



# CERTIFICATION

## AOAC Research Institute *Performance Tested Methods*<sup>SM</sup>

Certificate No.  
**061502**

The AOAC Research Institute hereby certifies the method known as:

### **GlutenTox® Pro**

manufactured by

**Hygiena Diagnóstica España**  
**P. I. Parque Plata, Calle Cañada Real 31-35**  
**Camas, Sevilla 41900**  
**Spain**

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*<sup>SM</sup> 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*<sup>SM</sup> 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

December 03, 2024

Expiration Date

December 31, 2025

<b>AUTHORS</b> ORIGINAL VALIDATION: Miguel A. Síglez, Bárbara Nocea, María del Mar Pérez, Eva M <sup>a</sup> García, Laura León, Carlos Galera MODIFICATION DECEMBER 2018: Hygiene Diagnóstica España	<b>ORIGINAL SUBMITTING COMPANY</b> Biomedal, S. L. Avenida Américo Vespucio, 5-E, 1 <sup>a</sup> M-12 41092 Sevilla Spain	<b>CURRENT COMPANY</b> Hygiene Diagnóstica España P. I. Parque Plata, Calle Cañada Real 31-35 Camas, Sevilla 41900 Spain
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<b>METHOD NAME</b> GlutenTox <sup>®</sup> Pro	<b>CATALOG NUMBERS</b> KIT 3000 (KT-5660; 25 analysis); KIT 3001 (KT-5288; 5 analysis)
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<b>INDEPENDENT LABORATORY</b> Q.Laboratories, Inc. 1400 Harrison Ave Cincinnati, OH 45214 USA
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<b>APPLICABILITY OF METHOD</b> Target analyte – Gluten.  Matrixes – bread, rice flour, paté, rolled oat, yogurt, food-grade painted wood, plastic, rubber, sealed ceramic, stainless steel  Performance claims - The GlutenTox <sup>®</sup> Pro test kit is a quick and easy to use screening method for the detection of gluten in raw or cooked foods and on environmental surfaces. The method is specific and reliable and provides sensitive and accurate test results comparable to AOAC OMA 2012.01.	<b>REFERENCE METHOD</b>  AOAC Official Methods of Analysis (OMA) 2012.01 “Gliadin as a Measure of Gluten in Foods Containing Wheat, Rye, and Barley” (11)
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<b>ORIGINAL CERTIFICATION DATE</b> June 26, 2015	<b>CERTIFICATION RENEWAL RECORD</b> Renewed through December 2025.
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<b>METHOD MODIFICATION RECORD</b>	<b>SUMMARY OF MODIFICATION</b>
1. December 2018 Level 2	1. Purchase and location change from Biomedal Avenida Américo Vespucio, 5-E, 1 <sup>a</sup> M-12, 41092 Sevilla, Spain to Hygiene Diagnóstica España P. I. Parque Plata, Calle Cañada Real 31-35, 41900 Camas, Sevilla, Spain.
2. November 2019 Level 1	2. Editorial changes.
3. December 2024 Level 1	3. Editorial changes.

Under this AOAC <i>Performance Tested Methods</i> <sup>SM</sup> License Number, 061502 this method is distributed by: NONE	Under this AOAC <i>Performance Tested Methods</i> <sup>SM</sup> License Number, 061502 this method is distributed as: NONE
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**PRINCIPLE OF THE METHOD (1)**

The GlutenTox<sup>®</sup>Pro method is an immunochromatographic assay for the detection of gluten in food and beverages (with non-hydrolyzed gluten) with different composition and levels of processing, from raw materials to processed food. In addition, the GlutenTox Pro Test Kit can be used to control the cleanliness of food production zones through surface analysis, a prerequisite to prevent the risk of cross-contamination in the final product.

**DISCUSSION OF THE VALIDATION STUDY (1)**

The GlutenTox Pro method did not show cross-reactivity to any of the compounds included in the list of *Validation Procedures for Quantitative Gluten ELISA Methods: AOAC Allergen Community Guidance and Best Practices*<sup>4</sup> used in the production of gluten-free products. The GlutenTox Pro assay also did not show any interference, when tested with the compounds from the list in the presence of gluten. No unexpected results were obtained however gum-type samples can be difficult to analyze due to the thick paste formed when added to the extraction solution provided in the GlutenTox Pro test kit. A warning to this type of samples has been included in the instructions for use.

The GlutenTox Pro test kit performed as expected in the selected food matrixes (rice flour, bread, rolled oat, pâté and yogurt) and test conditions (spike level and detection threshold combinations), 5 ppm being the lowest concentration of gluten that can be detected with the kit.

In all matrixes tested, the GlutenTox Pro method demonstrated 100 % specificity [probability of detection (POD) 0.00, confidence interval (CI) 0.00-0.11] at 0 ppm spiked level of gluten and 100 % sensitivity (POD 1.00, CI 0.89-1.00) at each spiked level of gluten and threshold level combinations. No false negative results were obtained in the food matrix study. The assay did not experience hook effect at any threshold level tested when the rice flour matrix was spiked at very high spiked levels of gluten (10,000 ppm).

In the incurred sample study, the incurred residue target level was approximately 25 ppm of gluten, the initial spiking level in the uncooked matrix was 50 ppm of gluten and a 78.2 % recovery was obtained when tested with the AOAC OMA 2012.01 method<sup>11</sup> (recovery could be between 50-150%).

The GlutenTox<sup>®</sup>Pro test kit performed as expected in the incurred bread sample and the results obtained in the incurred matrix study were consistent with those obtained in the selected food matrix study with bread. In both studies, false negative and/or overestimated results were not observed.

The results obtained when the GlutenTox Pro test kit was tested with the selected environmental surfaces (food-grade painted wood, plastic, rubber, sealed ceramic and stainless steel) demonstrated a 100 % specificity (POD 0.00, CI 0.00-0.11) at the unspiked level of gluten contamination and a 100 % sensitivity (POD 1.00, CI 0.89-1.00) at the high level of gluten contamination (400 ng/16 cm<sup>2</sup>), in each of the environmental surfaces analyzed.

At the low level of gluten contamination (16 ng/16 cm<sup>2</sup>), the GlutenTox Pro assay was able to detect as little as 16 ng of gluten when analyzed with the environmental surface matrixes.

**Table 3: GlutenTox Pro Test Kit Incurred Matrix (Bread) – POD Results (1)**

Matrix	Gluten Spiked Level*	Detection Threshold (ppm)	N <sup>a</sup>	Candidate			Ave. AOAC OMA 2012.01 results, ppm gluten, N=3	Variance (σ <sup>2</sup> )
				x <sup>b</sup>	POD <sub>c</sub> <sup>c</sup>	95% CI <sup>d</sup>		
Incurred Matrix (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		
		40	30	0	0.00	0.00, 0.11		
	39.1 ppm	5	30	30	1.00	0.89, 1.00	39.1	1.2
		10	30	30	1.00	0.89, 1.00		
		20	30	30	1.00	0.89, 1.00		
		40	30	0	0.00	0.00, 0.11		

\*Gluten Spiked Level results after cooking the bread

<sup>a</sup>N = Number of test portions

<sup>b</sup>x = Number of positive test portions

<sup>c</sup>POD<sub>c</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>d</sup>95% Confidence Intervals

**Table 4: GlutenTox Pro Test Kit for Rice Flour – POD Results (1)**

Matrix	Gluten Spiked Level	GlutenTox Pro Detection Threshold (ppm gluten)	N <sup>a</sup>	Candidate			Ave. AOAC OMA 2012.01 results, ppm gluten, N=3	Variance (σ <sup>2</sup> )
				x <sup>b</sup>	POD <sub>c</sub> <sup>c</sup>	95% CI <sup>d</sup>		
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		
		40	30	0	0.00	0.00, 0.11		
	3 ppm	5	30	11	0.37	0.22, 0.54	3.9	0.2
		10	30	0	0.00	0.00, 0.11		
		20	30	0	0.00	0.00, 0.11		
		40	30	0	0.00	0.00, 0.11		
	8 ppm	5	30	30	1.00	0.89, 1.00	8.8	0.2
		10	30	3	0.10	0.03, 0.26		
		20	30	0	0.00	0.00, 0.11		
		40	30	0	0.00	0.00, 0.11		
	15 ppm	5	30	30	1.00	0.89, 1.00	14.5	0.3
		10	30	30	1.00	0.89, 1.00		
		20	30	0	0.00	0.00, 0.11		
		40	30	0	0.00	0.00, 0.11		
	25 ppm	5	30	30	1.00	0.89, 1.00	21.5	1.8
		10	30	30	1.00	0.89, 1.00		
		20	30	30	1.00	0.89, 1.00		

	45 ppm	40	30	0	0.00	0.00, 0.11	38.0	1.1
		5	30	30	1.00	0.89, 1.00		
		10	30	30	1.00	0.89, 1.00		
		20	30	30	1.00	0.89, 1.00		
		40	30	30	1.00	0.89, 1.00		
	10,000 ppm	5	10	10	1.00	0.72, 1.00	8061.0	-
		10	10	10	1.00	0.72, 1.00		
		20	10	10	1.00	0.72, 1.00		
		40	10	10	1.00	0.72, 1.00		
		40	10	10	1.00	0.72, 1.00		

<sup>a</sup>N = Number of test portions

<sup>b</sup>x = Number of positive test portions

<sup>c</sup>POD<sub>c</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>d</sup>95% Confidence Intervals

**Table 6: GlutenTox Pro Test Kit for Bread – POD Results (1)**

Matrix	Gluten Spiked Level	GlutenTox Pro Detection Threshold (ppm gluten)	N <sup>a</sup>	Candidate			Ave. AOAC OMA 2012.01 results, ppm gluten, N=3	Variance (σ <sup>2</sup> )
				x <sup>b</sup>	POD <sub>c</sub> <sup>c</sup>	95% CI <sup>d</sup>		
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		
		40	30	0	0.00	0.00, 0.11		
	3 ppm	5	30	0	0.00	0.00, 0.11	2.3	0.1
		10	30	0	0.00	0.00, 0.11		
		20	30	0	0.00	0.00, 0.11		
		40	30	0	0.00	0.00, 0.11		
	8 ppm	5	30	30	1.00	0.89, 1.00	7.2	0.1
		10	30	0	0.00	0.00, 0.11		
		20	30	0	0.00	0.00, 0.11		
		40	30	0	0.00	0.00, 0.11		
	15 ppm	5	30	30	1.00	0.89, 1.00	14.0	1.5
		10	30	30	1.00	0.89, 1.00		
		20	30	0	0.00	0.00, 0.11		
		40	30	0	0.00	0.00, 0.11		
	25 ppm	5	30	30	1.00	0.89, 1.00	21.1	2.5
		10	30	30	1.00	0.89, 1.00		
		20	30	30	1.00	0.89, 1.00		
		40	30	0	0.00	0.00, 0.11		
	45 ppm	5	30	30	1.00	0.89, 1.00	38.5	2.4
		10	30	30	1.00	0.89, 1.00		
		20	30	30	1.00	0.89, 1.00		
		40	30	30	1.00	0.89, 1.00		

<sup>a</sup>N = Number of test portions

<sup>b</sup>x = Number of positive test portions

<sup>c</sup>POD<sub>c</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>d</sup>95% Confidence Intervals

**Table 7: GlutenTox Pro Test Kit for Rolled Oat – POD Results (1)**

Matrix	Gluten Spiked Level	GlutenTox Pro Detection Threshold (ppm gluten)	N <sup>a</sup>	Candidate			Ave. AOAC OMA 2012.01 results, ppm gluten, N=3	Variance (σ <sup>2</sup> )
				x <sup>b</sup>	POD <sub>c</sub>	95% CI <sup>d</sup>		
Rolled oat	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		
		40	30	0	0.00	0.00, 0.11		
	3 ppm	5	30	0	0.00	0.00, 0.11	2.7	0.0
		10	30	2	0.07	0.02, 0.21		
		20	30	0	0.00	0.00, 0.11		
		40	30	0	0.00	0.00, 0.11		
	8 ppm	5	30	30	1.00	0.89, 1.00	8.3	1.7
		10	30	0	0.00	0.00, 0.11		
		20	30	0	0.00	0.00, 0.11		
		40	30	0	0.00	0.00, 0.11		
	15 ppm	5	30	30	1.00	0.89, 1.00	12.6	1.0
		10	30	30	1.00	0.89, 1.00		
		20	30	0	0.00	0.00, 0.11		
		40	30	0	0.00	0.00, 0.11		
	25 ppm	5	30	30	1.00	0.89, 1.00	20.4	3.4
		10	30	30	1.00	0.89, 1.00		
		20	30	30	1.00	0.89, 1.00		
		40	30	0	0.00	0.00, 0.11		
45 ppm	5	30	30	1.00	0.89, 1.00	41.0	3.5	
	10	30	30	1.00	0.89, 1.00			
	20	30	30	1.00	0.89, 1.00			
	40	30	30	1.00	0.89, 1.00			

<sup>a</sup>N = Number of test portions

<sup>b</sup>x = Number of positive test portions

<sup>c</sup>POD<sub>c</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>d</sup>95% Confidence Intervals

**Table 8: GlutenTox Pro Test Kit for Pâté – POD Results (1)**

Matrix	Gluten Spiked Level	GlutenTox Pro Detection Threshold (ppm gluten)	N <sup>a</sup>	Candidate			Ave. AOAC OMA 2012.01 results, ppm gluten, N=3	Variance (σ <sup>2</sup> )
				x <sup>b</sup>	POD <sub>c</sub>	95% CI <sup>d</sup>		
Pâté	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		
		40	30	0	0.00	0.00, 0.11		
	3 ppm	5	30	0	0.00	0.00, 0.11	3.0	0.7
		10	30	9	0.30	0.17, 0.48		
		20	30	0	0.00	0.00, 0.11		
		40	30	0	0.00	0.00, 0.11		
	8 ppm	5	30	30	1.00	0.89, 1.00	9.2	0.4
		10	30	0	0.00	0.00, 0.11		
		20	30	0	0.00	0.00, 0.11		
		40	30	0	0.00	0.00, 0.11		
	15 ppm	5	30	30	1.00	0.89, 1.00	16.1	0.4
		10	30	30	1.00	0.89, 1.00		
		20	30	0	0.00	0.00, 0.11		
		40	30	0	0.00	0.00, 0.11		
	25 ppm	5	30	30	1.00	0.89, 1.00	27.6	36.8
		10	30	30	1.00	0.89, 1.00		
		20	30	30	1.00	0.89, 1.00		
		40	30	0	0.00	0.00, 0.11		
45 ppm	5	30	30	1.00	0.89, 1.00	41.0	18.9	
	10	30	30	1.00	0.89, 1.00			
	20	30	30	1.00	0.89, 1.00			
	40	30	30	1.00	0.89, 1.00			

<sup>a</sup>N = Number of test portions

<sup>b</sup>x = Number of positive test portions

<sup>c</sup>POD<sub>c</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>d</sup>95% Confidence Intervals

**Table 9: GlutenTox Pro Test Kit for Yogurt – POD Results (1)**

Matrix	Gluten Spiked Level	GlutenTox Pro Detection Threshold (ppm gluten)	N <sup>a</sup>	Candidate			Ave. AOAC OMA 2012.01 results, ppm gluten, N=3	Variance (σ <sup>2</sup> )
				x <sup>b</sup>	POD <sub>c</sub> <sup>c</sup>	95% CI <sup>d</sup>		
Yogurt	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		
		40	30	0	0.00	0.00, 0.11		
	3 ppm	5	30	0	0.00	0.00, 0.11	3.2	0.0
		10	30	0	0.00	0.00, 0.11		
		20	30	0	0.00	0.00, 0.11		
		40	30	0	0.00	0.00, 0.11		
	8 ppm	5	30	30	1.00	0.89, 1.00	9.3	0.0
		10	30	0	0.00	0.00, 0.11		
		20	30	0	0.00	0.00, 0.11		
		40	30	0	0.00	0.00, 0.11		
	15 ppm	5	30	30	1.00	0.89, 1.00	16.6	2.4
		10	30	30	1.00	0.89, 1.00		
		20	30	0	0.00	0.00, 0.11		
		40	30	0	0.00	0.00, 0.11		
	25 ppm	5	30	30	1.00	0.89, 1.00	24.9	0.5
		10	30	30	1.00	0.89, 1.00		
		20	30	30	1.00	0.89, 1.00		
		40	30	0	0.00	0.00, 0.11		
45 ppm	5	30	30	1.00	0.89, 1.00	38.2	1.5	
	10	30	30	1.00	0.89, 1.00			
	20	30	30	1.00	0.89, 1.00			
	40	30	30	1.00	0.89, 1.00			

<sup>a</sup>N = Number of test portions

<sup>b</sup>x = Number of positive test portions

<sup>c</sup>POD<sub>c</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>d</sup>95% Confidence Intervals

**Table 10: GlutenTox Pro Test Kit Environmental Surface– POD Results (1)**

Matrix (16 cm <sup>2</sup> )	Amount of Spiked Gluten (ng/16 cm <sup>2</sup> )	N <sup>a</sup>	Candidate		
			x <sup>b</sup>	POD <sub>c</sub> <sup>c</sup>	95% CI <sup>d</sup>
Food-grade painted wood	Blank 0	5	0	0.00	0.00, 0.43
	Low 16	30	25	0.83	0.66, 0.93
	High 400	5	5	1.00	0.57, 1.00
Plastic	Blank 0	5	0	0.00	0.00, 0.43
	Low 16	30	23	0.77	0.59, 0.88
	High 400	5	5	1.00	0.57, 1.00
Rubber	Blank 0	5	0	0.00	0.00, 0.43
	Low 16	30	26	0.87	0.70, 0.95
	High 400	5	5	1.00	0.57, 1.00
Sealed Ceramic	Blank 0	5	0	0.00	0.00, 0.43
	Low 16	30	25	0.83	0.66, 0.93
	High 400	5	5	1.00	0.57, 1.00
Stainless steel	Blank 0	5	0	0.00	0.00, 0.43
	Low 16	30	21	0.70	0.52, 0.83
	High 400	5	5	1.00	0.57, 1.00

<sup>a</sup>N = Number of test portions

<sup>b</sup>x = Number of positive test portions

<sup>c</sup>POD<sub>c</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>d</sup>95% Confidence Intervals

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