



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
071801

The AOAC Research Institute hereby certifies the method known as:

Q+ MAX® for Aflatoxin Quantitative Test with Aqueous Extraction

manufactured by

Neogen Corporation
620 Leshar Place
Lansing, MI 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, appearing to read "Bradley A. Stawick".

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

Issue Date
Expiration Date

January 15, 2024
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METHOD NAME Q+ MAX® for Aflatoxin Quantitative Test with Aqueous Extraction	CATALOG NUMBER 8088
INDEPENDENT LABORATORY Q Laboratories, Inc. 1400 Harrison Avenue Cincinnati, OH 45214 USA	APPLICABILITY OF METHOD Target Analytes – Mycotoxin aflatoxin (B₁, B₂, G₁, and G₂) Matrixes – (10 g) - Corn, almonds, walnuts, peanut, and pistachios Performance claims – Detection of total aflatoxin ranging from 3 ppb to 300 ppb in corn, almonds, walnuts, peanuts, and pistachios after aqueous extraction. Further dilution with water is required for levels >50 ppb. Average LOD for all matrixes is 2 ppb and LOQ is 7 ppb. Method linearity was determined for each matrix at levels ranging from 0 to 300 ppb.
ORIGINAL CERTIFICATION DATE July 18, 2018	CERTIFICATION RENEWAL RECORD Renewed annually through December 2024.
METHOD MODIFICATION RECORD 1. November 2018 Level 1 2. November 2019 Level 1 3. July 2021 Level 2	SUMMARY OF MODIFICATION 1. Editorial/clerical changes. 2. Editorial/clerical changes. 3. Reduction in the inert filling agent in MAX 1.
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PRINCIPLE OF THE METHOD (1)

Reveal Q+ MAX for Aflatoxin is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. The extract is wicked through a reagent zone, which contains antibodies specific for aflatoxin conjugated to colloidal gold particles. If aflatoxin is present, it will be captured by the antibody-particle complex. The aflatoxin-antibody-particle complex is then wicked onto a membrane, which contains a zone of aflatoxin conjugated to a protein carrier. This zone captures any uncomplexed antibody-particle, allowing the particles to concentrate and form a visual line (test line). As the level of aflatoxin in a sample increases, free aflatoxin in test extract will complex with the antibody-gold particles. This allows less antibody-gold to be captured in the test zone. Therefore, as the concentration of aflatoxin in the sample increases, the test line density decreases. Algorithms programmed into the readers convert these line densities into a quantitative result displayed in parts per billion (ppb). The membrane also contains a control zone where an immune complex present in the reagent zone is captured by a second antibody, forming a visible line (control line). The control line will always form regardless of the presence of aflatoxin, ensuring the strip is functioning properly.

DISCUSSION OF THE VALIDATION STUDY (1)

The results presented here demonstrate that the Reveal Q+ MAX for Aflatoxin adequately detects aflatoxin in contaminated corn, peanuts, almonds, walnuts, and pistachios. Results for naturally contaminated aflatoxin in corn were confirmed by an independent laboratory. The linearity curve generated has a R² value of 0.985. High cross-reactivity was reported for all four aflatoxin analytes with no cross-reactivity observed on any of the closely related non-aflatoxin compounds at a high concentration in the presence and absence of aflatoxin. The LOD for all matrixes is 2 ppb and the LOQ is 7 ppb. Finally, results of the robustness testing showed that the assay can withstand minor deliberate alterations to multiple assay parameters and still yield accurate results for corn. As for almond, it is recommended that the end users stays within the defined test portion parameters. Furthermore, Reveal Q+ MAX for Aflatoxin offers the advantage of minimal labor, bench space, hardware requirements, disposable materials, and rapid results within 6 minutes following sample extraction.

Table 4: Recoveries of Added Aflatoxin (ppb) from Matrixes (1)

Matrix	Level (ppb)	N ^a	Mean (ppb)	Sr (ppb)	RSDr(%)	Recovery (%)	Bias (ppb)
Corn	0	6	2.3	0.2	10.2	0.0	0.0
	5.2	6	5.5	0.3	6.2	105.8	0.3
	21.0	6	22.4	3.5	15.5	106.5	1.4
	51.6	6	39.3	3.6	9.1	76.2	-12.3
	103.6	6	94.1	7.0	7.5	91.0	-9.3
	282.0	6	296.5	23.5	7.9	105.1	14.5
Corn ^b	Mean			6.4	9.4	80.8	-0.9
	0	5	<2	0.0	0.0	0.0	0.0
	5	5	5.2	0.9	17.1	103.2	0.2
	20	5	19.1	3.7	19.6	95.5	-0.9
	50	5	54.3	6.9	12.7	108.6	4.3
	100	5	89.3	13.7	15.5	89.3	-10.7
Pistachios	300	5	321.7	42.9	13.3	107.2	21.7
	Mean			11.3	13.0	84.0	2.4
	0	6	2.3	0.3	14.8		
	5	6	4.8	0.5	10.0	95.7	-0.2
	20	6	17.4	0.9	5.0	86.8	-2.6
	50	6	37.3	3.1	8.2	74.6	-12.7
Almonds	100	6	93.0	14.5	15.6	93.0	-7.0
	300	6	325.6	20.4	6.3	108.5	25.6
	Mean			6.6	10.0	91.7	0.6
	0	6	2.4	0.3	11.0		
	5	6	5.7	0.4	7.5	113.0	0.6
	20	6	25.3	1.8	7.0	126.3	5.3
Walnuts	50	6	52.5	2.9	5.5	105.0	2.5
	100	6	115.6	13.8	11.9	115.6	15.6
	300	6	374.0	53.3	14.3	124.7	74.0
	Mean			12.1	9.5	116.9	19.6
	0	6	2.1	0.2	10.1		
	5	6	5.4	0.2	4.0	107.3	0.4
Peanuts	20	6	16.7	1.0	6.0	83.7	-3.3
	50	6	43.0	3.7	8.6	86.0	-7.0
	100	6	105.6	18.7	17.7	105.6	5.6
	300	6	292.1	26.6	9.1	97.4	-7.9
	Mean			8.4	9.3	96.0	-2.4
	0	6	2.2	0.3	14.9		
Peanuts	5	6	3.7	0.5	13.7	74.7	-1.3
	20	6	16.8	1.3	7.8	84.1	-3.2
	50	6	36.1	2.9	8.1	72.2	-13.9
	100	6	79.0	8.3	10.5	79.0	-21.0
	300	6	316.3	10.4	3.3	105.4	16.3
	Mean			4.0	9.7	83.1	-4.6

^aN= Number of test portions.^bTrial performed by independent laboratory.**Table 5: Results of LOD and LOQ Testing (1)**

Matrix	N ^a	Mean (ppb)	Sr (ppb) ^d	LOD (ppb)	LOQ (ppb)
Corn	10	2.2	0.2	2.6	7.9
Corn ^b	10	<2		<2	6.0
Pistachios	10	2.0	0.1	2.2	6.5
Almonds	10	2.0	0.1	2.2	6.7
Walnuts	10	2.1	0.2	2.5	7.6
Peanuts	10	2.2	0.2	2.7	8.0

^aN= Number of test portions.^bTrial performed by independent laboratory.**REFERENCES CITED**

1. Le, Q.N, Roman, B., Driksna, D., Gilbert, L., Gonzales, K., Klein, F., and Donofrio, R., Evaluation of the Reveal Q+ MAX® for Detection of Total Aflatoxin in Corn, Almonds, Pistachios, Walnuts, and Peanuts, AOAC Performance Tested MethodsSM certification number 071801.
2. CAST (Council for Agricultural Science Technology) (2003) *Mycotoxins: Risk in Plant, Animal, and Human System*, Ames, IA, No. 139
3. Centers for Disease Control and Prevention (2017) *National Biomonitoring Program*, Toxins <https://www.cdc.gov/biomonitoring/toxins.html> (December 2017)
4. United States Department of Agriculture (2016), *Design Criteria and Test Performance Specifications for Quantitative Aflatoxin Test Kits*. (2016). USDA-FGIS Kansas City, MO https://www.gipsa.usda.gov/fgis/metheqp/aflatoxins_criteria.pdf