



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
042301

The AOAC Research Institute hereby certifies the method known as:

GlutenTox ELISA Rapid G12

manufactured by

Hygiene Diagnóstica España
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This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*SM Program and found to perform as stated in the applicability of the method. 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.

A handwritten signature in black ink that reads "Scott Coates".

Scott Coates, Senior Director
Signature for AOAC Research Institute

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|-----------------|-------------------|
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| METHOD NAME GlutenTox ELISA Rapid G12 | CATALOG NUMBER KIT3075 |
| INDEPENDENT LABORATORY Q Laboratories 1930 Radcliff Drive Cincinnati, OH 45204 | APPLICABILITY OF METHOD Target analyte – Gluten from wheat, barley, and rye flour Matrixes – (0.5 g) – soy flour, corn bread, seasoning mix, rolled oats, evaporated milk, and gluten free baked bread Performance claims – The GlutenTox ELISA Rapid G12 test kit is designed to detect and quantify gluten in processed and non-processed foods listed above at a range of 1.2 - 200 mg/kg gluten. This range of quantitation is suitable for proposed gluten-free monitoring in the United States and is compliant with current EU regulations and Codex Alimentarius definitions. |
| ORIGINAL CERTIFICATION DATE April 12, 2023 | CERTIFICATION RENEWAL RECORD Renewed annually through December 2024. |
| METHOD MODIFICATION RECORD NONE | SUMMARY OF MODIFICATION NONE |
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PRINCIPLE OF THE METHOD (1)

The GlutenTox ELISA Rapid G12 method is a sandwich ELISA assay that can be used to detect and quantify gluten in food samples.

To solubilize the gluten present in the sample's matrix, the extraction solution (UGES) provided in the kit is added to the food sample.

After the extraction, the sample's extract is added to a multi-well plate coated with a monoclonal anti-gliadin antibody (G12) that specifically recognizes the most immunogenic fraction of gluten. After the washing steps, the addition of a second monoclonal anti-gliadin antibody conjugated to HRP (A1-HRP) and the substrate solution (TMB) will allow to measure the signal (color change). GlutenTox ELISA Rapid G12 is a direct method. The higher the concentration of gluten present in the sample, the more intense the signal will be.

DISCUSSION OF THE VALIDATION STUDY (1)

The GlutenTox ELISA Rapid G12 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*” (1) or those added to the AOAC Research Institute Performance Tested MethodsSM program: *PTM validation of the GlutenTox ELISA Rapid G12 Kit in Select Foods*, version 10, 27 Sep 2021 used in the production of gluten-free products. Four compounds that had tested positive in the prescreening evaluation with the AOAC OMA 2012.01 method (6) (oat flour, romano bean flour, fava bean flour and Lima bean flour) also tested >LOQ with the GlutenTox ELISA Rapid G12 test kit and were not retested. For the sake of finding out if the positivity of these matrices was due to a gluten contamination during the manufacturing process or to a cross-reaction, further analysis was carried out using the same matrices in bean format (and rolled oats) and grinding them in the lab before performing the tests to minimize the risk of a gluten contamination. Definitely, the GlutenTox ELISA Rapid test kit did not show cross reactivity with these matrices. Therefore, it can be confirmed that the previous positive results were due to a gluten contamination. The GlutenTox ELISA Rapid G12 assay also did not show any interference, when tested with the required compounds for testing in the presence of gluten. No unexpected results were obtained (the lima bean matrix included in the interference study was that in bean format and subsequently ground).

The GlutenTox ELISA Rapid G12 test kit performed as expected when 6 additional wheat flour varieties were tested in rice flour and positive results were obtained in all wheat cultivars analyzed. However, with the Einkorn Wheat Flour (*Triticum monococcum*) variety, a recovery result lower than expected was obtained. Further studies would be needed to determine if this is due to a lower gluten:protein ratio.

The GlutenTox ELISA Rapid G12 test kit performed as expected in the selected food matrixes (gluten free soy flour, corn bread, seasoning mix, rolled oats and evaporated milk), spike levels of gluten with wheat flour, and in both Hygiene (method developer) and the independent laboratory (only the corn bread and seasoning mix matrixes were tested), obtaining comparable results.

In all matrixes tested at different spike levels with barley and rye flours, the GlutenTox ELISA Rapid G12 assay performed as expected (meeting performance claims for recovery and repeatability, mainly with barley flour) or showing slight (<28%) to moderate (46% or 85%) overestimation depending on the matrix, source of gluten contaminant and gluten concentration.

Results obtained in the method developer incurred matrix study with wheat, barley and rye flours indicate that the assay performed as expected (meeting performance claims for recovery and repeatability, mainly with wheat and rye flours) or showing slight (25% or 37%) to moderate (49%) overestimation depending on the source of gluten contaminant and gluten concentration. These data are comparable to those obtained in the incurred sample study of the independent laboratory where the GlutenTox ELISA Rapid G12 method performed as expected with wheat and rye flours and showed a slight (11%) to moderate (77%) overestimation with barley at 20 mg/kg and 30 mg/kg spike levels of gluten, respectively.

Nevertheless, this occasional overestimation of gluten from barley or rye is less important factor in gluten analysis for the people suffering from celiac disease, since possible problems from false negatives or underestimations could be much worse.

No false negative results were observed in the entire validation study.

The GlutenTox ELISA Rapid G12 assay performed as expected in the calibration study in all dilutions. To minimize the trend of a non-random pattern found in the higher analyte concentrations of each dilution, a suitable dilution should be made according to the expected amount of gluten in the sample.

The intermediate precision study demonstrated that the design 2b and the contribution of the Analyst/Day/Calibration as a single confounded factor to the variance were appropriate when the GlutenTox ELISA Rapid G12 assay was tested with the incurred bread matrix.

In this study the overall RSD for the method was in accordance with the acceptance criteria, even was mathematically reduced by over 4% when the variance of the ELISA component was divided by the number of replicates tested (two ELISA wells per test portion).

The overall LOQ_{est} validated of the GlutenTox ELISA Rapid G12 test kit by the method developer in the selected matrixes performed as expected, showing an excellent correlation with the overall LOD-LOQ_{est} (according to the standard deviation of blank samples). These results are in line and are consistent with the LOD and LOQ values obtained from the independent laboratory (calculated from the linear regression model) using three matrixes and four spike concentration levels of gluten from wheat flour (LOD = 0.4 mg/kg gluten and LOQ = 1.2 mg/kg gluten).

Table 2: GlutenTox ELISA Rapid G12-Food matrix study with Wheat Flour from candidate and from independent laboratory (analyst 1 and analyst 2) (1)

| Matrix | Target Contamination Level (mg/kg) | Candidate | | | | |
|-----------------------------------|------------------------------------|-------------------------------------|----------|--------|----------------|------------------|
| | | Mean Concentration Obtained (mg/kg) | Recovery | Bias | S _r | RSD _r |
| Soy flour | 0 | -0.05 | < LOD | -0.05 | 0.187 | -410.941 |
| | 5 | 4.51 | 90.25 | -0.49 | 0.648 | 14.361 |
| | 10 | 7.63 | 76.32 | -2.37 | 1.031 | 13.519 |
| | 20 | 19.46 | 97.30 | -0.54 | 1.719 | 8.833 |
| Corn Bread | 0 | -0.14 | < LOD | -0.14 | 0.271 | -190.988 |
| | 5 | 6.20 | 123.95 | 1.20 | 0.730 | 11.793 |
| | 10 | 9.77 | 97.70 | -0.23 | 0.684 | 7.001 |
| | 20 | 20.18 | 100.91 | 0.18 | 0.687 | 3.408 |
| Seasoning Mix | 0 | 0.07 | < LOD | 0.07 | 0.190 | 280.276 |
| | 5 | 4.37 | 87.39 | -0.63 | 0.222 | 5.099 |
| | 10 | 9.51 | 95.08 | -0.49 | 0.725 | 7.635 |
| Rolled Oats | 0 | -0.05 | < LOD | -0.05 | 0.170 | -370.012 |
| | 5 | 5.70 | 113.96 | 0.70 | 0.359 | 6.307 |
| | 10 | 9.13 | 91.31 | -0.87 | 1.126 | 12.333 |
| | 20 | 20.85 | 104.26 | 0.85 | 1.005 | 4.822 |
| Evaporated Milk | 0 | 0.28 | < LOD | 0.28 | 0.029 | 10.294 |
| | 5 | 3.13 | 62.54 | -1.87 | 0.035 | 1.107 |
| | 10 | 6.58 | 65.82 | -3.42 | 0.107 | 1.624 |
| | 20 | 14.31 | 71.56 | -5.69 | 0.543 | 3.796 |
| Independent laboratory, analyst 1 | | | | | | |
| Matrix | Target Contamination Level (mg/kg) | Mean Concentration Obtained (mg/kg) | Recovery | Bias | S _r | RSD _r |
| Corn Bread | 0 | 0.328 | / | 0.328 | 0.015 | 4.482 |
| | 5 | 4.755 | 95.10 | -0.245 | 0.520 | 10.938 |
| | 10 | 7.643 | 76.43 | -2.357 | 0.570 | 7.452 |
| | 20 | 20.877 | 104.39 | 0.877 | 2.473 | 11.844 |
| Seasoning Mix | 0 | 0.197 | / | 0.197 | 0.029 | 14.638 |
| | 5 | 3.979 | 79.589 | -1.021 | 0.182 | 4.582 |
| | 10 | 11.578 | 115.780 | 1.578 | 0.369 | 3.189 |
| 20 | 21.268 | 106.340 | 1.268 | 1.320 | 6.206 | |
| Independent laboratory, analyst 2 | | | | | | |
| Matrix | Target Contamination Level (mg/kg) | Mean Concentration Obtained (mg/kg) | Recovery | Bias | S _r | RSD _r |
| Corn Bread | 0 | 0.293 | / | 0.293 | 0.029 | 9.814 |
| | 5 | 4.683 | 93.667 | -0.317 | 0.182 | 3.894 |
| | 10 | 7.561 | 75.617 | -2.438 | 0.566 | 7.486 |
| | 20 | 20.916 | 104.580 | 0.916 | 2.431 | 11.623 |
| Seasoning Mix | 0 | 0.163 | / | 0.163 | 0.035 | 21.443 |
| | 5 | 4.165 | 78.833 | -1.073 | 0.164 | 4.165 |
| | 10 | 11.452 | 114.517 | 1.452 | 0.314 | 2.739 |
| | 20 | 20.984 | 104.922 | 0.984 | 1.559 | 7.430 |

Table 3: GlutenTox ELISA Rapid G12-Food matrix study with Barley and Rye Flours from candidate (1)

| Matrix/Contaminant | Target Contamination Level (mg/kg) | Candidate | | | | |
|---------------------------------|------------------------------------|-------------------------------------|----------|--------|----------------|------------------|
| | | Mean Concentration Obtained (mg/kg) | Recovery | Bias | S _r | RSD _r |
| Soy flour Barley flour | 0 | 0.098 | < LOD | 0.098 | 0.059 | 60.345 |
| | 10 | 15.374 | 153.736 | 5.374 | 0.978 | 6.359 |
| | 20 | 31.779 | 158.897 | 11.779 | 1.276 | 4.014 |
| Corn Bread Barley flour | 0 | 0.098 | < LOD | 0.098 | 0.041 | 41.214 |
| | 10 | 13.083 | 130.832 | 3.083 | 2.051 | 15.675 |
| | 20 | 34.097 | 170.484 | 14.097 | 4.684 | 13.738 |
| Seasoning Mix Barley flour | 0 | 0.093 | < LOD | 0.093 | 0.098 | 105.809 |
| | 10 | 8.067 | 80.674 | -1.933 | 0.123 | 1.529 |
| | 20 | 17.307 | 86.534 | -2.693 | 0.896 | 5.176 |
| Rolled Oats Barley flour | 0 | 0.114 | < LOD | 0.114 | 0.068 | 59.686 |
| | 10 | 13.076 | 130.760 | 3.076 | 1.198 | 9.162 |
| | 20 | 28.776 | 143.880 | 8.776 | 2.656 | 9.231 |
| Evaporated Milk Barley flour | 0 | 0.039 | < LOD | 0.039 | 0.023 | 59.237 |
| | 10 | 18.737 | 187.370 | 8.737 | 1.408 | 7.516 |
| | 20 | 43.888 | 219.441 | 23.888 | 3.981 | 9.071 |

| Matrix/Contaminant | Target Contamination Level (mg/kg) | Candidate | | | | |
|------------------------------|------------------------------------|-------------------------------------|----------|--------|----------------|------------------|
| | | Mean Concentration Obtained (mg/kg) | Recovery | Bias | S _r | RSD _r |
| Soy flour Rye flour | 0 | 0.138 | < LOD | 0.138 | 0.040 | 28.893 |
| | 10 | 18.206 | 182.064 | 8.206 | 3.064 | 16.829 |
| | 20 | 35.376 | 176.878 | 15.376 | 1.501 | 4.244 |
| Corn Bread Rye flour | 0 | 0.234 | < LOD | 0.234 | 0.074 | 31.512 |
| | 10 | 19.220 | 192.199 | 9.220 | 1.378 | 7.171 |
| | 20 | 55.572 | 277.861 | 35.572 | 7.895 | 14.206 |
| Seasoning Mix Rye flour | 0 | 0.086 | < LOD | 0.086 | 0.054 | 62.831 |
| | 10 | 18.524 | 185.243 | 8.524 | 3.054 | 16.484 |
| | 20 | 33.961 | 169.806 | 13.961 | 6.723 | 19.797 |
| Rolled Oats Rye flour | 0 | 0.125 | < LOD | 0.125 | 0.107 | 86.002 |
| | 10 | 13.890 | 138.901 | 3.890 | 1.803 | 12.977 |
| | 20 | 32.382 | 161.912 | 12.382 | 2.613 | 8.069 |
| Evaporated Milk Rye flour | 0 | 0.125 | < LOD | 0.125 | 0.095 | 76.082 |
| | 10 | 18.129 | 181.292 | 8.129 | 0.183 | 1.008 |
| | 20 | 36.231 | 181.157 | 16.231 | 0.952 | 2.628 |

Table 4: GlutenTox ELISA Rapid G12 – Incurred matrix study from candidate and from independent laboratory (analyst 1 and analyst 2) (1)

| Matrix/Contaminant | Target Contamination Level (mg/kg) | Candidate | | | | |
|-----------------------------------|------------------------------------|-------------------------------------|----------|---------|----------------|------------------|
| | | Mean Concentration Obtained (mg/kg) | Recovery | Bias | S _r | RSD _r |
| Baked Bread Wheat flour | 0 | 0.084 | < LOD | 0.084 | 0.033 | 39.11 |
| | 20 | 13.976 | 69.882 | -6.024 | 1.125 | 8.047 |
| | 30 | 21.276 | 70.921 | -8.724 | 1.909 | 8.974 |
| Baked Bread Barley flour | 0 | 0.080 | < LOD | 0.080 | 0.077 | 95.75 |
| | 20 | 41.143 | 205.717 | 21.143 | 7.191 | 17.478 |
| | 30 | 67.282 | 224.273 | 37.282 | 8.953 | 13.307 |
| Baked Bread Rye flour | 0 | 0.078 | < LOD | 0.078 | 0.041 | 52.89 |
| | 20 | 37.540 | 187.699 | 17.540 | 6.943 | 18.494 |
| | 30 | 43.646 | 145.486 | 13.646 | 7.553 | 17.305 |
| Independent laboratory, analyst 1 | | | | | | |
| Matrix/Contaminant | Target Contamination Level (mg/kg) | Mean Concentration Obtained (mg/kg) | Recovery | Bias | S _r | RSD _r |
| Baked Bread Wheat flour | 0 | 0.141 | / | 0.141 | 0.035 | 24.992 |
| | 20 | 10.576 | 52.881 | -9.424 | 1.077 | 10.179 |
| | 30 | 16.650 | 55.501 | -13.350 | 1.639 | 9.846 |
| Baked Bread Barley flour | 0 | 0.141 | / | 0.141 | 0.035 | 24.992 |
| | 20 | 33.151 | 165.755 | 13.151 | 5.121 | 15.446 |
| | 30 | 79.338 | 264.460 | 49.338 | 17.988 | 26.673 |
| Baked Bread Rye flour | 0 | 0.141 | / | 0.141 | 0.035 | 24.992 |
| | 20 | 27.954 | 139.768 | 7.954 | 5.651 | 20.214 |
| | 30 | 40.598 | 135.325 | 10.578 | 5.024 | 12.374 |
| Independent laboratory, analyst 2 | | | | | | |
| Matrix/Contaminant | Target Contamination Level (mg/kg) | Mean Concentration Obtained (mg/kg) | Recovery | Bias | S _r | RSD _r |
| Baked Bread Wheat flour | 0 | 0.096 | / | 0.096 | 0.032 | 32.787 |
| | 20 | 10.654 | 53.271 | -9.346 | 1.082 | 10.156 |
| | 30 | 16.749 | 55.828 | -13.251 | 1.596 | 9.528 |
| Baked Bread Barley flour | 0 | 0.096 | / | 0.096 | 0.032 | 32.787 |
| | 20 | 33.385 | 166.927 | 13.385 | 5.189 | 15.543 |
| | 30 | 80.039 | 266.796 | 50.039 | 18.356 | 22.934 |
| Baked Bread Rye flour | 0 | 0.096 | / | 0.096 | 0.032 | 32.787 |
| | 20 | 27.746 | 138.728 | 7.746 | 6.021 | 21.700 |
| | 30 | 41.013 | 136.709 | 11.013 | 5.109 | 12.457 |

Table 5 GlutenTox ELISA Rapid G12.- LOD-LOQ_{est} study (1)

| Blank matrixes: Concentration (mg/kg gluten) | | | | | | |
|--|-----------|------------|-------------|---------------|-----------------|--|
| Replicate | Soy flour | Corn bread | Rolled oats | Seasoning mix | Evaporated milk | |
| 1 | 0.256 | 0.237 | 0.373 | 0.419 | 0.468 | |
| 2 | 0.444 | 0.419 | 0.298 | 0.174 | 0.241 | |
| 3 | 0.453 | 0.481 | 0.390 | 0.381 | 0.392 | |
| 4 | 0.319 | 0.281 | 0.022 | 0.423 | 0.121 | |
| 5 | 0.436 | 0.499 | 0.369 | 0.219 | 0.361 | |
| 6 | 0.104 | 0.231 | 0.361 | 0.436 | 0.348 | |
| 7 | 0.440 | 0.445 | 0.161 | 0.407 | 0.263 | |
| 8 | 0.478 | 0.423 | 0.407 | 0.306 | 0.348 | |
| 9 | 0.256 | 0.192 | 0.419 | 0.361 | 0.122 | |
| 10 | 0.436 | 0.423 | 0.269 | 0.011 | 0.383 | |
| Mean | 0.362 | 0.363 | 0.307 | 0.314 | 0.305 | |
| SDr | 0.123 | 0.115 | 0.127 | 0.139 | 0.116 | |
| LOD: Mean + 3.3 SDr | 0.768 | 0.742 | 0.725 | 0.772 | 0.686 | |
| Overall LOD = 0.738 mg/kg | | | | | | |
| LOQ _{est} : Mean + 10 SDr | 1.592 | 1.512 | 1.575 | 1.702 | 1.460 | |
| Overall LOQ _{est} = 1.568 mg/kg | | | | | | |

Table 6 GlutenTox ELISA Rapid G12.- LOQ_{est} validation (1)

| Replicate | Spiked matrixes at LOQ _{est} : Concentration (mg/kg gluten) | | | | |
|-----------|--|------------|-------------|---------------|-----------------|
| | Soy flour | Corn bread | Rolled oats | Seasoning mix | Evaporated milk |
| 1 | 1.434 | 1.475 | 2.207 | 1.396 | 1.011 |
| 2 | 1.456 | 1.541 | 1.541 | 1.545 | 1.102 |
| 3 | 1.493 | 2.009 | 2.001 | 1.707 | 1.140 |
| 4 | 1.697 | 1.668 | 2.089 | 1.249 | 1.179 |
| 5 | 1.438 | 1.781 | 1.893 | 1.348 | 1.179 |
| 6 | 1.181 | 2.014 | 2.226 | 1.951 | 1.058 |
| 7 | 1.420 | 1.602 | 1.908 | 1.747 | 1.089 |
| 8 | 1.574 | 1.517 | 1.810 | 1.712 | 1.110 |
| 9 | 1.285 | 1.640 | 1.614 | 1.519 | 1.071 |
| 10 | 1.848 | 1.682 | 1.898 | 1.541 | 1.007 |
| Mean | 1.483 | 1.693 | 1.919 | 1.571 | 1.095 |
| SDr | 0.191 | 0.190 | 0.226 | 0.211 | 0.061 |
| RSD, % | 12.857 | 11.214 | 11.804 | 13.450 | 5.554 |
| Recovery% | 93 | 112 | 122 | 92 | 75 |

Overall LOQ_{est} = 1.552 mg/kg

Table 7. GlutenTox ELISA Rapid G12.- LOD-LOQ_{est} study (1)

| Replicate | Blank baked bread matrixes (incurred): Concentration (mg/kg gluten) | | |
|--|---|--------------------|-----------------|
| | Wheat flour study | Barley flour study | Rye flour study |
| 1 | 0.063 | 0.189 | 0.091 |
| 2 | 0.073 | 0.006 | 0.069 |
| 3 | 0.146 | 0.051 | 0.116 |
| 4 | 0.022 | 0.144 | 0.003 |
| 5 | 0.105 | 0.055 | 0.112 |
| 6 | 0.073 | 0.153 | 0.005 |
| 7 | 0.105 | 0.013 | 0.096 |
| 8 | 0.078 | 0.010 | 0.088 |
| 9 | 0.104 | 0.176 | 0.107 |
| 10 | 0.073 | 0.004 | 0.096 |
| Mean | 0.084 | 0.080 | 0.078 |
| SDr | 0.033 | 0.077 | 0.041 |
| LOD: Mean + 3.3 SDr | 0.193 | 0.333 | 0.215 |
| Overall LOD = 0.247 mg/kg | | | |
| LOQ _{est} : Mean + 10 SDr | 0.414 | 0.845 | 0.493 |
| Overall LOQ _{est} = 0.584 mg/kg | | | |

Table 8. GlutenTox ELISA Rapid G12 – Selectivity study. (1)

| Compounds | GlutenTox ELISA Rapid G12 | | |
|-----------------------|---------------------------|-----------------------|-------------|
| | Un-spiked | 20 ppm | |
| | Result (mg/kg gluten) | Result (mg/kg gluten) | Correctness |
| Almond Flour | Below LOQ | 17.69 | -12% |
| Amaranth flour | Below LOQ | 18.27 | -11% |
| Arrowroot | Below LOQ | 18.86 | -6% |
| Black bean flour | Below LOQ | 19.89 | -1% |
| Brown rice flour | Below LOQ | 19,82 | -1% |
| Buckwheat flour | Below LOQ | 21.06 | 5% |
| Chestnut flour | Below LOQ | 21.09 | 5% |
| Coconut flour | Below LOQ | 23.24 | 16% |
| Ground Coffee | Below LOQ | 19.76 | -1% |
| Corn starch/meal | Below LOQ | 20.54 | 3% |
| Dried fruits | Below LOQ | 18.91 | -5% |
| Egg powder | Below LOQ | 20.37 | 2% |
| Fava bean flour | 9.82 | 24.06 | 19% |
| Fava beans, ground* | Below LOQ | 18.35 | -8% |
| Flax seed flour | Below LOQ | 22.09 | 10% |
| Green pea flour | Below LOQ | 19.69 | -2% |
| Guar gum (1:10) | Below LOQ | 21.85 | 8% |
| Hazelnut flour | Below LOQ | 19.18 | -5% |
| Lentil flour | Below LOQ | 19.09 | -5% |
| Lima bean flour | 297 | - | - |
| Lima beans, ground* | Below LOQ | 16.82 | -16% |
| Milk powder | Below LOQ | 20.44 | 1% |
| Milk (whole, liquid) | Below LOQ | 14.82 | -26% |
| Millet flour | Below LOQ | 20.35 | 3% |
| Oat flour | 2.79 | 17.38 | -13% |
| Oats, rolled* | Below LOQ | 20.85 | 4% |
| Parsley flakes | Below LOQ | 22.14 | 10% |
| Pork sausage | Below LOQ | 19.76 | -3% |
| Potato starch | Below LOQ | 23.71 | 19% |
| Quinoa flour | Below LOQ | 19.18 | -4% |
| Romano bean flour | 6.06 | 23.68 | 18% |
| Romano beans, ground* | Below LOQ | 18.53 | -7% |
| Sorghum flour | Below LOQ | 19.72 | -1% |
| Soya flour | Below LOQ | 22.76 | 13% |
| Sweet rice flour | Below LOQ | 19.85 | -1% |
| Tapioca flour | Below LOQ | 19.11 | -4% |
| Ground Tea | Below LOQ | 15.39 | -23% |
| White bean flour | Below LOQ | 15.68 | -22% |
| White rice flour | Below LOQ | 18.64 | -7% |
| Xanthan gum (1:20) | Below LOQ | 18.47 | -9% |
| Yellow pea flour | Below LOQ | 24.17 | 19% |

*Indicates commodities ground into meal from bean/oat material and re-tested.

Table 9. GlutenTox ELISA Rapid G12 – Selectivity study. Rice flour spiked at 20 mg/kg gluten from other sources of gluten (1)

| Compounds | GlutenTox ELISA Rapid G12 | | |
|--|---------------------------|-----------------------|-------------|
| | Un-spiked | 20 ppm | |
| | Result (mg/kg gluten) | Result (mg/kg gluten) | Correctness |
| Einkorn Wheat Flour (<i>Triticum monococcum</i>) | - | 7.22 | -64% |
| Khorasan Wheat flour (<i>Triticum turgidum</i>) | - | 18.29 | -9% |
| Spelt Wheat Flour (<i>Triticum spelta</i>) | - | 23.84 | 18% |
| Triticale Flour (xTriticosecale) | - | 8.39 | -59% |
| Durum Wheat Flour (<i>Triticum durum</i>) | - | 23.81 | 19% |
| Emmer Wheat Flour (<i>Triticum dicoccon</i>) | - | 22.72 | 4% |

REFERENCES CITED

1. Salagre, C., Lopez, A., and Galera, C., Validation of the GlutenTox® ELISA Rapid G12 for Determination of Gluten in Select Non-Heat Processed Matrixes and Heat Processed Matrixes, AOAC *Performance Tested*SM certification number 042301. Approved April 12, 2023.