Comparison of ELISA Performance in the Detection and Quantification of Gluten in Common Food Matrices

Introduction

Detection and quantification of Gluten in food products poses many challenges. First, the gluten contamination can take place at any level in food production from the field to the shelf during harvesting, transportation and processing. Second, in locations such as bakeries, where gluten is present ubiquitously, it is important to have separate production lines. Lastly, food must contain < 20 mg/kg (ppm) of gluten to be considered “gluten-free”.

In order to assess the levels of gluten in food products, analysis is typically performed using antibody-based enzyme-linked immunosorbent assays (ELISAs), to provide quantitative results in hours. This method is mainly based on the R5 and G12 gluten antibodies.

In all methods for gluten analysis in any given sample, the gluten first has to be extracted from the sample’s matrix. This is the case for ELISA methods too; the extracted samples are added to antibody-coated wells on the plate (capture antibody) where gliadin will bind to the antibody during an incubation period. After washing, a second antibody, which is enzyme-labeled (detection antibody), is added. Unbound antibody is washed away and substrate is added. Color develops and a stopping reagent is added. The optical densities of the controls, standard curve, and the sample are plotted against the curve to calculate the exact concentration of gliadin in parts per million (ppm).

What follows is a comparison of four common ELISA methods for gluten detection in a variety of food matrices (including extraction steps). The ELISA kits are GlutenTox® ELISA Rapid G12, AgraQuant®Gluten G12™, Ridascreen® Gliadin and Veratox® Gliadin R5.

Methods

Summary of ELISA Methods Tested

Kit ➤	AgraQuant® Gluten G12™	Veratox® for Gliadin R5	Ridascreen® Gliadin	GlutenTox® ELISA Rapid G12
Reference	10002002 (COKAL0248)	8510	R7001	KIT3075
Antibody	G12/G12	R5/R5	R5/R5	G12/A1
Method	Sandwich	Sandwich	Sandwich	Sandwich
Standards	Ready to use	Ready to use	Ready to use	Ready to use
	Gliadin	Gliadin	Gliadin	Gliadin
LOD	2 ppm gluten	2.5 ppm gliadin (5 ppm gluten)	0.5 ppm gliadin - 1 ppm gluten (matrix dependent)	0.3 ppm gluten / 0.15 ppm gliadin
LOQ	4 ppm gluten	2.5 ppm	2.5 ppm gliadin - 5 ppm gluten	0.6 ppm gluten / 0.3 ppm gliadin
Range	4 ppm - 200 ppm	2.5 - 40 ppm	5 - 80 ppm gluten	0.6 ppm - 200 ppm
Sample extraction time	110 min	20 min - 120 min (heat-processed)	120 min	50 min
Analysis time	60 min + washes	30 min + washes	90 min + washes	90 min + washes
Total time	170 min +	50 - 150 min +	210 min +	140 min +
Extraction solution	Contains toxic reagents	WARNING: This product can expose you to chemicals including thimerosal, which is known in the State of California to cause birth defects or other reproductive harm.	Contains toxic reagents - beta-mercaptoethanol (not provided in the kit)	UGES (Universal Gluten Extraction Solution) Non-toxic (provided)
Sample	0.25 g	1 g	0.25 g	0.5 g
Limitations	High LOD/LOQ	Requires renaturing cocktail for heat-processed samples	The extraction cocktail is not provided with the kit. Samples with chocolate, coffee, cocoa, chestnut flour, buckwheat, millet and spices need skimmed milk powder	

Methods

Sample Extraction

For solid samples or complex matrices, extraction is essential for the proper detection and quantification of gliadin/gluten. Each sample was spiked with the appropriate level of gluten and then was extracted once. Extracted samples were then tested in duplicate, obtaining two data points per sample and gluten contamination level. The sample extraction procedures for each kit varied somewhat and are summarized below.

AgraQuant® Gluten G12™ Sample: solid or liquid	**Ridascreen® Gliadin** Sample: solid or liquid	**Veratox® for Gliadin R5** Sample: heat-processed or unknown origin	**Veratox® for Gliadin R5** Sample: non-heat-processed	**GlutenTox® ELISA Rapid G12** Sample: solid/semi-solid/liquid non-heat or heat processed
Add extraction buffer and vortex	Add cocktail and mix	Add renaturing cocktail solution and vortex 30 sec	Add extraction additive and ethanol and vortex 30 sec	Add extraction solution and vortex
Incubate 40 min at 50°C. Vortex at regular intervals	Incubate 40 min at 50°C. Vortex at regular intervals	Incubate 40 min at 50°C	Incubate 10 min at RT on orbital shaker or rotator	Incubate 40 min at 50°C. Mix/vortex periodically
Cool, add ethanol, vortex	Cool, add ethanol, shake	Cool, add ethanol, vortex		
Incubate 60 min at RT in lab shaker. Vortex if clumping	Incubate 60 min at RT with shaking/rotating	Incubate 60 min at RT with shaking on a rotator		
Centrifuge 10 min at 2000 x g	Centrifuge 10 min at 2500 x g	Centrifuge 10 min at 2500 x g	Centrifuge 10 min at 2500 x g	If solid, centrifuge 10 min at 2500 x g
Dilute 1:10 and vortex	Dilute 1:12.5 and mix	Dilute 1:12.5 and vortex	Dilute 1:50 and vortex	Dilute, if necessary, 1:50 to 1:200

Test Procedure

For detection and quantification of gliadin/gluten by ELISA, each kit has similar steps for sample dilution, antibody binding, and visualization and quantification, taking from 90 to 180 minutes depending on antibody and substrate incubation times. Each assay has three incubations and three washes before the addition of stop solution so the results can be read on a microtiter plate reader at a specified wavelength.

1. Add sample
2. Wash microplate
3. Add detection antibody
4. Wash microplate
5. Add substrate
6. Read microplate
7. Calculate results

Results and Discussion

Extraction Solution

GlutenTox ELISA Rapid G12 uses a patented (ES2 393 412 A1) UGES (extraction solution) based on reducing, disaggregating, and solubilizing reagents in an aqueous ethanol solution. UGES is an odorless extraction solution and has no toxicity to the user and the environment in contrast to the extraction solution used in the other kits. (1)

Kit Performance

Summary

The overall standard ELISA duration including gluten extraction is faster when GlutenTox® ELISA Rapid G12 is used.

Comparison

When each of these four kits were used to test a wide variety of matrices containing gluten at concentrations of 5 ppm to 80 ppm, similar results were obtained. Values were calculated as a % recovery of the gluten amount present (**Table 1**). **Note:** The Veratox® Gliadin R5 kit was not used (n/d) for matrices containing ≥80 ppm of gluten as it is above the range of quantification for the Neogen® kit. A second Veratox Gliadin kit was also used in the study (Kit 8480, data not shown. All recovery date obtained with this kit was >150%).

Table 1: Gluten/Gliadin Recovery Per Matrix (% Recovery)

Average Recovery per Matrix				
Matrix	**AgraQuant® Gluten G12™**	**Ridascreen® Gliadin**	**Veratox®**	**GlutenTox® Rapid G12**
Bread	141%	138%	117%	115%
Extruded Rice Crackers	153%	132%	209%	137%
Soy Flour	125%	120%	175%	133%
Rolled Oats	146%	136%	127%	109%
Seasoning Mix	143%	117%	190%	111%
Chicken & Turkey Sausages	148%	142%	125%	120%
Chocolate	73%	102%	n/d	76%
Yogurt	127%	120%	n/d	101%
Biscuits	153%	103%	n/d	117%
Soy Sauce	109%	107%	n/d	111%

Detection

When evaluating spiked gluten levels in the various matrices shown in Table 1, results are similar among all the kits tested. When comparing the average recovery per level of gluten spiked in any matrix, results again were comparable among spike levels (**Table 2**). However, the GlutenTox® Rapid G12 assay showed results closest to the gluten spiked levels overall.

Table 2: Average Recovery Per Gluten Spiked Level

Average Recovery per Gluten Spiked Level				
Gluten Spiked Level	**AgraQuant® Gluten G12™**	**Ridascreen® Gliadin**	**Veratox®**	**GlutenTox® Rapid G12**
5 ppm	99.1%	123%	179%	114%
10 ppm	108%	115%	149%	114%
20 ppm	115%	117%	140%	114%
40 ppm	154%	118%	160%	110%
80 ppm	191%	135%	n/a	113%

Conclusions

This study compares four gluten test kits - two that utilize the R5 antibody and two kits that use the G12 antibody - for the determination of gluten in a wide range of spiked levels in a wide variety of matrix types. The results in Table 1 demonstrate the equivalency of each antibody and test kit for the determination of gluten in matrices including bread, chocolate, soy and poultry. In addition, when analyzed collectively over a range of spiked gluten levels, the GlutenTox® ELISA Rapid G12 kit provided gluten value results most closely matching the actual spiked levels. This demonstrates that the GlutenTox® ELISA Rapid G12 kit is a suitable choice for the determination of gluten in food products in food handling facilities and performs as accurately as the currently endorsed reference method (i.e., R5 ELISA). The main advantages of the GlutenTox method are:

1) It performs faster in comparison to the other methods.
2) It does not use potentially dangerous reagents such as the cocktail solution of the R5 method.

References

(1) Segura, V.; Díaz, J.; Ruiz-Carnicer, Á.; Muñoz-Suano, A.; Carrillo-Carrión, C.; Sousa, C.; Cebolla, Á.; Comino, I. Rapid, Effective, and Versatile Extraction of Gluten in Food with Application on Different Immunological Methods. *Foods* 2021, *10*, 652, doi:10.3390/foods10030652.