Comparison of Manual and Fully Automated ELISA Test Procedures using Hygiena® GlutenTox® ELISA Rapid G12 Assay

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INTRODUCTION:

Automation has increasingly become an essential requirement for laboratories due to time and cost constraints. It plays an important role in the automation of ELISA assays, especially in high-throughput or regulated laboratory environments. Manual ELISA workflows are time-consuming and prone to variability due to human error, which can affect reproducibility and data quality. Automated systems streamline key steps—such as reagent dispensing, incubation timing, washing, and plate reading—ensuring consistency, improving throughput, and reducing hands-on time. As assay complexity and sample volumes grow, automation enhances both efficiency and reliability, making it a useful choice for a laboratory's workflows.

PURPOSE:

The purpose of this study was to compare the performance of a gluten ELISA assay using a manual protocol and a fully automated platform.

METHOD:

A high-level concentration of gluten was prepared from wheat flour to artificially inoculate five matrices: soy flour, cornbread, rolled oats, seasoning mix and evaporated milk. After the gluten was added, matrices were thoroughly mixed until fully incorporated. Serial dilutions were created to obtain final contamination levels of 0, 5, 10 and 20 ppm. From each level, 6 x 0.5 g test portions were extracted with the extraction solution (UGES) (1), at room temperature (soy flour, rolled oats, seasoning mix and evaporated milk) or at 50ºC in a water bath (cornbread), for 40 minutes, and tested following the ELISA assay instructions using both manual and automation protocols.

Reference Material
Material used for contamination (wheat flour) was procured from a retail source and was independently characterized for total protein content.

Conversion factor of wheat used:
• Wheat: 0.80
High-concentration stocks of gluten from wheat flour were prepared in each food matrix. Each stock underwent a mixing process repeated four times to achieve a homogeneous distribution of the contaminant within the food matrix. Using a stepwise dilution scheme, these stocks were diluted to prepare gluten-contaminated matrix batches for each level being tested (0, 5, 10 and 20 ppm of gluten from wheat flour). Every contaminated matrix batch went through the same mixing process (as the high concentration stocks) four times.

RESULTS:

The concentration of gluten in each replicate sample was obtained from the OD_{450} measurements compared to the standards.

The evaluation of sample extracts for gluten concentration and recovery produced average values for each matrix, as shown in Tables 1-5.

All calculated results from the samples were at the targeted inoculation levels (0, 5, 10 and 20 ppm) with high reproducibility and within the AOAC performance requirements for recovery, ranging from 50 – 150% (2).

Results between manual and automated test procedures were assessed using the relative standard deviation (RSD).

All percentages for repeatability for the same samples were within acceptable limits in all cases (<20%), most of them within the range of 0.07 to 4.7% (in all matrices, at different concentrations of gluten), with the highest variation being 12% (rolled oats and corn bread, at 5 ppm gluten) (Tables 1-5).

Table 1. Comparison of Automated and Manual ELISA Test Procedures with Soy Flour (Mean Values).

Soy Flour									
Gluten Concentration (ppm)	Automated				Manual				Automated vs Manual
	Concentration (ppm)	SDr*	% Recovery	SDr	Concentration (ppm)	SDr	% Recovery	SDr	RSDr†
0	<LOQ**	-	<LOQ	-	<LOQ	-	<LOQ	-	-
5	5.641	0.544	112.810	10.875	5.891	0.211	117.810	4.220	**3.066**
10	10.467	0.766	104.670	7.656	11.725	1.029	117.252	10.287	**8.018**
20	20.700	1.055	103.500	5.275	22.720	1.731	113.600	8.654	**6.579**

SDr** = standard deviation of repeatability; †**RSDr** = relative standard deviation of repeatability; **LOQ* = limit of quantification.

Table 2. Comparison of Automated and Manual ELISA Test Procedures with Rolled Oats (Mean Values).

Rolled Oats									
Gluten Concentration (ppm)	Automated				Manual				Automated vs Manual
	Concentration (ppm)	SDr*	% Recovery	SDr	Concentration (ppm)	SDr	% Recovery	SDr	RSDr†
0	<LOQ**	-	<LOQ	-	<LOQ	-	<LOQ	-	-
5	6.811	0.716	136.220	14.322	5.698	0.359	113.956	7.187	**12.585**
10	10.532	1.029	105.318	10.295	9.131	1.126	91.315	11.262	**10.071**
20	20.724	3.562	103.618	17.809	20.851	1.006	104.256	5.028	**0.434**

SDr** = standard deviation of repeatability; †**RSDr** = relative standard deviation of repeatability; **LOQ* = limit of quantification.

Table 3. Comparison of Automated and Manual ELISA Test Procedures with Evaporated Milk (Mean Values).

Evaporated Milk									
Gluten Concentration (ppm)	Automated				Manual				Automated vs Manual
	Concentration (ppm)	SDr*	% Recovery	SDr	Concentration (ppm)	SDr	% Recovery	SDr	RSDr†
0	<LOQ**	-	<LOQ	-	<LOQ	-	<LOQ	-	-
5	3.264	0.096	65.273	1.924	3.127	0.035	62.539	0.693	**3.026**
10	6.575	0.149	65.752	1.491	6.582	0.107	65.821	1.069	**0.074**
20	14.381	0.403	71.906	2.015	14.313	0.543	71.564	2.716	**0.337**

SDr** = standard deviation of repeatability; †**RSDr** = relative standard deviation of repeatability; **LOQ* = limit of quantification.

Table 4. Comparison of Automated and Manual ELISA Test Procedures with Cornbread (Mean Values).

Cornbread									
Gluten Concentration (ppm)	Automated				Manual				Automated vs Manual
	Concentration (ppm)	SDr*	% Recovery	SDr	Concentration (ppm)	SDr	% Recovery	SDr	RSDr†
0	<LOQ**	-	<LOQ	-	<LOQ	-	<LOQ	-	-
5	7.417	1.245	148.340	24.902	6.197	0.731	123.949	14.618	**12.668**
10	10.443	0.820	104.432	8.195	9.770	0.684	97.704	6.841	**4.707**
20	19.673	2.490	98.365	12.449	20.181	0.688	100.907	3.439	**1.804**

SDr** = standard deviation of repeatability; †**RSDr** = relative standard deviation of repeatability; **LOQ* = limit of quantification.

Table 5. Comparison of Automated and Manual ELISA Test Procedures with Seasoning Mix (Mean Values).

Seasoning Mix									
Gluten Concentration (ppm)	Automated				Manual				Automated vs Manual
	Concentration (ppm)	SDr*	% Recovery	SDr	Concentration (ppm)	SDr	% Recovery	SDr	RSDr†
0	<LOQ**	-	<LOQ	-	<LOQ	-	<LOQ	-	-
5	5.017	0.520	100.337	10.392	4.369	0.223	87.387	4.457	**9.756**
10	9.875	0.824	98.747	8.238	9.508	0.726	95.081	7.260	**2.675**
20	20.812	1.219	104.062	6.095	21.050	1.155	105.252	5.774	**0.804**

SDr** = standard deviation of repeatability; †**RSDr** = relative standard deviation of repeatability; **LOQ* = limit of quantification.

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CONCLUSION:

The AOAC RI PTM #042301 certified GlutenTox ELISA Rapid G12 assay (3) showed that it can be consistently used in a fully automated ELISA analyzer with reproducible quality.
This ELISA method is recommended for laboratories demanding automated ELISA solutions for the detection and quantification of gluten in food samples.