



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.

050901

The AOAC Research Institute hereby certifies the method known as:

Veratox® Aflatoxin

manufactured by

Neogen Corporation

620 Leshar Place

Lansing, Michigan 48912 USA

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*SM Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*SM certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads "Scott Coates".

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date December 26, 2023

Expiration Date December 31, 2024

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METHOD NAME Veratox® Aflatoxin	CATALOG NUMBER 8030
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INDEPENDENT LABORATORY
U.S. Department of Agriculture
Grain Inspection Packers and Stockyards Administration (GIPSA)
Technical Service Division
Kansas City, MO USA

APPLICABILITY OF METHOD Target analyte – Aflatoxin (total = B ₁ , B ₂ , G ₁ , G ₂) Matrixes – Corn (50 g) Performance claims – Based on internal validation study: <i>Precision.</i> —Less than 21% CV average of all levels tested. <i>Accuracy.</i> —94.85% agreement with HPLC with >95% aflatoxin recovery observed. <i>Cross-reactivity with closely related compound.</i> —None. <i>LOD.</i> —1.4 ppb. <i>Range of quantitation.</i> —This assay has a range of quantitation between 5.0 and 50 ppb without additional dilution, and range can be extended through further dilution with 70% methanol.	REFERENCE METHOD <i>Official Methods of Analysis (2007) 18th Ed., 2nd Rev., AOAC INTERNATIONAL, Gaithersburg, MD, Method 994.08 (2)</i>
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ORIGINAL CERTIFICATION DATE May 2009	CERTIFICATION RENEWAL RECORD Renewed annually through 2024.
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METHOD MODIFICATION RECORD 1. December 2020 Level 1 2. August 2022 Level 2	SUMMARY OF MODIFICATION 1. Editorial changes for clarity. 2. Change in substrate composition for regulation and labelling requirements.
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Under this AOAC <i>Performance Tested Methods</i> SM License Number, 050901 this method is distributed by: NONE	Under this AOAC <i>Performance Tested Methods</i> SM License Number, 050901 this method is distributed as: NONE
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PRINCIPLE OF THE METHOD (1)
Veratox for aflatoxin is a direct competitive ELISA in a microwell format that allows the users to obtain exact concentrations in parts per billion (ppb). Free aflatoxin in the samples and controls is allowed to compete with enzyme-labeled aflatoxin (conjugate) for the antibody-binding sites. After a wash step, a substrate is added, which reacts with the bound conjugate to produce a blue color. More blue color means less aflatoxin. The test is read in a microwell reader to yield optical densities. The optical densities of the controls form the standard curve, and the sample optical densities are plotted against the curve to calculate the exact concentration of aflatoxin.

DISCUSSION OF THE VALIDATION STUDY (1)
Given the observed accuracy over several levels of naturally contaminated reference corn compared to HPLC and the degree of reproducibility observed in both the internal and collaborating laboratory data set, it is recommended that the Veratox for aflatoxin method be granted *Performance Tested Method* status for corn.

Table 3. Direct comparison of HPLC to Veratox aflatoxin ELISA^{a, b} (2)

HPLC	ELISA
0.0	0.0
5.6	5.4
8.1	9.2
10.0	10.2
13.0	15.7
14.1	12.7
17.1	15.8
19.4	19.8
20.4	24.8
20.7	23.5
22.6	22.9
24.9	32.0
33.8	32.1
38.5	28.9
70.1	80.7

^a $n = 3$ for each sample (averages shown). LOD for HPLC = 0.1 ppb. All results in ppb.

^b LOD for **994.08** = 0.1 ppb aflatoxin B1. LOD for Veratox method = 1.4 ppb total aflatoxin.

Table 5. Limit of detection using aflatoxin-free corn^a (1)

Replicate	Veratox	HPLC
1	0.4	NDA ^b
2	0.2	NDA
3	0.8	NDA
4	0.9	NDA
5	1.6	NDA
6	0.3	NDA
7	0.4	NDA
8	0.3	NDA
9	0.5	NDA
10	1.0	NDA
Mean	0.6	<0.01
SD	0.4	NA
Mean + 2SD	1.4	NA

^a LOD of HPLC = 0.1 ppb; all values in ppb.

^b NDA = No detectable amount.

Table 13. Results of independent laboratory trials with the Veratox aflatoxin ELISA^{a, b} (1)

	5.0 ppb	20.0 ppb
Analyst A		
1	3.5	20.9
2	4.3	20.7
3	4.7	20.8
4	5.7	26.2
5	9.0	25.8
6	7.1	25.0
7	6.6	26.7
Analyst B		
1	6.0	18.1
2	5.0	19.9
3	6.5	18.3
4	5.6	24.0
5	6.2	25.1
6	7.8	24.2
7	6.8	33.0
Analyst C		
1	6.5	22.8
2	6.6	22.2
3	6.2	21.4
4	6.5	18.7
5	7.1	24.8
6	8.1	23.8
7	7.2	26.9
Mean	6.3	23.3
SD	1.3	3.6
CV, %	20.1	15.2

^a All results in ppb.

^b HPLC Method **994.08** also performed by independent laboratory: result for 5 ppb sample = 5.83 ppb; result for 20 ppb sample = 20.69 ppb.

REFERENCES

1. Lupo, A., Roebuck, C., Dutcher, M., Kennedy, J., and Abouzied, M., Validation Study of a Rapid ELISA for Detection of Aflatoxin in Corn, AOAC Research Institute *Performance Tested Methods*SM certification number 050901.
2. *Official Methods of Analysis* (2007) 18th Ed., 2nd Rev., AOAC INTERNATIONAL, Gaithersburg, MD, Method 994.08
3. Fowley, M., Gray, R.L., Roman, B., and Donofrio, R., Level 2 Modification Study to Validate Multiple Veratox® ELISA Methods, AOAC *Performance Tested Methods*SM certification number 050901. Approved August 3, 2022.