

# Validation of the GlutenTox® Sticks Plus for Cube Kit Using Various Corn Samples Spiked with Wheat Flour

## Introduction

GlutenTox® Sticks Plus for Cube is a rapid, gluten detection and quantification kit for food and beverage products. This kit contains lateral-flow cassettes with the G12/A1 anti-gliadin antibodies that specifically recognize the 33-mer gluten peptide known to trigger celiac disease. The cassettes are scanned with the Hygiena® Cube Reader, which is an easy-to-use instrument that provides quantitative data for gluten.

This report provides an overview of a validation study showing that results from the test method (GlutenTox Sticks Plus for Cube) are consistent with results from the reference method (GlutenTox® ELISA Rapid G12 kit) for quantification of gluten. The GlutenTox ELISA Rapid G12 kit uses the same G12/A1 antibodies as the test method and has AOAC Research Institute *PTM*<sup>SM</sup> certification (Certificate no. 042301) [1].

Gluten was quantified from unspiked and spiked corn samples using rice flour without or with wheat flour at different concentrations, i.e., 0, 5 or 20 parts per million (ppm\*) of gluten from wheat flour. The higher concentration was chosen because  $\leq 20$  ppm is needed for “gluten-free” labeling in many regions, such as the EU, UK, US and Canada. The lower concentration (5 ppm gluten) was chosen because it is near the limit of quantification (LOQ) of the GlutenTox Sticks Plus for Cube kit (LOQ = 3 ppm gluten).

## Materials and Methods

### Samples

Unspiked samples of corn flour, semolina and grits tested negative for gluten (i.e., <LOQ) using the GlutenTox ELISA Rapid G12 kit (LOQ = 1.2 ppm gluten). Rice flour or a mix of rice flour and different amounts of wheat flour were added to the corn samples (Table 1). The rice flour is naturally gluten-free, while wheat flour naturally contains gluten. The amounts added represented 0, 5 or 20 ppm of gluten in the samples tested.

**Table 1. Study Samples.**

Matrix	Sample Number	Grind	Concentration of Gluten in Samples
Corn flour	F2	Fine	0 ppm 5 ppm 20 ppm
Corn semolina	B2	Medium	
Corn grits	C3	Coarse	
Corn grits	C4	Coarse	

\* ppm = milligrams of gluten per kilogram of food (mg/kg)



## Methods

All kits were used as instructed with the dilution sizes specified in Table 2. In brief, the spiked samples were extracted during a 40-minute incubation at room temperature (20 to 25 °C) and then centrifuged for 10 minutes at 2,500 x g. Each extract was tested in duplicate with each method (i.e., two cassettes with the GlutenTox Sticks Plus for Cube and two wells with the GlutenTox ELISA Rapid G12 kit).

**Table 2. Overview of Gluten Testing Kits.**

Kit*	Product No.	Kit Format	Dilutions Tested in This Study	Method
GlutenTox Sticks Plus for Cube <sup>†</sup>	KIT3007	Lateral flow assay	1:30	Test
GlutenTox ELISA Rapid G12	KIT3075	ELISA	1:20 (0 and 5 ppm gluten) 1:50 (20 ppm gluten)	Reference

\* All kits were manufactured by Hygiena Diagnóstica España.

† The Hygiena Cube Reader (Product No. ASY3206) was used to scan the GlutenTox Sticks Plus lateral flow cassettes.

## Results and Discussion

Table 3 shows the average quantification results of gluten detected in unspiked samples and samples spiked with 5 or 20 ppm of gluten. All unspiked samples produced results that were <LOQ for both kits.

When samples spiked with 5 and 20 ppm of gluten were analyzed, results obtained with the GlutenTox Sticks Plus for Cube and the GlutenTox ELISA Rapid G12 assays showed comparable results, as the percentage of the coefficient of variation (% CV) was 20% or less. In samples spiked with 5 ppm gluten and tested using the ELISA and lateral flow assays, the close similarity in the results (several in the 1 – 2 % CV range) is especially remarkable, demonstrating strong similarities in gluten quantification.

**Table3. Average Quantification (ppm) of Gluten in Unspiked and Spiked Corn Samples.**

Kit	0 ppm Gluten*				5 ppm Gluten				20 ppm Gluten			
	Flour F2	Semolina B2	Grits C3	Grits C4	Flour F2	Semolina B2	Grits C3	Grits C4	Flour F2	Semolina B2	Grits C3	Grits C4
GlutenTox Sticks Plus for Cube	—	—	—	—	5.6	5.65	6.75	5.95	16.5	15.85	13.65	17.7
GlutenTox ELISA Rapid G12	—	—	—	—	5.7	4.66	6.53	5.81	19.26	19.26	18.04	23.00
% CV	—	—	—	—	1%	14%	2%	2%	11%	14%	20%	18%

\* — = not detected (<LOQ)



Table 4 shows the average percent recovery in unspiked samples and samples spiked with 5 or 20 ppm of gluten. All recoveries are in the acceptable range of 50 – 150% (Range: GlutenTox Sticks Plus for Cube, 68 – 135% and GlutenTox ELISA Rapid G12, 90 – 131%) [1 – 2].

**Table 4. Average Recovery (%) for Gluten in Unspiked and Spiked Corn Samples.**

Kit	0 ppm Gluten*				5 ppm Gluten				20 ppm Gluten			
	Flour F2	Semolina B2	Grits C3	Grits C4	Flour F2	Semolina B2	Grits C3	Grits C4	Flour F2	Semolina B2	Grits C3	Grits C4
GlutenTox Sticks Plus for Cube	—	—	—	—	112	113	135	119	83	79	68	89
GlutenTox ELISA Rapid G12	—	—	—	—	114	93	131	116	96	96	90	115

\* — = not detected (<LOQ)

## Conclusions

The gluten quantification results showed a very strong correlation between the test method (lateral flow, GlutenTox Sticks Plus for Cube) and reference method (ELISA, GlutenTox ELISA Rapid G12) for all three types of corn matrices (flour, semolina and grits), with and without gluten (0, 5 or 20 ppm). No false negatives or false positives were observed with either of the methods used in this study.

Therefore, the GlutenTox Sticks Plus for Cube method can be used for gluten quantification for routine product testing of corn matrices. Compared to using ELISA kits, the food and beverage industry will find that GlutenTox Sticks Plus for Cube provides faster results with a simpler protocol while delivering similar results. Nevertheless, for gluten-free labeling purposes, sample results should be confirmed periodically for gluten by an ISO 17025-accredited laboratory.

## References

- Galera C, Salagre C and López A. (2023) Validation of the GlutenTox® ELISA Rapid G12 Test Kit for Determination of Gluten in Select Non-Heat-Processed Matrixes and Heat-Processed Matrixes: AOAC *Performance Tested Method*<sup>SM</sup> 042301. *J AOAC Int.* 106(6): 1478 – 1504.
- AOAC International (2023) Guidelines for Validation of Quantitative Gluten Methods, with Specific Examples for ELISA Assays, First Round. <https://griegler-aoac-org.cld.bz/AOAC-Gluten-Quantitative-Validation-Guidance-Round1-Nov2023>.