for Total Aflatoxin kit satisfies the performance criteria defined in the EU directive 519/2014 from 16 May 2014 specifying the methods of sampling procedure and analysis for the official control of Fusarium toxins in foodstuffs.

The following table summarizes the performance criteria listed in the directive – RSDr (intra assay variability), RSDR (inter assay variability) and recovery rate demonstrated by the kit.

Neogen Kit	Part No.	Range of Concentration µg/Kg	RSDr	RSDR	Recovery	Correlation to HPLC
Veratox HS MAX for Aflatoxin	8032	1–10	5.4%	6.1%	90–110%	0.9985

### CONCLUSION

Throughout a wide range of tests, Veratox HS MAX for Total Aflatoxin demonstrated a good correlation to the HPLC reference method and superior test robustness. This coupled with its reproducibility, make Veratox HS MAX for Total Aflatoxin a good choice for the rapid and accurate detection of aflatoxin contamination in corn at levels between 1–10 ppb.



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