CERTIFICATION

AOAC Research Institute

Performance Tested Methods^{SM}

Certificate No.

061403

The AOAC Research Institute hereby certifies the method known as:

AgraStrip® Gluten G12® Test Kit

manufactured by

Romer Labs Diagnostic GmbH
Technopark 1
3430 Tulln, Austria

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*^{SM} Program. 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.

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

Issue Date	November 21, 2024
Expiration Date	December 31, 2025

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SUBMITTING COMPANY
Romer Labs
Technopark 1
3430 Tulln, Austria

CURRENT COMPANY
Romer Labs Division Holding GmbH
Technopark 5
3430 Tulln, Austria

METHOD NAME
AgraStrip® Gluten G12® Test Kit

CATALOG NUMBERS
Original catalog numbers: COKAL0200AS
Updated catalog number: 10001995

INDEPENDENT LABORATORY
Q Laboratories, inc.
1400 Harrison Ave.
Cincinnati, OH 45214
USA

APPLICABILITY OF METHOD
Target analyte – Gluten

Matrixes – Ice cream, dark chocolate, cookies, bread and rice flour, stainless steel

Performance claims – The AgraStrip® Gluten G12® assay was rigorously evaluated during this study and demonstrates its suitability as an AOAC PTM-certified gluten detection method.

REFERENCE METHOD

AOAC OMA 2012.01

ORIGINAL CERTIFICATION DATE
June 10, 2014

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2025.

METHOD MODIFICATION RECORD
1. **November 2018 Level 1**
2. **May 2019 Level 1**
3. **February 2020 Level 1**
4. **November 2020 Level 1**
5. **November 2023 Level 1**

SUMMARY OF MODIFICATION
1. **Editorial/clerical changes.**
2. **Updated catalog numbers.**
3. **Editorial/clerical changes.**
4. **Editorial/clerical changes.**
5. **Editorial/clerical changes.**

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NONE

PRINCIPLE OF THE METHOD (1)
The AgraStrip® Gluten G12® test kit is a lateral flow immunochromatographic assay for the detection of gluten. The test kit is designed to detect the presence of gluten in foods with varying compositions and levels of processing, from raw foods/ingredients to finished product testing. Additionally, the test kit is designed to detect the presence of gluten on surfaces through swab testing.

DISCUSSION OF THE VALIDATION STUDY (1)

The results of this study demonstrate that the AgraStrip Gluten G12 test kit will neither cross-react with a broad spectrum of gluten-free food compounds, nor will those compounds interfere with a positive result. Environmental surface spikes were recovered as expected, demonstrating no false positive results, fractional recovery just below the LOD, and recovery at high spike concentration. The assay demonstrated consistent results between different production lots, as well as between different kits within the same lot. Ongoing stability studies show that the AgraStrip Gluten G12 kit is stable over a period of 3 months, as well as over a period of 50 days during accelerated stability at 42°C. Results of the robustness study indicated a zero-minute protein extraction is not advisable, nor is halving the amount of extraction buffer used, as this effectively doubles the gluten concentration in the extract. Five-minute extraction, over-filling the extraction buffer, and varying the AgraStrip incubation time did not significantly affect the test outcome.

Food matrix testing indicates the AgraStrip Gluten G12 assay is capable of detecting the presence of 5, 10 and 20 ppm gliadin spike levels in gluten-free rice flour, cookies and bread at the respective 5, 10 and 20 ppm assay thresholds. Per the validation outline, gliadin was spiked at 0, 3, 8, 15 and 25 ppm levels, though it comprises only ~50% of the total protein content of gluten. The AgraStrip Gluten G12 antibodies detect the presence of gluten through a gliadin epitope, however the assay is designed to reflect gluten content (while AOAC OMA 2012.01 measures gliadin content). Therefore, food matrix testing where gliadin was spiked at 3, 8, 15 and 25 ppm represents gluten concentrations of approximately 6, 16, 30 and 50 ppm in the AgraStrip Gluten G12 assay. During incurred matrix testing, no spike recovery at the 20 ppm threshold was observed, despite the sample having measured at ~15 ppm gliadin, or 30 ppm gluten. Because AOAC OMA 2012.01 has a %RSD ranging from 22 – 52% [5], the range for the amount of gliadin in the baked bread allows for the possibility that the amount of gliadin in the baked bread could have been below the 20 ppm threshold. The AgraStrip Gluten G12 assay has been observed to perform reliably when testing heat-treated samples, due to the highly stable sequential epitope which the G12 antibody detects. The high gliadin concentrations observed during the gluten-free ice cream testing reflect the difficulty of spiking complete wheat gluten standard into a food matrix containing water. The gluten particles drew water in, bloomed, and tended to adhere to tube walls and pipet tips, as well as agglutinate. This agglutinative effect caused by the water present in ice cream likely produced heterogeneity of gluten concentrations observed with that matrix. A gliadin spike into the ice cream likely would have produced better homogeneity. Because a fat, cocoa butter, was used to suspend and dilute the WGS for chocolate, and chocolate had far less water content, better homogeneity was achieved, and the results more accurately reflect detection of the spike levels of wheat gluten standard, despite the high levels of tannins present in 70% cacao dark chocolate tested. Still, at the 8 ppm threshold in chocolate 2 false negative results were observed. These results were due to the chocolate hardening in the bottom of the extraction tube before it could be fully mixed with the extraction buffer. These results do not indicate a failure of the assay to detect gluten, but rather reflect the challenge of spiking and manipulating, within such a large study, a matrix having chocolate's physical properties. Over the entire study no false positive results were observed in any blank sample.

Table 5. Food matrix testing results for gliadin-spiked rice flour. (1)

Matrix	Gliadin (Gluten) Spike Concentration	AgraStrip® Detection Threshold (ppm Gluten)	N[a]	Candidate			Avg AOAC OMA 2012.01 Results (ppm Gliadin) N=3
				x[b]	POD_C[c]	95% CI[d]	
Rice Flour	0 ppm	5	30	0	0.00	0.00, 0.11	<2.5
		10	30	0	0.00	0.00, 0.11	
		20	30	0	0.00	0.00, 0.11	
	3 ppm (6)	5	30	12	0.40	0.25, 0.58	3.55
		10	30	0	0.00	0.00, 0.11	
		20	30	0	0.00	0.00, 0.11	
	8 ppm (16)	5	30	30	1.00	0.89, 1.00	4.98
		10	30	13	0.43	0.27, 0.61	
		20	30	3	0.10	0.03, 0.26	
	15 ppm (30)	5	30	30	1.00	0.89, 1.00	13.47
		10	30	30	1.00	0.89, 1.00	
		20	30	30	1.00	0.56, 0.86	
	25 ppm (50)	5	30	30	1.00	0.89, 1.00	23.50
		10	30	30	1.00	0.89, 1.00	
		20	30	30	1.00	0.89, 1.00	

[a]N = Number of test portions
[b]x = Number of positive test portions
[c]POD_C = Candidate method confirmed positive outcomes divided by the total number of trials
[d]95% Confidence Intervals

Table 6. Food matrix testing for gliadin-spiked gluten-free cookies. (1)

Matrix	Gliadin (Gluten) Spike Concentration	AgraStrip® Detection Threshold (ppm Gluten)	N[a]	Candidate			Avg AOAC OMA 2012.01 Results (ppm Gliadin) N=3
				x[b]	POD_C[c]	95% CI[d]	
Cookies	0 ppm	5	30	0	0.00	0.00, 0.11	0.30
		10	30	0	0.00	0.00, 0.11	
		20	30	0	0.00	0.00, 0.11	
	3 ppm (6)	5	30	28	0.93	0.79, 0.98	2.65
		10	30	26	0.87	0.70, 0.95	
		20	30	0	0.00	0.00, 0.11	
	8 ppm (16)	5	30	30	1.00	0.89, 1.00	8.28
		10	30	30	1.00	0.89, 1.00	
		20	30	4	0.13	0.05, 0.30	
	15 ppm (30)	5	30	30	1.00	0.89, 1.00	11.67
		10	30	30	1.00	0.89, 1.00	
		20	30	29	0.97	0.83, 1.00	
	25 ppm (50)	5	30	30	1.00	0.89, 1.00	17.75
		10	30	30	1.00	0.89, 1.00	
		20	30	30	1.00	0.89, 1.00	

[a]N = Number of test portions
[b]x = Number of positive test portions
[c]POD_C = Candidate method confirmed positive outcomes divided by the total number of trials
[d]95% Confidence Intervals

Table 7. Food matrix testing for gluten-free bread. (1)

Matrix	Gliadin (Gluten) Spike Concentration	AgraStrip® Detection Threshold (ppm Gluten)	N[a]	Candidate			Avg AOAC OMA 2012.01 Results (ppm Gliadin) N=3
				x[b]	POD_C[c]	95% CI[d]	
Bread	0 ppm	5	30	0	0.00	0.00, 0.11	<2.5
		10	30	0	0.00	0.00, 0.11	
		20	30	0	0.00	0.00, 0.11	
	3 ppm (6)	5	30	30	1.00	0.89, 1.00	2.32
		10	30	13	0.43	0.27, 0.61	
		20	30	0	0.00	0.00, 1.00	
	8 ppm (16)	5	30	30	1.00	0.89, 0.11	7.60
		10	30	28	0.93	0.79, 0.98	
		20	30	3	0.10	0.03, 0.26	
	15 ppm (30)	5	30	30	1.00	0.89, 1.00	13.37
		10	30	30	1.00	0.89, 1.00	
		20	30	29	0.97	0.83, 1.00	
	25 ppm (50)	5	30	30	1.00	0.89, 1.00	18.75
		10	30	30	1.00	0.89, 1.00	
		20	30	30	1.00	0.89, 1.00	

[a]N = Number of test portions
[b]x = Number of positive test portions
[c]POD_C = Candidate method confirmed positive outcomes divided by the total number of trials
[d]95% Confidence Intervals

Table 8. Food matrix testing for WGS-spiked gluten-free ice cream. (1)

Matrix	WGS Spike Gluten Concentration	AgraStrip® Detection Threshold (ppm Gluten)	N[a]	Candidate			Avg AOAC OMA 2012.01 Results (ppm Gliadin) N=3
				x[b]	POD$_C$[c]	95% CI[d]	
Ice Cream	0 ppm	5	30	0	0.00	0.00, 0.11	<2.5
		10	30	0	0.00	0.00, 0.11	
		20	30	0	0.00	0.00, 0.11	
	3 ppm	5	30	30	1.00	0.89, 1.00	6.18
		10	30	28	0.93	0.79, 0.98	
		20	30	0	0.00	0.00, 0.11	
	8 ppm	5	30	30	1.00	0.89, 1.00	21.42
		10	30	30	1.00	0.89, 1.00	
		20	30	10	0.33	0.19, 0.51	
	15 ppm	5	30	30	1.00	0.89, 1.00	38.17
		10	30	30	1.00	0.89, 1.00	
		20	30	30	1.00	0.89, 1.00	
	25 ppm	5	30	30	1.00	0.89, 1.00	30.43
		10	30	30	1.00	0.89, 1.00	
		20	30	30	1.00	0.89, 1.00	

[a]N = Number of test portions
[b]x = Number of positive test portions
[c]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[d]95% Confidence Intervals

Table 9. Food matrix testing for WGS-spiked gluten-free chocolate. (1)

Matrix	WGS Spike Gluten Concentration	AgraStrip® Detection Threshold (ppm Gluten)	N[a]	Candidate			Avg AOAC OMA 2012.01 Results (ppm Gliadin) N=3
				x[b]	POD$_C$[c]	95% CI[d]	
Chocolate	0 ppm	5	30	0	0.00	0.00, 0.11	<2.5
		10	30	0	0.00	0.00, 0.11	
		20	30	0	0.00	0.00, 0.11	
	3 ppm	5	30	3	0.10	0.03, 0.26	4.36
		10	30	0	0.00	0.00, 0.11	
		20	30	0	0.00	0.00, 0.11	
	8 ppm	5	30	28	0.93	0.79, 0.98	5.61
		10	30	7	0.23	0.12, 0.41	
		20	30	1	0.03	0.00, 0.17	
	15 ppm	5	30	30	1.00	0.89, 1.00	13.15
		10	30	30	1.00	0.89, 1.00	
		20	30	17	0.57	0.39, 0.73	
	25 ppm	5	30	30	1.00	0.89, 1.00	32.87
		10	30	30	1.00	0.89, 1.00	
		20	30	30	1.00	0.89, 1.00	

[a]N = Number of test portions
[b]x = Number of positive test portions
[c]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[d]95% Confidence Intervals

Table 10. Food matrix testing for hook effect at high WGS spike concentration in rice flour. (1)

Matrix	WGS Spike Gluten Concentration	AgraStrip® Detection Threshold (ppm Gluten)	N[a]	Candidate			Avg AOAC OMA 2012.01 Results (ppm Gliadin) N=3
				x[b]	POD_C[c]	95% CI[d]	
Rice Flour Hook Effect	10,000 ppm	5	10	10	1.00	0.72, 1.00	6,640
		10	10	10	1.00	0.72, 1.00	
		20	10	10	1.00	0.72, 1.00	

[a]N = Number of test portions
[b]x = Number of positive test portions
[c]POD_C = Candidate method confirmed positive outcomes divided by the total number of trials
[d]95% Confidence Intervals

REFERENCES CITED

1. Radcliffe, S., Sutzko, M., Jiang, Z., Freitag, D., Swoboda, C., Frank, L., and Rogers, A., Evaluation of the AgraStrip® Gluten G12® Test for the Detection of Gluten in Food and Surfaces, AOAC *Performance Tested Methods*SM certification number 061403.
2. Popping, B., Diaz-Amigo, C. and Hoenicke, K., *Molecular Biological and Immunological Techniques and Applications for Food Chemists. Wiley, 2009. p. 368.*