FOOD COMPOSITION AND ADDITIVES

GlutenTox® Pro Test for the Detection of Gluten in Select Foods and Surfaces

Performance Tested Method[SM] 061502

Abstract

The GlutenTox® Pro Test is an immunochromatographic test for the detection of gluten in foods and on surfaces with varying compositions and levels of processing, from raw foods/ingredients to final product testing. The Method Developer evaluation for the validation of the GlutenTox Pro Test Kit (Biomedal Diagnostics, Sevilla, Spain) for the detection of gluten in foods and on surfaces was conducted at Biomedal, S.L., Camas, Sevilla, Spain. The GlutenTox Pro test method was evaluated by testing the following: cross-reactivity, interference, specificity and sensitivity, robustness, stability, lot-to-lot variation, food matrix, and environmental surface. To evaluate the performance of the GlutenToxPro test for the detection of gluten, 10 matrixes were selected: rice flour, bread/biscuit, rolled oat, pâté, and yogurt (and a second bread matrix for incurred sampled testing) for the food matrix study and food-grade painted wood, plastic, rubber, sealed ceramic, and stainless steel for the environmental surface matrix study. For the food matrix study, 30 replicates were evaluated at six spiked levels of gluten (0, 3, 8, 15, 25, and 45 ppm) against four detection thresholds (5, 10, 20, and 40 ppm) for each food matrix. Additionally, 10 replicates were evaluated at a concentration of 10 000 ppm using all four detection thresholds only for rice flour matrix. Three replicates of each concentration level of gluten were analyzed using paired samples by the AOAC OMA **2012.01** reference method for each food matrix. For the environmental surface study, 30 replicates were evaluated at a low spike level of gluten (16 ng/16 cm^2), five replicates at a high spike level of gluten (400 ng/16 cm^2), and five replicates at an unspiked control level (0 ng/16 cm^2) for each surface matrix. Upon completion of testing, the probability of detection values and confidence intervals were calculated and plotted versus the concentration level as determined by the reference method when applicable. An independent laboratory evaluation of the GlutenTox Pro Test Kit with rice flour and stainless steel environmental surface was conducted at Q Laboratories, Inc. (Cincinnati, OH). The GlutenTox Pro Test Kit demonstrated reliability as an effective rapid method for the detection of gluten in food matrixes (LOD 5 ppm gluten; threshold limits 5, 10, 20, and 40 ppm gluten) and on environmental surfaces (amount of detection 16 ng/16 cm^2).

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The method was independently tested, evaluated, and certified by the AOAC Research Institute as a *Performance Tested Method*[SM]. *See* http://www.aoac.org/testkits/steps.html for information on certification.

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Scope of Method

The target analyte of the GlutenTox® Pro Test Kit is gluten, and the study was performed with five selected food matrixes: rice flour, bread/biscuit, rolled oats, pâté, and yogurt and five environmental surfaces: food-grade painted wood, plastic, rubber, sealed ceramic, and stainless steel.

This validation outline evaluated the performance of the GlutenTox Pro test method by testing the following: cross-reactivity, interference, specificity and sensitivity, robustness, stability, lot-to-lot variation, food matrix, and environmental surface, following the *AOAC Guidelines for Validation of Binary Chemistry Methods* (1) and the *Validation Procedures for Quantitative Gluten ELISA Methods: AOAC Allergen Community Guidance and Best Practices* (2).

Definitions

Where appropriate, definitions have been taken from international standards, and the source is noted. Definitions include the following:

(a) *Probability of detection (POD)*.—The proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration dependent. POD estimate is calculated as the number of positive outcomes divided by the total number of trials.

(b) *Qualitative binary method*.—A method of analysis with two possible outcomes.

(c) *Sample*.—A small portion or quantity taken from a population or lot that is an ideally representative selection of the whole (ISO 3534-2:2006) (3).

(d) *Specificity*.—The proportion of samples without analyte giving negative results in binary test methods.

(e) *Sensitivity*.—The proportion of samples with analyte giving positive results in binary test methods.

Gluten is a mixture of water insoluble proteins [prolamins (gliadins in wheat, hordeins in barley, secalins in rye, etc.) and glutelins] found in the seeds of cereals that can cause adverse health effects to people intolerant to gluten. Oats can be tolerated by most but not all people who are intolerant to gluten (4). People with celiac disease suffer from a permanent intolerance to gluten.

Celiac disease is a disorder that damages the small intestine causing atrophy of the intestinal villi, which interferes with the absorption of nutrients such as proteins, lipids, carbohydrates, mineral salts, and vitamins. This disease is caused by an inappropriate response of the immune system to gluten from wheat, barley, rye, and, to a lesser extent, oat (5, 6) leading to diarrhea, vitamin and mineral deficiencies, anemia, and thin bones (osteoporosis). Celiac disease affects people of all ages.

Currently, the only treatment for celiac disease sufferers is a strict lifelong gluten-free diet that presents great difficulties because gluten, in addition to being present in many foods, may also be found in food additives and preservatives.

According to the Codex Alimentarius Commission and EC Regulation 41/2009 on the composition and labeling of foodstuffs suitable for people intolerant to gluten, food can be considered as "gluten-free" if its gluten content does not exceed 20 parts per million (ppm or mg of gluten/kg of food).

Principle of the Method

The GlutenTox 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.

General Information

The test consists of an extraction stage using a simple procedure that is common to all types of food. The detection step is based on the reaction of the 33mer-like immunotoxic peptides of gluten in the sample with the colored conjugates (monoclonal anti-gliadin 33mer antibody (7, 8)/red microsphere) previously fixed on the stick. This complex spreads by capillarity through the stick. If the result is positive, a RED line appears in the result zone of the stick. The absence of RED line indicates a negative result. Whether or not gluten is present the mixture of the conjugate moves through the stick up to the control region where anti-mouse monoclonal antibodies have been immobilized, and when the test was properly realized a BLUE line (control line) will appear.

These rapid tests are especially useful in routine monitoring to ensure that products comply with a program of Hazard Analysis and Critical Control Point, and to ensure proper labeling. They also allow quick decisions and corrective actions in case there is any risk of contamination along the production chain.

Materials and Methods

Food for Cross-Reactivity Study

The cross-reactivity study was performed to ensure that the GlutenTox Pro test kit does not produce positive results when tested on common compounds that do not contain any gluten. The list of compounds recommended by *Validation Procedures for Quantitative Gluten ELISA Methods: AOAC Allergen Community Guidance and Best Practices* (2) was prescreened using the AOAC OMA **2012.01** method (9) to detect natural contamination prior to the study.

Food for Interference Study

The interference study was performed to ensure that the compounds (particularly those from the aforementioned list used in the production of gluten-free products) do not produce unexpected results from the GlutenTox Pro test kit when tested in the presence of gluten. Prescreening of the matrixes was done as part of the cross-reactivity study.

Gluten Free Food for Testing

(a) *Rice flour*.—Hacendado (Mercadona Supermarket, Madrid, Spain); Batch 013015, expiration date 12-2014.

(b) *Bread*.—Gluten-free baguette, Beiker (Mercadona Supermarket); Batch 09.05.14 BC.

(c) *Oat*.—Rolled oats, Trader Joe's, Batch 12-23-14, expiration date 12-2014.

(d) *Pâté*.—Ibérico, Hacendado, Batch 000212, expiration date 01-2017.

(e) *Yogurt*.—Dannon (Mercadona Supermarket); Batch 17:19 2V904, expiration date 02-14.

All matrixes were mixed or crushed and stored at 4°C in conical polypropylene tubes until their time of use.

Environmental Surfaces

(a) *Stainless steel*.—Stainless steel tray, 27 × 18 cm, IKEA (Seville, Spain). Ref. 500.558.63.

(b) *Rubber*.—Ethylene vinyl acetate sheet, 30 × 20 cm, stationer's shop.

(c) *Plastic*.—Polyethylene tray, 34 × 17 cm, IKEA; Ref. 401.649.47.

(d) *Ceramic*.—Crystal tray, 27 × 18 cm, IKEA; Ref. 600.587.62.

(e) *Food grade painted wood*.—Formica plywood, 30 × 30 cm, Leroy-Merlin (Seville, Spain); Ref. 16437953.

Test Kit Information

The general test kit information includes the following:

(a) *Kit name*.—GlutenTox Pro.

(b) *Cat. No*.—KT-5660 (25 analyses); KT-5288 (five analyses).

Test Kit Components

Materials provided with each GlutenTox Pro Test Kit:

(a) *GlutenTox PRO stick (x25) in a tube*.

(b) *Plastic pipet (x50)*.

(c) *Disposable plastic spoons (x25)*.

(d) *Yellow cap bottle with extraction solution (x25)*.

(e) *Blue-cap bottle with dilution solution (x25)*.

(f) *Instruction leaflet*.

Additional supplies and reagents (not included in the test kit):

(a) *Mortar or any other utensil to grind the sample*.

(b) *Nonpowdered disposable gloves*.

(c) *Alcohol (ethanol)*.

(d) *Wide-mouth specimen cups*.—60 mL polypropylene (PP). *Conical tube*.—50 mL PP.

(e) *Weighing dishes*.—89 × 89 mm polystyrene.

(f) *Spatula with spoon*.—Stainless steel spoon.

Apparatus

(a) *Analytical balance*.—Range: 0.001–220 g.

(b) *Analytical balance*.—Range: 0.00001–225 g.

(c) *Household breadmaker*.

(d) *Drying oven*.

(e) *Centrifuge*.—Home blender.

(f) *Digital timer*.

Safety Precautions

To avoid contamination that could interfere with the analysis, the use of nonpowdered disposable gloves is recommended. Once the GlutenTox PRO stick has been removed from the tube, it must be used as soon as possible under strict clean conditions. Close the tube after removing the stick. Do not use material from the kit after the expiration date. Do not drink any solution (liquid) from the kit (the extraction solution contains alcohol). The product must be stored at a temperature ranging from 2 to 30°C/35.6 to 86°F during the shelf life of the kit. All components of the GlutenTox Pro test kit may be disposed of in ordinary trash.

General Preparation

Each sample should be homogenized appropriately according to the sample matrix prior to sampling. Liquids should be shaken vigorously, semi-liquids/doughy matrixes should be mixed with a clean spoon or tooth pick; and solids should be ground using a mortar and pestle and/or meat grinder.

Note 1: Before using the kit, clean the utensils and the areas with which the sample will be in contact with soap and water and rinse well. After cleaning, it is highly recommended to wipe them with a clean cloth dampened with alcohol.

Note 2: Extreme caution should be taken to ensure that samples are homogenous prior to testing.

GlutenTox Pro Test Kit Procedure for Foods

The analysis of each matrix sample was conducted according to the following steps:

Use the provided spoon with leveled amounts of sample depending on the kind of food. Use two spoonfuls for flour, fine powders, and fine crumbs (e.g., corn flour, rice flour, milk powder, spices, bread, cookies, cakes, and snacks, etc.) and one spoonful for liquids, sauces, meat, fish, and cold meat (e.g., milk, juice, condensed milk, yogurt, soup, gravy, sauce, cream, meat, fish, pâté, sausage, canned meat, and fish, etc.). If you have an appropriate scale, weigh 1 g of sample instead of using the spoon.

Open one extraction bottle with yellow cap. Add the contents of the spoon or the 1 g to the extraction bottle with yellow cap.

Close the bottle. Shake vigorously for at least 2 min and let the contents settle for about 5 min so that the solids fall to the bottom of bottle. Settling time will depend on the type of sample.

Open one dilution bottle with blue cap. Using a disposable plastic pipet, take approximately 1 mL of extracted sample from the extraction bottle with yellow cap. Add 10, 4, 2, or 1 extraction solution drops to the dilution bottle with blue cap according to your required threshold/limits, which are 5, 10, 20, or 40 ppm of gluten, respectively. Mix softly for at least 15 s.

Add with a new disposable plastic pipet 5–10 drops from the dilution bottle with blue cap in the same inverted blue cap. Put the cotton wool side of the GlutenTox PRO stick in contact with the liquid present in the blue cap until all the liquid is absorbed. Let it stand in the blue cap. Timing for the color development must be started when the test stick is placed in the blue cap.

Wait 10 min to see the final results. If there is a high concentration of gluten, the result may appear in less than 1–2 min.

GlutenTox Pro Test Kit Procedure for Environmental Surfaces

The analysis of each environmental surface was conducted according to the following steps:

Rub the cotton wool side of the GlutenTox PRO stick against a surface of at least 16 cm^2/2.46 in^2.

After rubbing the environmental surface with the test stick, no extraction step must be performed.

Open a dilution bottle with blue cap and place inverted cap on a clean surface. Place 5–10 drops with a new disposable plastic pipet from the dilution bottle into the blue cap. Put the cotton wool side of the GlutenTox PRO stick in contact with the liquid present in the blue cap until all liquid is absorbed.

Wait 10 min to see the final results. If there is a high concentration of gluten, the result may appear in less than 1–2 min.

Interpretation of Results

Negative: A single BLUE line (control line) appears in the central part of the stick (control zone)

Positive: In addition to the control line (BLUE), a RED line (result line) appears in the result zone. The intensity of the red line in the result zone will vary depending on the gluten concentration present in the sample.

Invalid: The control line (BLUE) does not appear, whether or not the result line appears (RED).

Reference Materials

Gliadin standard from the Prolamin Working Group (PWG).—In the validation of the GlutenTox Pro Test Kit a PWG-gliadin solution was the unique reference standard used for contamination. PWG-gliadin is a reference material that has been produced under guidance of the PWG with protein content 91.4% and gliadin content 88.2%. The characteristics of this standard are described in van Eckert et al. (10). The PWG-gliadin was obtained from 28 European wheat cultivars; it was extracted with 60% ethanol and was concentrated and lyophilized. The GlutenTox Pro test kit is based on a monoclonal antigliadin 33mer antibody (7, 8) that recognizes the 33mer peptide from gliadin.

Preparation of Validation Materials

Preparation of PWG stock spiking solution.—The spiking solution was prepared using purified gliadin (88.2% gliadin content) obtained from the PWG. This standard was dissolved at 6 mg/mL in 60% ethanol (v/v); 136 mg of PWG-gliadin was weighed into a PP tube, and 20 mL of 60% ethanol were then added. This solution was used for all spiking tests.

Preparation of spiked food test samples.—The samples were spiked with PWG gliadin, which was first dissolved at 6 mg/mL in 60% ethanol (v/v) to obtain a stock solution. This was diluted 1:10 in 60% ethanol (v/v), and the spiking solution was further diluted with different amounts of 60% ethanol (v/v) to obtain different gliadin concentrations (3.6, 9.6, 18, 30, and 54 µg/mL); 0.5 mL of each gliadin concentration was added to 1.2 g of matrix to achieve the final gluten spiked levels (3, 8, 15, 25, and 45 ppm).

Note: To calculate the final gluten spiked levels, each gliadin concentration must be adjusted multiplying by a factor of 2.

The rice flour matrix study performed by the independent laboratory involved a spiking strategy similar to that of Biomedal, although each final gluten spiked level was prepared by spiking a bulk lot of rice flour with a PWG gliadin working standard. Samples were allowed to dry for 18 h at room temperature (24 ± 2°C).

Preparation of gluten free bread and incurred bread.—The gluten free bread sample was prepared by cutting, chopping, or grinding to a finely ground texture with a Thermomix® crusher (Seville Branch, Seville, Spain). The bread sample was added into a cup crusher and crushed for 10 cycles of 1 min each, inverting the cup crusher between each cycle.

Sample portions (1.2 g) of the bread matrix were weighed into a wide-mouth specimen tube with a screw-top-cap. All replicates of the test sample were blind-coded by an analyst not involved in the validation prior to analysis performed by another analyst.

PWG-gliadin spiking solution (0.5 mL) at the appropriate concentration level for each spiked level of gluten (0, 3, 8, 15, 25, and 45 ppm) was added to the bread sample and homogenized. Once spiked, the blind-coded samples were held for 24 h at 4°C before analysis.

For the evaluation of the GlutenTox Pro method with incurred samples, two breads were prepared with two spiked levels of gluten, 0 and 50 ppm, in a household breadmaker as follows:

(a) *Ingredients*.—

(1) Gluten-free Chickpea flour (Las Panaeras, Batch 140); 115 g.

(2) Gluten-free Buckwheat flour (Naturkostaus der Heide, Batch 23.10.14G); 115 g.

(3) Baking powder (Royal, Batch 1293); 4 g.

(4) Deionized water; 140 g (140 mL).

(b) *Procedure*.—

(1) A 115 g portion of each gluten-free flour was weighed on an analytical balance and added into the household breadmaker.

(2) A 4 g portion of baking powder was weighed and added to the flour mixture.

(3) A 140 mL volume of deionized water was added, with or without the spiking solution, the BASIC program option in the household breadmaker display was chosen, and the start button was pressed.

(4) Timing of BASIC program: First Kneading: 10 min; First Rising: 20 min; Second Kneading: 15 min; Second Rising: 20 min; Forming: 30 s; Last Rising: 55 min; Baking: 60 min. Total: 3 h.

(5) Samples were left to dry in a drying oven for 3 days at 42°C (weight of the dried material 0.243 kg), frozen, and ground to a fine particle size before being portioned into 1.2 g sample size in wide-mouth specimen cups. Then, the samples were held for 24 h at 4°C before analysis.

Note: To spike the bread with 50 ppm of gluten, 1 mL of the PWG-gliadin solution at a concentration of 6 mg/mL in 60% ethanol (v/v) was added to 139 mL of deionized water.

To prepare the unspiked bread, 1 mL of 60% ethanol (v/v) was added to 139 mL of deionized water.

Preparation of environmental surface test samples.—All environmental surfaces were treated in the same manner as follows:

Prior to spiking, the testing surfaces were washed with soap and water, cleaned with 70% ethanol, rinsed thoroughly with deionized water, and dried.

1.57" (4 cm) × 1.57"(4 cm) test areas (2.46 in.^{2}/16 cm^2) were marked off with a permanent marker (40 replicate 1.57" × 1.57" test areas/environmental surface were evaluated for the presence of gluten).

Thirty 1.57" × 1.57" test areas were spiked at a low concentration level of contamination to produce fractional positive results (7–23 positive replicates or 25–75% positive results). Test areas were spiked by diluting a 60 µg/mL PWG-gliadin solution (previously prepared from PWG-gliadin at a concentration of 6 mg/mL) in 60% ethanol and pipetting 20 µL aliquots of a final 400 ng/mL PWG-gliadin solution onto each test area to produce 8 ng/16 cm^2 gliadin (16 ng/16 cm^2 gluten).

Five test areas were spiked at high concentration level to produce all positive results, pipetting 20 µL aliquots of a

10 μg/mL PWG-gliadin solution in 60% ethanol onto each test area to produce 200 ng/16 cm^2 gliadin (400 ng/16 cm^2 gluten).

Five test areas were spiked with 20 μL of a blank solution (60% ethanol) onto each test area.

Surfaces were allowed to dry for 18 h at room temperature (24 ± 2°C).

Note: To calculate the final gluten spiked levels, each gliadin concentration must be adjusted multiplying by a factor of 2.

Note: All test samples were blind-coded and spiked at appropriate gluten levels by an analyst who was not involved in the validation study prior to analysis performed by another analyst.

1" × 1" (6.45 cm^2) test areas prepared by the independent laboratory were spiked by pipetting a 20 μL aliquot of an 882 μg/mL concentration of the Gliadin-PWG standard, prepared in 60% ethanol, onto the test areas to produce 17.6 μg/6.45 cm^2 gliadin (35.2 μg/6.45 cm^2 gluten) for each high contamination level test area. The low level concentration was prepared by transferring 4 mL of the 882 μg/mL standard into 16 mL of 60% ethanol. A 20 μL aliquot of this diluted standard was pipetted into the test areas to produce 3.5 μg/6.45 cm^2 gliadin (7 μg/6.45 cm^2 gluten) for each low inoculation level test area. Surfaces were allowed to dry for 18 h at room temperature (24 ± 2°C).

Validation Study

The reference method AOAC OMA **2012.01** (9) was used to verify the gluten levels of the three test samples of each spiked level for each food matrix. Variance (σ^2) was also calculated.

This validation study of the GlutenTox Pro Test Kit (Biomedal Diagnostics, Sevilla, Spain) for the detection of gluten in foods and surfaces was conducted under the AOAC Research Institute *Performance Tested Method*[SM] Program: *GlutenTox Pro Test for the Detection of Gluten in Select Foods and Surfaces, Version 11.1, February 3, 2014* following the *AOAC INTERNATIONAL Guidelines for Validation of Qualitative Binary Chemistry Methods* (1) and the *Validation Procedures for Quantitative Gluten ELISA Methods: AOAC Allergen Community Guidance and Best Practices* (2) in conjunction with the instructions for use (Ed. 4-October 2013) included with the test kit. Method Developer studies were conducted in the laboratory of Biomedal, S.L. and included cross-reactivity, interference and product consistency studies, robustness testing, stability study, lot-to-lot variation study, and food matrix and environmental surface studies for all claimed matrixes. The independent laboratory study was conducted by Q Laboratories, Inc. and included a matrix study for rice flour and stainless steel of the claimed food and surface matrixes, respectively.

Results

Cross-Reactivity Study

For the study, each matrix was prepared according to the GlutenTox Pro test kit package insert, blind-coded, and tested once using the 40 ppm threshold level.

As regards sample preparation, when adding the guar gum or xanthan gum matrixes to the extraction solution provided in the GlutenTox Pro test kit, a paste was formed making it difficult to take approximately 1 mL of the extracted sample as indicated in the package insert. The results are shown in Table 1.

Table 1. GlutenTox Pro cross reactivity results

	Detection threshold level		
	40 ppm		
Compounds	Test line	Control line	Result
Almond flour	−	+	Negative
Amaranth flour	−	+	Negative
Arrowroot	−	+	Negative
Black bean flour	−	+	Negative
Brown rice flour	−	+	Negative
Buckwheat flour	−	+	Negative
Chestnut flour	−	+	Negative
Coconut flour	−	+	Negative
Coffee	−	+	Negative
Corn starch/meal	−	+	Negative
Dried fruits	−	+	Negative
Egg powder	−	+	Negative
Fava bean flour	−	+	Negative
Flax seed flour	−	+	Negative
Garfava flour	−	+	Negative
Green pea flour	−	+	Negative
Guar gum	−	+	Negative
Hazelnut flour	−	+	Negative
Lentil flour	−	+	Negative
Lima bean flour	−	+	Negative
Meats	−	+	Negative
Milk powder	−	+	Negative
Millet flour	−	+	Negative
Oat flour	−	+	Negative
Potato starch	−	+	Negative
Quinoa flour	−	+	Negative
Romano bean flour	−	+	Negative
Sesame flour	−	+	Negative
Sorghum flour	−	+	Negative
Soya flour	−	+	Negative
Spices	−	+	Negative
Sweet rice flour	−	+	Negative
Tapioca flour	−	+	Negative
Tea	−	+	Negative
White bean flour	−	+	Negative
White rice flour	−	+	Negative
Xanthan gum	−	+	Negative
Yellow pea flour	−	+	Negative

All matrixes prescreened for AOAC OMA **2012.01** method (9) produced a value of <2.5 ppm.

All matrixes tested using the GlutenTox Pro test kit gave negative results for the cross-reactivity study.

Interference Study

Each matrix was prepared according to the GlutenTox Pro test kit package insert. Spikes, using the gliadin-containing

Table 2. GlutenTox Pro 15 ppm spiked level of gluten for interference study

	Detection threshold level					
	10 ppm			20 ppm		
Compounds	Test line	Control line	Result	Test line	Control line	Result
Almond flour	+	+	Positive	−	+	Negative
Amaranth flour	+	+	Positive	−	+	Negative
Arrowroot	+	+	Positive	−	+	Negative
Black bean flour	+	+	Positive	−	+	Negative
Brown rice flour	+	+	Positive	−	+	Negative
Buckwheat flour	+	+	Positive	−	+	Negative
Chestnut flour	+	+	Positive	−	+	Negative
Coconut flour	+	+	Positive	−	+	Negative
Coffee	+	+	Positive	−	+	Negative
Corn starch/meal	+	+	Positive	−	+	Negative
Dried fruits	+	+	Positive	−	+	Negative
Egg powder	+	+	Positive	−	+	Negative
Fava bean flour	+	+	Positive	−	+	Negative
Flax seed flour	+	+	Positive	−	+	Negative
Garfava flour	+	+	Positive	−	+	Negative
Green pea flour	+	+	Positive	−	+	Negative
Guar gum	+	+	Positive	−	+	Negative
Hazelnut flour	+	+	Positive	−	+	Negative
Lentil flour	+	+	Positive	−	+	Negative
Lima bean flour	+	+	Positive	−	+	Negative
Meats	+	+	Positive	−	+	Negative
Milk powder	+	+	Positive	−	+	Negative
Millet flour	+	+	Positive	−	+	Negative
Oat flour	+	+	Positive	−	+	Negative
Potato starch	+	+	Positive	−	+	Negative
Quinoa flour	+	+	Positive	−	+	Negative
Romano bean flour	+	+	Positive	−	+	Negative
Sesame flour	+	+	Positive	−	+	Negative
Sorghum flour	+	+	Positive	−	+	Negative
Soya flour	+	+	Positive	−	+	Negative
Spices	+	+	Positive	−	+	Negative
Sweet rice flour	+	+	Positive	−	+	Negative
Tapioca flour	+	+	Positive	−	+	Negative
Tea	+	+	Positive	−	+	Negative
White bean flour	+	+	Positive	−	+	Negative
White rice flour	+	+	Positive	−	+	Negative
Xanthan gum			No result obtained			No result obtained
Yellow pea flour	+	+	Positive	−	+	Negative

reference material (PWG gliadin), were tested at the 15 ppm level of gluten and at two different detection threshold levels (10 and 20 ppm).

For the spike level of gluten and detection threshold combination, each blind-coded test portion was analyzed once by the GlutenTox Pro method.

As regards sample preparation, when adding the guar gum or xanthan gum matrixes to the extraction solution provided in the GlutenTox Pro test kit, a paste was formed making it difficult to take approximately 1 mL of the extracted sample as indicated in the package insert. The results are shown in Table 2.

For the 15 ppm spike level, all matrixes tested using the GlutenTox Pro test kit gave positive results for the 10 ppm threshold level and produced negative results for the 20 ppm threshold level in the interference study.

Xanthan gum matrix was not able to produce any results when tested since it was not possible to take any volume of the sample extract. When the test was performed adding one spoonful of the matrix (normal = two spoonfuls) to the extraction bottle the expected results were obtained using 10 drops of the extracted sample for the 10 ppm threshold level (normal = 5 drops) and four drops of the extracted sample for the 20 ppm threshold level (normal = 2 drops). At these two threshold levels, positive and negative results were obtained, respectively, using a spike level of gluten of 15 ppm.

Incurred Sample Study

The incurred sample study was conducted in the same fashion as the food matrix study but only on bread. Two initial spike levels of gluten were used (0 and 50 ppm) in the uncooked matrix; all detection threshold levels were tested (5, 10, 20, and 40 ppm) at these two concentration levels of gluten. For each spike level and detection threshold combination, 30 blind-coded replicate test portions were analyzed by the GlutenTox Pro method. For the AOAC OMA **2012.01** method (9), three replicates were analyzed at each spike level.

Two gluten free bread mixes, previously spiked at each contamination level (0 and 50 ppm spiked level of gluten), were prepared. The results are shown in Table 3.

For the analysis of incurred bread, the POD was calculated for each spiked level of gluten for the GlutenTox Pro method (Least Cost Formulations, Ltd, AOAC Binary Data Interlaboratory Study Workbook (2011) http://lcfltd.com/aoac/aoac-binary-v2-2.xls (11).

At 0 and 50 ppm spike levels of gluten, the AOAC OMA **2012.01** method (9) produced average values of <2.5 and 39.1 [1.2; variance ($\sigma 2$)] ppm gluten, respectively, after baking.

For the 0 ppm spike level, there were 0 observed positive samples and a candidate method confirmed positive outcomes divided by the total number of trials (POD_C) value of 0.00 for all four detection threshold levels for the GlutenTox Pro test kit.

For the 39.1 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5, 10, and 20 ppm threshold levels and 0 observed positive samples and a POD_C value of 0.00 for the 40 ppm threshold level for the GlutenTox Pro test kit.

Matrix Study

The matrix study was performed to test the ability of the GlutenTox Pro test kit to detect the gliadin-containing reference material (PWG gliadin) in each of the five selected food matrixes (rice flour, bread/biscuit, rolled oat, pâté, and yogurt). Spikes were tested at six different levels of gluten (0, 3, 8, 15, 25, and 45 ppm) and at a 10 000 ppm spike level (only for rice flour matrix). At

Table 3. GlutenTox Pro Test Kit incurred matrix (bread)–POD results

				Candidate				
Matrix	Gluten spiked level, ppm[a]	Detection threshold, ppm	N[b]	x[c]	POD_C[d]	95% CI[e]	Average AOAC OMA[f] **2012.01** results, ppm gluten, N = 3	Variance (σ^2)
Incurred matrix(bread)	0	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	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		

[a] Gluten spike level results after cooking the bread.
[b] N = Number of test portions.
[c] x = Number of positive test portions.
[d] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.
[e] 95% Confidence intervals.
[f] OMA = *Official Methods of Analysis*.

each spike level, the test method was assayed using four different detection threshold levels (5, 10, 20, and 40 ppm gluten). For each spike level and detection threshold combination, 30 blind-coded replicate test portions were analyzed by the GlutenTox Pro method. For the 10000 ppm level, only 10 blind-coded replicates were analyzed by the candidate method at each of the four detection threshold levels. Replicates were taken from a singular lot of bulk material. Each spike level was prepared by spiking samples directly (using the spiking solution) with their respective ppm level of gluten. All matrixes were prescreened using the AOAC OMA **2012.01** method (9) to detect natural contamination prior to the study start-up.

Rice Flour

For rice flour, a summary of results is presented in Table 4 and Figure 1.

At 0, 3, 8, 15, 25, and 45 ppm spike levels of gluten, the AOAC OMA **2012.01** method (9) produced average values of <2.5, 3.9 [0.2 (σ2)], 8.8 [0.2 (σ2)], 14.5 [0.3 (σ2)], 21.5 [1.8 (σ2)], and 38.0 [1.1 (σ2)] ppm gluten, respectively.

For the 0 ppm spike level, there were 0 observed positive samples and a POD_C value of 0.00 for all four detection threshold levels for the GlutenTox Pro test kit.

For the 3 ppm spike level, there were 11 observed positive samples and a POD_C value of 0.37 for the 5 ppm threshold level and 0 observed positive samples and a POD_C value of 0.00 for the 10, 20, and 40 ppm threshold levels. For the 8 ppm spike level there were 30 and three observed positive samples and POD_C values of 1.00 and 0.10 for the 5 and 10 ppm threshold levels, respectively. There were 0 observed positive samples and a POD_C value of 0.00 for the 20 and 40 ppm threshold levels.

For the 15 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5 and 10 ppm threshold levels and 0 observed positive samples and a POD_C value of 0.00 for the 20 and 40 ppm threshold levels.

For the 25 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5, 10, and 20 ppm threshold levels and 0 observed positive samples and a POD_C value of 0.00 for the 40 ppm threshold level.

For the 45 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5, 10, 20, and 40 ppm threshold levels. Test samples spiked at 10000 ppm gluten showed no hook effect at all four threshold levels for the GlutenTox Pro test kit. Results shown in Table 4 indicate positive results at all four threshold levels.

For rice flour tested by the independent laboratory, a summary of results is presented in Table 5.

At 0, 3, 8, 15, 25, and 45 ppm spike levels of gluten, the AOAC OMA **2012.01** method (9) produced average values of <2.5, 2.9 (0.0), 7.6 (0.1), 15.2 (0.5), 23.0 (0.7), and 46.0 (12.5) ppm gluten, respectively.

For the 0 ppm spike level, there were 0 observed positive samples and a POD_C value of 0.00 for all four detection threshold levels for the GlutenTox Pro test kit.

For the 3 ppm spike level, there were two observed positive samples and a POD_C value of 0.07 for the 5 ppm threshold level and 0 observed positive samples and a POD_C value of 0.00 for the 10, 20, and 40 ppm threshold levels.

For the 8 ppm spike level, there were 30 and two observed positive samples and POD_C values of 1.00 and 0.07 for the 5 and 10 ppm threshold levels, respectively. There were 0 observed positive samples and a POD_C value of 0.00 for the 20 and 40 ppm threshold levels.

For the 15 ppm spike level, there were 30 observed positive samples for the 5 and 10 ppm threshold levels and four observed positive samples for the 20 ppm threshold level. A POD_C value of 1.00 was obtained for the 5 and 10 threshold levels, and 0.13 for the 20 ppm threshold level. There were 0 observed positive samples and a POD_C value of 0.00 for the 40 ppm threshold level.

For the 25 ppm spike level, there were 30 observed positive samples and a POD value of 1.00 for the 5, 10, and 20 ppm threshold levels and 0 observed positive samples and a POD_C value of 0.00 for the 40 ppm threshold level.

Table 4. GlutenTox Pro Test Kit for rice flour–POD results

Matrix	Gluten spike level, ppm	GlutenTox Pro detection threshold, ppm gluten	N[a]	Candidate			Average AOAC OMA[e] **2012.01** results, ppm gluten, N = 3	Variance (σ^2)
				x[b]	POD_C[c]	95% CI[d]		
Rice flour	0	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	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	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	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	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		
		40	30	0	0.00	0.00, 0.11		
	45	5	30	30	1.00	0.89, 1.00	38.0	1.1
		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		
	10000	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		

[a] N = Number of test portions.

[b] x = Number of positive test portions.

[c] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.

[d] 95% Confidence intervals.

[e] OMA = *Official Methods of Analysis*.

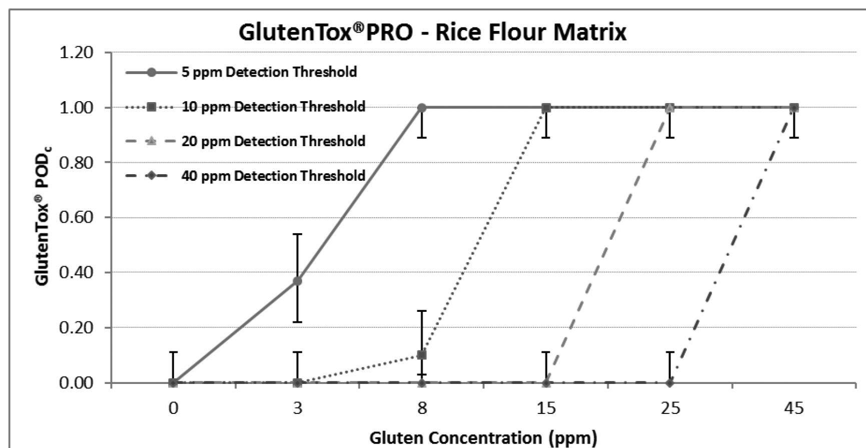

Figure 1. GlutenTox Pro POD versus gluten concentration for rice flour.

Table 5. Independent laboratory GlutenTox ProTest Kit for rice flour–POD results

				Candidate				
Matrix	Gluten spike level, ppm	GlutenTox Pro detection threshold, ppm gluten	N[a]	x[b]	POD_C[c]	95% CI[d]	Average AOAC OMA[e] **2012.01** results, ppm gluten, N = 3	Variance (σ^2)
Rice flour	0	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	5	30	2	0.07	0.02, 0.21	2.9	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	5	30	30	1.00	0.89, 1.00	7.6	0.1
		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		
	15	5	30	30	1.00	0.00, 0.43	15.2	0.5
		10	30	30	1.00	0.89, 1.00		
		20	30	4	0.13	0.05, 0.30		
		40	30	0	0.00	0.00, 0.11		
	25	5	30	30	1.00	0.89, 1.00	23.0	0.7
		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	5	30	30	1.00	0.89, 1.00	46.0	12.5
		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		
	10000	5	10	10	1.00	0.72, 1.00		
		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		

[a] N = Number of test portions.

[b] x = Number of positive test portions.

[c] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.

[d] 95% Confidence intervals.

[e] OMA = *Official Methods of Analysis*.

For the 45 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5, 10, 20, and 40 ppm threshold levels.

Test samples spiked at 10 000 ppm gluten showed no hook effect at all four threshold levels. Positive results were obtained at all four threshold levels.

Figure 2 is a plot of difference in probability of detection values (dPOD) for rice flour between the candidate and independent laboratories. There is no significant difference between data from both laboratories except for the value of 0.78 at 3 ppm gluten spike level within the 5 ppm detection threshold. This is likely due to differences in analyst interpretation of test lines when the gluten concentration is at levels close to the LOD (or to the threshold limits).

Bread

For bread, a summary of results is presented in Table 6 and Figure 3.

At 0, 3, 8, 15, 25, and 45 ppm spike levels of gluten, the AOAC OMA **2012.01** method (9) produced average values of <2.5, 2.3 (0.1), 7.2 (0.1), 14.0 (1.5), 21.1 (2.5), and 38.5 (2.4) ppm gluten, respectively.

For the 0 and 3 ppm spike levels, there were 0 observed positive samples and a POD_C value of 0.00 for all four detection threshold levels for the GlutenTox Pro test kit.

For the 8 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5 ppm threshold level and 0 observed positive samples and a POD_C value of 0.00 for the 10, 20, and 40 ppm threshold levels.

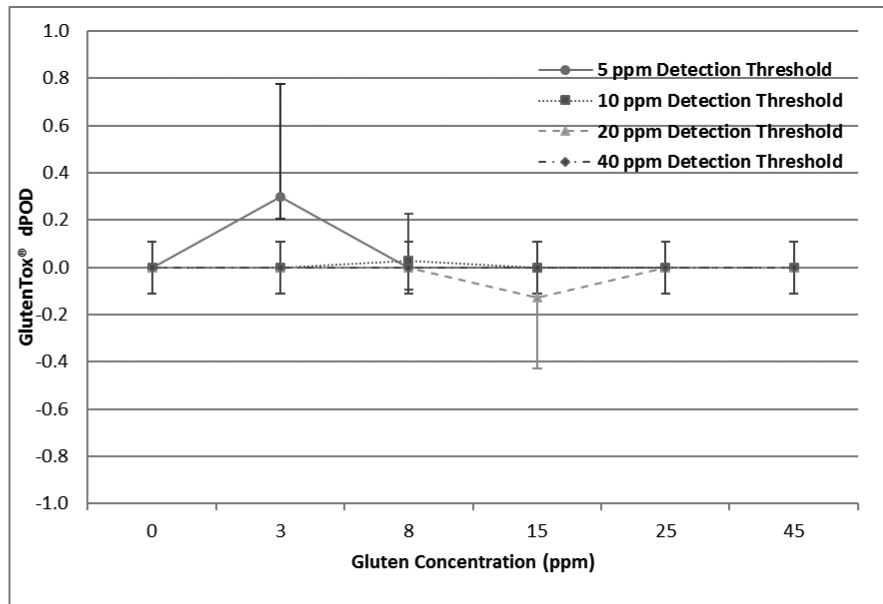

Figure 2. dPOD of GlutenTox Pro POD results between candidate and independent laboratories versus gluten concentration for rice flour.

Table 6. GlutenTox Pro Test Kit for bread–POD results

				Candidate				
Matrix	Gluten spike level, ppm	GlutenTox Pro detection threshold, ppm gluten	N[a]	x[b]	POD_C[c]	95% CI[d]	Average AOAC OMA[e] **2012.01** results, ppm gluten, N = 3	Variance (σ^2)
Bread	0	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	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	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	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	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	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		

[a] N = Number of test portions.

[b] x = Number of positive test portions.

[c] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.

[d] 95% Confidence intervals.

[e] OMA = *Official Methods of Analysis*.

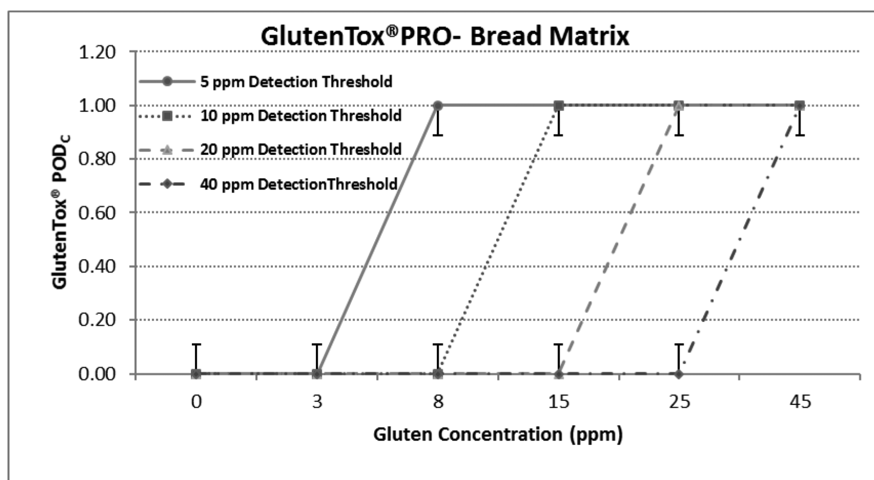

Figure 3. GlutenTox Pro POD versus gluten concentration for bread.

For the 15 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5 and 10 ppm threshold levels and 0 observed positive samples and a POD_C value of 0.00 for the 20 and 40 ppm threshold levels.

For the 25 ppm spike level, there were 30 observed positive samples and a POD value of 1.00 for the 5, 10, and 20 ppm threshold levels and 0 observed positive samples and a POD_C value of 0.00 for the 40 ppm threshold level.

For the 45 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5, 10, 20, and 40 ppm threshold levels.

Rolled Oat

For rolled oat, a summary of results is presented in Table 7 and Figure 4.

At 0, 3, 8, 15, 25, and 45 ppm spike levels of gluten, the AOAC OMA **2012.01** method (9) produced average values of <2.5, 2.7 (0.0), 8.3 (1.7), 12.6 (1.0), 20.4 (3.4), and 41.0 (3.5) ppm gluten, respectively.

For the 0 ppm spike level, there were 0 observed positive samples and a POD_C value of 0.00 for all four detection threshold levels for the GlutenTox Pro test kit.

For the 3 ppm spike level, there were 0 observed positive samples and a POD_C value of 0.00 for the 5, 20, and 40 ppm threshold levels and two observed positive samples and a POD_C value of 0.07 for the 10 ppm threshold level. These occasional overestimated results appear to be related to the homogeneity of the spiked samples (which were spiked directly with the spiking solution) rather than the habitual kit performance.

For the 8 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5 ppm threshold level and 0 observed positive samples and a POD_C value of 0.00 for the 10, 20, and 40 ppm threshold levels.

For the 15 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5 and 10 ppm threshold levels and 0 observed positive samples and a POD_C value of 0.00 for the 20 and 40 ppm threshold levels.

For the 25 ppm spike level, there were 30 observed positive samples and a POD value of 1.00 for the 5, 10, and 20 ppm threshold levels and 0 observed positive samples and a POD_C value of 0.00 for the 40 ppm threshold level.

For the 45 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5, 10, 20, and 40 ppm threshold levels.

Pâté

For pâté, a summary of results is presented in Table 8 and Figure 5.

At 0, 3, 8, 15, 25, and 45 ppm spike levels of gluten, the AOAC OMA **2012.01** method (9) produced average values of <2.5, 3.0 (0.7), 9.2 (0.4), 16.1 (0.4), 27.6 (36.8), and 41.0 (18.9) ppm gluten, respectively.

For the 0 ppm spike level, there were 0 observed positive samples and a POD_C value of 0.00 for all four detection threshold levels for the GlutenTox Pro test kit.

For the 3 ppm spike level, there were 0 observed positive samples and a POD_C value of 0.00 for the 5, 20, and 40 ppm threshold levels and nine observed positive samples and a POD_C value of 0.30 for the 10 ppm detection threshold level. These occasional overestimated results appear to be related to the homogeneity of the spiked samples (which were spiked directly with the spiking solution) rather than the habitual kit performance. The possibility of a matrix effect in this case is also discarded since no positive results were observed when gluten concentrations were at levels close to the LOD (or to the threshold limits).

For the 8 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5 ppm threshold level and 0 observed positive samples and a POD_C value of 0.00 for the 10, 20, and 40 ppm threshold levels.

For the 15 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5 and 10 ppm threshold levels and 0 observed positive samples and a POD_C value of 0.00 for the 20 and 40 ppm threshold levels.

For the 25 ppm spike level, there were 30 observed positive samples and a POD value of 1.00 for the 5, 10, and 20 ppm threshold levels and 0 observed positive samples and a POD_C value of 0.00 for the 40 ppm threshold level.

Table 7. GlutenTox Pro Test Kit for rolled oat–POD results

Matrix	Gluten spike level, ppm	GlutenTox Pro detection threshold, ppm gluten	Candidate				Average AOAC OMA[e] **2012.01** results, ppm gluten, N = 3	Variance (σ^2)
			N[a]	x[b]	POD_C[c]	95% CI[d]		
Rolled oat	0	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	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	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	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	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	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		

[a] N = Number of test portions.

[b] x = Number of positive test portions.

[c] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.

[d] 95% Confidence intervals.

[e] OMA = *Official Methods of Analysis*.

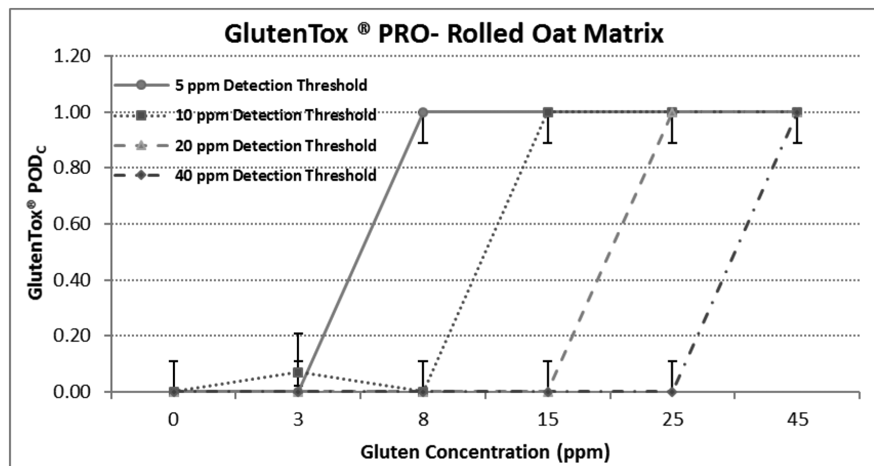

Figure 4. GlutenTox Pro POD versus gluten concentration for rolled oat.

Table 8. GlutenTox ProTest Kit for pâté–POD results

Matrix	Gluten spike level, ppm	GlutenTox Pro detection threshold, ppm gluten	N[a]	Candidate x^b	Candidate POD_C[c]	Candidate 95% CI[d]	Average AOAC OMA[e] **2012.01** results, ppm gluten, N = 3	Variance (σ^2)
Pâté	0	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	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	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	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	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	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		

[a] N = Number of test portions.

[b] x = Number of positive test portions.

[c] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.

[d] 95% Confidence intervals.

[e] OMA = *Official Methods of Analysis*.

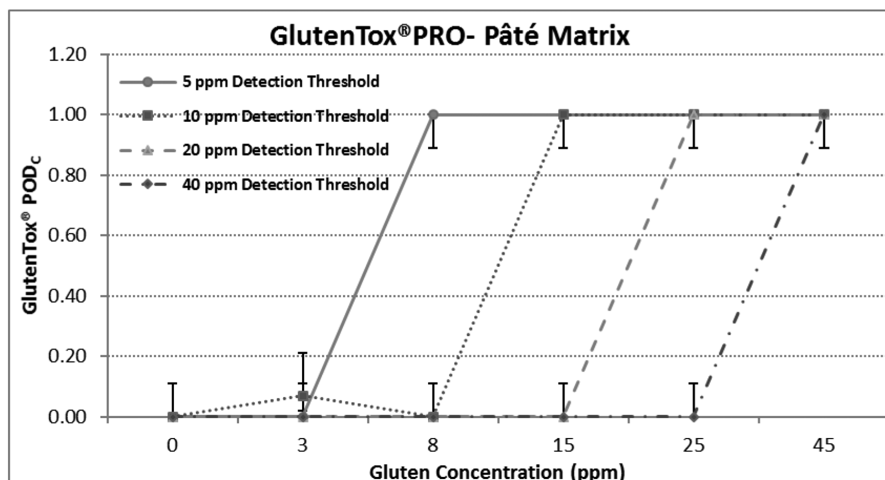

Figure 5. GlutenTox Pro POD versus gluten concentration for pâté.

For the 45 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5, 10, 20, and 40 ppm threshold levels.

Yogurt

For yogurt, a summary of results is presented in Table 9 and Figure 6.

At 0, 3, 8, 15, 25, and 45 ppm spike levels of gluten, the AOAC OMA **2012.01** method (9) produced average values of <2.5, 3.2 (0.0), 9.3 (0.0), 16.6 (2.4), 24.9 (0.5), and 38.2 (1.5) ppm gluten, respectively.

For the 0 ppm and 3 ppm spike levels, there were 0 observed positive samples and a POD_C value of 0.00 for all four detection threshold levels for the GlutenTox Pro test kit.

For the 8 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5 ppm threshold level and 0 observed positive samples and a POD_C value of 0.00 for the 10, 20, and 40 ppm threshold levels.

For the 15 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5 and 10 ppm threshold levels and 0 observed positive samples and a POD_C value of 0.00 for the 20 and 40 ppm threshold levels.

For the 25 ppm spike level, there were 30 observed positive samples and a POD value of 1.00 for the 5, 10, and 20 ppm threshold levels and 0 observed positive samples and a POD_C value of 0.00 for the 40 ppm threshold level.

For the 45 ppm spike level, there were 30 observed positive samples and a POD_C value of 1.00 for the 5, 10, 20, and 40 ppm threshold levels.

Environmental Surface Study

The environmental surface study was performed to test the ability of the GlutenTox Pro test kit to detect the gliading-containing reference material (PWG gliadin) on each of five environmental surface matrixes (food-grade painted wood, plastic, rubber, sealed ceramic, and stainless steel).

Table 9. GlutenTox Pro Test Kit for yogurt–POD results

Matrix	Gluten spike level, ppm	GlutenTox Pro detection threshold, ppm gluten	N[a]	Candidate x[b]	Candidate POD_C[c]	Candidate 95% CI[d]	Average AOAC OMA **2012.01** results, ppm gluten, N = 3[e]	Variance (σ^2)
Yogurt	0	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	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	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	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	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	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		

[a] N = Number of test portions.

[b] x = Number of positive test portions.

[c] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.

[d] 95% Confidence intervals.

[e] OMA = *Official Methods of Analysis*.

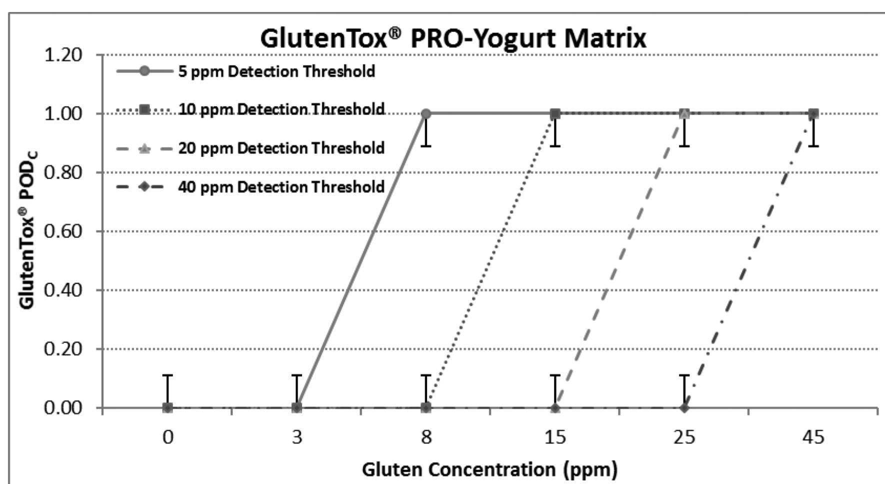

Figure 6. GlutenTox Pro POD versus gluten concentration for yogurt.

The results of each environmental surface matrix analysis are presented in Table 10. The requirement of fractional positive results (at a low spiked level) was obtained for each surface matrix test portion analyzed by the GlutenTox Pro test kit: food-grade painted wood, plastic, rubber, sealed ceramic, and stainless steel test portions produced 25 (83%), 23 (77%), 26 (87%), 25 (83%), and 21 (70%) observed positive samples and POD_C values of 0.83, 0.77, 0.87, 0.83, and 0.70, respectively.

Table 10. GlutenTox ProTest Kit environmental surface–POD results

Matrix (16 cm^2)	Amt of spiked gluten,ng/16 cm^2	N[a]	Candidate		
			x[b]	POD_C[c]	95% CI[d]
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

[a] N = Number of test portions.

[b] x = Number of positive test portions.

[c] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.

[d] 95% Confidence intervals.

All samples analyzed at the unspiked level were negative, and at the high spike level were positive for each surface matrix tested.

For the evaluation of stainless steel by the independent laboratory, 40 replicate 1" × 1" (6.45 cm^2) test areas were evaluated for the presence of gluten. The candidate used a different surface area (16 cm^2) since it had previously been used in an internal validation (12) as indicated in the package insert.

The results obtained with the GlutenTox Pro test kit (shown in Table 11) were all negative for the unspiked sample portions, all positive for the high spike level, and for fractional recovery, the independent laboratory recorded 21 positive.

Figure 7 shows plots of dPOD of stainless steel surface testing results between the candidate and independent laboratories. There is no significant difference between the internal validation data and independent laboratory data at the unspiked, low, and high spike levels of gluten for the stainless steel surface.

Product Consistency (Lot-to-Lot)

Consistency among three different lots of the GlutenTox Pro test kit [191246 (exp. date June, 2015), 110247 (exp. date

Table 11. Independent laboratory GlutenTox ProTest Kit for stainless steel–POD results

Matrix (6.45 cm^2)	Amt of spiked gluten	N[a]	Candidate		
			X[b]	POD_C[c]	95% CI[d]
Stainless steel	0	5	0	0.00	0.00, 0.43
	Low (7 μg/6.45 cm^2)	30	21	0.70	0.52, 0.83
	High (35.2 μg/6.45 cm^2)	5	5	1.00	0.57, 1.00

[a] N = Number of test portions.

[b] x = Number of positive test portions.

[c] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.

[d] 95% Confidence intervals.

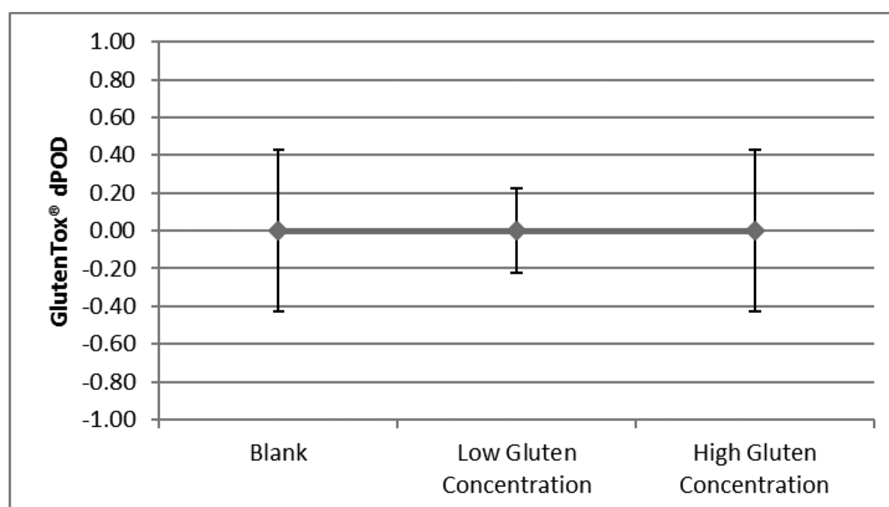

Figure 7. dPOD of GlutenTox Pro POD results between candidate and independent laboratories versus gluten concentration levels for stainless steel.

July, 2015), and 110647 (exp. date April, 2016)] was examined in rice flour for lot-to-lot variability. Spikes, using the gliadin-containing reference material (PWG gliadin), were tested at two different levels of gluten (0 and 15 ppm). At each spike level, every lot of the test method was assayed using two different detection threshold levels (10 and 20 ppm) and for each kit lot, spike level and detection threshold combination, 10 blind-coded replicate test portions were analyzed.

For product consistency, results are presented in Table 12. For the 0 ppm spike level, there were 0 observed positive samples and a POD_C value of 0.00 for the two detection threshold levels for all lots.

For the 15 ppm spike level, there were 10 and 0 observed positive samples and POD_C values of 1.00 and 0.00 for the 10 and 20 ppm threshold levels, respectively, for all lots.

Test Kit Variation Study

Variation among three test kits of the same production lot (191246) of GlutenTox Pro test kit was examined for rice flour. Spikes, using the gliadin-containing reference material (PWG gliadin), were tested at two different levels of gluten (0 and 15 ppm). At each spike level, every kit of a single lot of the GlutenTox Pro test method was assayed using two different detection threshold levels (10 and 20 ppm) and for each kit (of a single lot), spike level, and detection threshold combination, 10 blind-coded replicate test portions were analyzed. Test kit variation results are presented in Table 13.

For the 0 ppm spike level, there were 0 observed positive samples and a POD_C value of 0.00 for the two detection threshold levels for all kits of the single lot.

Table 12. GlutenTox Pro Test Kit product consistency–POD results

					Candidate		
Matrix	Kit Lot	Gluten spiked level, ppm	Detection threshold, ppm	N[a]	x[b]	POD_C[c]	95% CI[d]
Rice flour	Kit Lot No. 1 (191246)	Blank, 0	10	10	0	0.00	0.00, 0.28
			20	10	0	0.00	0.00, 0.28
	Kit Lot No. 1 (191246)	15	10	10	10	1.00	0.72, 1.00
			20	10	0	0.00	0.00, 0.28
	Kit Lot No. 2 (110247)	Blank, 0	10	10	0	0.00	0.00, 0.28
			20	10	0	0.00	0.00, 0.28
	Kit Lot No. 2 (110247)	15	10	10	10	1.00	0.72, 1.00
			20	10	0	0.00	0.00, 0.28
	Kit Lot No. 3 (110647)	Blank, 0	10	10	0	0.00	0.00, 0.28
			20	10	0	0.00	0.00, 0.28
	Kit Lot No. 3 (110647)	15	10	10	10	1.00	0.72, 1.00
			20	10	0	0.00	0.00, 0.28

[a] N = Number of test portions.

[b] x = Number of positive test portions.

[c] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.

[d] 95% Confidence intervals.

Table 13. GlutenTox Pro Test Kit Test Kit variation–POD results

					Candidate		
Matrix	Kit Lot	Gluten spiked level, ppm	Detection threshold, ppm	N[a]	x[b]	POD_C[c]	95% CI[d]
Rice flour	Kit 1.1 Lot No. 1 (191246)	Blank, 0	10	10	0	0.00	0.00, 0.28
			20	10	0	0.00	0.00, 0.28
	Kit 1.1 Lot No. 1 (191246)	15	10	10	10	1.00	0.72, 1.00
			20	10	0	0.00	0.00, 0.28
	Kit 1.2 Lot No. 1 (191246)	Blank, 0	10	10	0	0.00	0.00, 0.28
			20	10	0	0.00	0.00, 0.28
	Kit 1.2 Lot No. 1 (191246)	15	10	10	10	1.00	0.72, 1.00
			20	10	3	0.30	0.11, 0.60
	Kit 1.3 Lot No. 1 (191246)	Blank, 0	10	10	0	0.00	0.00, 0.28
			20	10	0	0.00	0.00, 0.28
	Kit 1.3 Lot No. 1 (191246)	15	10	10	10	1.00	0.72, 1.00
			20	10	0	0.00	0.00, 0.28

[a] N = Number of test portions.

[b] x = Number of positive test portions.

[c] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.

[d] 95% Confidence intervals.

For the 15 ppm spike level, there were 10 observed positive samples and a POD_C value of 1.00 for the 10 ppm threshold level for all kits and 0 and three observed positive samples and POD_C values of 0.00 and 0.30 for the 20 ppm threshold level for two kits and for one kit, respectively, of the single lot.

Robustness Study

The robustness study was performed to evaluate the ability of the method to remain unaffected by small variations in procedural parameters that might be expected to occur when the method is performed by an end user. Three parameters important to the end user were chosen to be varied. The effects of perturbations in extraction time (shaking time; 0 or 5 min, normal = 2 min), amount of dilution sample solution into blue cap (3 or 15 drops, normal = 5–10 drops), and time to result (5 or 15 min, normal = 10 min) were examined for rice flour using the gliadin-containing reference material (PWG gliadin). A factorial design was used to test the ruggedness parameters. Spikes were tested at two different spike levels of gluten (0 and 15 ppm). At each spike level of gluten and for each of the eight treatment combinations, the test method was assayed using two different detection threshold levels (10 and 20 ppm).

For each spike level and detection threshold combination, 10 blind-coded replicate test portions were analyzed by the GlutenTox Pro method, but varying the parameters as indicated for each treatment combination. Robustness results are presented in Table 14.

Treatment combination 1.—For the 0 ppm and 15 ppm spike levels, there were 0 observed positive samples and a POD_C value of 0.00 for the two detection threshold levels for the GlutenTox Pro test kit.

Treatment combination 2.—For the 0 ppm spike level, there were 0 observed positive samples and a POD_C value of 0.00 for the two detection threshold levels.

For the 15 ppm spike level, there were 10 and 0 observed positive samples and POD_C values of 1.00 and 0.00 for the 10 and 20 ppm threshold levels, respectively.

Treatment combination 3.—For the 0 ppm and 15 ppm spike levels, there were 0 observed positive samples and a POD_C value of 0.00 for the two detection threshold levels.

Treatment combination 4.—For the 0 ppm spike level, there were 0 observed positive samples and a POD_C value of 0.00 for the two detection threshold levels.

For the 15 ppm spike level, there were 10 and 0 observed positive samples and POD_C values of 1.00 and 0.00 for the 10 and 20 ppm threshold levels, respectively.

Treatment combination 5.—For the 0 ppm spike level, there were 0 observed positive samples and a POD_C value of 0.00 for the two detection threshold levels.

For the 15 ppm spike level, there were four and 0 observed positive samples and POD_C values of 0.40 and 0.00 for the 10 and 20 ppm threshold levels, respectively.

Treatment combination 6.—For the 0 ppm spike level, there were 0 observed positive samples and a POD_C value of 0.00 for the two detection threshold levels. For the 15 ppm spike level, there were eight and 0 observed positive samples and POD_C values of 0.80 and 0.00 for the 10 and 20 ppm threshold levels, respectively.

Treatment combination 7.—For the 0 ppm spike level, there were 0 observed positive samples and a POD_C value of 0.00 for the two detection threshold levels.

For the 15 ppm spike level, there were five and 0 observed positive samples and POD_C values of 0.50 and 0.00 for the 10 and 20 ppm threshold levels, respectively.

Treatment combination 8.—For the 0 ppm spike level, there were 0 observed positive samples and a POD_C value of 0.00 for the two detection threshold levels. For the 15 ppm spike level, there were 10 and 0 observed positive samples and POD_C values of 1.00 and 0.00 for the 10 and 20 ppm threshold levels, respectively.

Table 14. GlutenTox Pro Test Kit robustness study–POD results

Treatment combination	Extraction time, min	Amt of dilution sample solution into blue cap, drops	Time to result, min	Contamination level, ppm	Detection threshold, ppm	Candidate			
						N[a]	x[b]	POD_C[c]	95% CI[d]
1	0	3	5	Blank, 0	10	10	0	0.00	0.00, 0.28
					20	10	0	0.00	0.00, 0.28
1	0	3	5	15	10	10	0	0.00	0.00, 0.28
					20	10	0	0.00	0.00, 0.28
2	0	3	15	Blank, 0	10	10	0	0.00	0.00, 0.28
					20	10	0	0.00	0.00, 0.28
2	0	3	15	15	10	10	10	1.00	0.72, 1.00
					20	10	0	0.00	0.00, 0.28
3	0	15	5	Blank, 0	10	10	0	0.00	0.00, 0.28
					20	10	0	0.00	0.00, 0.28
3	0	15	5	15	10	10	0	0.00	0.00, 0.28
					20	10	0	0.00	0.00, 0.28
4	0	15	15	Blank, 0	10	10	0	0.00	0.00, 0.28
					20	10	0	0.00	0.00, 0.28
4	0	15	15	15	10	10	10	1.00	0.72, 1.00
					20	10	0	0.00	0.00, 0.28
5	5	3	5	Blank, 0	10	10	0	0.00	0.00, 0.28
					20	10	0	0.00	0.00, 0.28
5	5	3	5	15	10	10	4	0.40	0.17, 0.69
					20	10	0	0.00	0.00, 0.28
6	5	3	15	Blank, 0	10	10	0	0.00	0.00, 0.28
					20	10	0	0.00	0.00, 0.28
6	5	3	15	15	10	10	8	0.80	0.49, 0.94
					20	10	0	0.00	0.00, 0.28
7	5	15	5	Blank, 0	10	10	0	0.00	0.00, 0.28
					20	10	0	0.00	0.00, 0.28
7	5	15	5	15	10	10	5	0.50	0.24, 0,76
					20	10	0	0.00	0.00, 0.28
8	5	15	15	Blank, 0	10	10	0	0.00	0.00, 0.28
					20	10	0	0.00	0.00, 0.28
8	5	15	15	15	10	10	10	1.00	0.72, 1.00
					20	10	0	0.00	0.00, 0.28

[a] N = Number of test portions.

[b] x = Number of positive test portions.

[c] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.

[d] 95% Confidence intervals.

Stability Studies

Stability of GlutenTox Pro test kit was examined for rice flour through accelerated studies based on the Arrhenius model (13), having to support a shelf life of 2 years. One lot of the GlutenTox Pro test kit contents were stored at 42°C (2–30°C is the normal storage temperature of the GlutenTox Pro test kit contents), and the accelerated stability study were performed testing the kit at specified time points (10, 20, 35, 50, and 90 days). Spikes, using the gliadin-containing reference material (PWG gliadin), were tested at two different spike levels of gluten (0 and 15 ppm). At each spike level of gluten and at each stability time point, the GlutenTox Pro test method was assayed using two different detection threshold levels (10 and 20 ppm) and for each spike level and detection threshold combination, 10 blind-coded replicate test portions were analyzed.

As accelerated stability provides only a rough estimate of shelf-life, real time data supporting the entire shelf life of the kit under normal storage conditions will be submitted prior to

Table 15. GlutenTox Pro Test Kit stability accelerated–POD results

Matrix	Storage temperature	Time points, days	Contamination level, ppm	Detection threshold, ppm	N[a]	Candidate		
						x[b]	POD_C[c]	95% CI[d]
Rice flour	42°C	10	Blank, 0	10	10	0	0.00	0.00, 0.28
				20	10	0	0.00	0.00, 0.28
		10	15	10	10	10	1.00	0.72, 1.00
				20	10	1	0.10	0.00, 0.40
		20	Blank, 0	10	10	0	0.00	0.00, 0.28
				20	10	0	0.00	0.00, 0.28
		20	15	10	10	10	1.00	0.72, 1.00
				20	10	0	0.00	0.00, 0.28
		35	Blank, 0	10	10	0	0.00	0.00, 0.28
				20	10	0	0.00	0.00, 0.28
		35	15	10	10	10	1.00	0.72, 1.00
				20	10	0	0.00	0.00, 0.28
		50	Blank, 0	10	10	0	0.00	0.00, 0.28
				20	10	0	0.00	0.00, 0.28
		50	15	10	10	10	1.00	0.72, 1.00
				20	10	0	0.00	0.00, 0.28
		90	Blank, 0	10	10	0	0.00	0.00, 0.28
				20	10	0	0.00	0.00, 0.28
		90	15	10	10	10	1.00	0.72, 1.00
				20	10	0	0.00	0.00, 0.28

[a] N = Number of test portions.

[b] x = Number of positive test portions.

[c] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.

[d] 95% Confidence intervals.

renewal at the end of the first year of certification. Accelerated stability results are presented in Table 15.

Stability time point: 10 days.—For the 0 ppm spike level, there were 0 observed positive samples and a POD_C value of 0.00 for the two detection threshold levels for the GlutenTox Pro test kit.

For the 15 ppm spike level, there were 10 and one observed positive samples and POD_C values of 1.00 and 0.10 for the 10 and 20 ppm threshold levels, respectively.

Stability time point: 20, 35, 50, and 90 days.—For the 0 ppm spike level, there were 0 observed positive samples and a POD_C value of 0.00 for the two detection threshold levels. For the 15 ppm spike level, there were 10 and 0 observed positive samples and POD_C values of 1.00 and 0.00 for the 10 and 20 ppm threshold levels, respectively.

Discussion

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* (2) 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 about this type of sample has been included in the instructions for use.

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

In all matrixes tested, the GlutenTox Pro method demonstrated 100% specificity [POD 0.00, confidence interval (CI) 0.00–0.11] at 0 ppm spike level of gluten and 100% sensitivity (POD 1.00, CI 0.89–1.00) at each spike level of gluten and threshold level combinations. No false-negative results were obtained in the food matrix study. The assay did not experience a hook effect at any threshold level tested when the rice flour matrix was spiked at very high 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 (9; recovery could be between 50 and 150%).

The GlutenTox Pro test kit performed as expected for 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^2) for each of the environmental surfaces analyzed.

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

The lot-to-lot data and the accelerated stability data (10, 20, 35, 50, and 90 days at 42°C) showed evidence that the GlutenTox Pro method is stable and can be consistently manufactured with reproducible quality.

Variation data among three test kits of a single lot of GlutenTox Pro test kits demonstrated no statistical differences in gluten detection. Occasional slight overestimations are irrelevant in gluten analysis compared to problems that could arise from false negatives or underestimations.

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

Robustness data indicated that the GlutenTox Pro assay remained unaffected by minor variations in procedural parameters with the exception of the amount of time that the test strip was left in the dilution sample solution before reading the result. Due to the test format, there must be sufficient time for the dilution sample solution to travel up the test strip, and this time cannot be shortened. The effect of decreasing the strip incubation time was not dependent of the amount of dilution sample solution used, but this effect was smaller when coupled with an increased sample extraction time.

When the test strip was left in a smaller amount of dilution sample solution, some invalid results appeared.

Conclusions

The GlutenTox 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 and should be granted *Performance Tested Method*^{SM} certification.

The GlutenTox Pro test kit is a stable and cost-effective kit recommended for consumers and industry. The instructions for use include the possibility of choosing different detection threshold levels of gluten according to the end user requirements.

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