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GlutenTox® SticksPlus for Reader

Quick test for the quantiication of gluten content in food and beverages with GlutenTox® Reader.

REF KIT3007 (KT-6476)

GlutenTox® SticksPlus for Reader

Contents

1. Intended Purpose

GlutenTox Sticks Plus for Reader is a rapid immunochromatographic test for the quantiication of gluten, which is harmful for celiac patients, in food, beverages and other consumer products.

2. Introduction

Celiac disease is a disorder that damages the small intestine causing the 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 (a mix of proteins found in cereals) from wheat, barley, rye, and to a lesser extent, from oat [ref. 1 and 2], leading to diarrhea, vitamin and mineral deiciencies, 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 dificulties 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 the 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*).

* Milligrams of gluten per kilo of food.

3. Test basis

GlutenTox Sticks Plus for Reader is an immunochromatographic (lateral low) test for the quantiication of gluten in foods with different composition and levels of processing, from raw materials to heat-processed food, beverages and other consumer products. It is based on the anti-gliadin G12 antibody, which speciically recognizes the 33-mer peptide, the most immunogenic fraction of gluten [ref. 3]. This rapid test is useful in routine monitoring of gluten presence, to guarantee that products comply with a program of Hazard Analysis and Critical Control Points (HACCP), and to ensure proper labeling. It also allows quick decisions and corrective actions in case there is any risk of contamination along the production chain.

In all methods used for gluten analysis in a given sample, the gluten irst has to be extracted from the sample’s matrix. Extraction is one of the most critical points of the testing process. The extraction solution provided in this kit, Universal Gluten Extraction Solution (UGES), is suited for all types of food thanks to the combination of denaturing agents, reducing agents and solubilizers.

For the analysis of food containing polyphenols, including tannins, such as chocolate, tea, coffee, wine, purple corn and corn iber, soy, berries, legumes like chickpeas and lentils, etc., it is necessary to use a special additive (**not included in this kit, see section 5**) that prevents the interference of the above mentioned compounds in the extraction process (see Figure 1). The same applies in the case of cosmetic products with antioxidant compounds such as vitamins A, C and E, carotenes, carotenoids, etc.

After the extraction, during the detection step, the gluten present in the sample reacts irst with the anti-gliadin G12 antibody [ref. 3] conjugated to red colored particles, previously placed on the stick. The resulting complexes spread by capillarity through the stick and react with a second anti-gliadin antibody, also previously immobilized on the stick. If the result is positive, a RED line appears in the Test Zone (T) of the cassette. The absence of the RED line indicates a negative result. Whether or not gluten is present, the sample moves through the stick up to the Control Zone (C) where, if the test was properly performed, a GREEN line will appear, due to the accumulation of green colored particles also included in the stick. The presence of this GREEN line indicates that: 1) the sample volume was enough, 2) the sample low was appropriate, and 3) the conjugate particles included in the test were properly released. If the GREEN line does not appear, the test should be considered invalid.

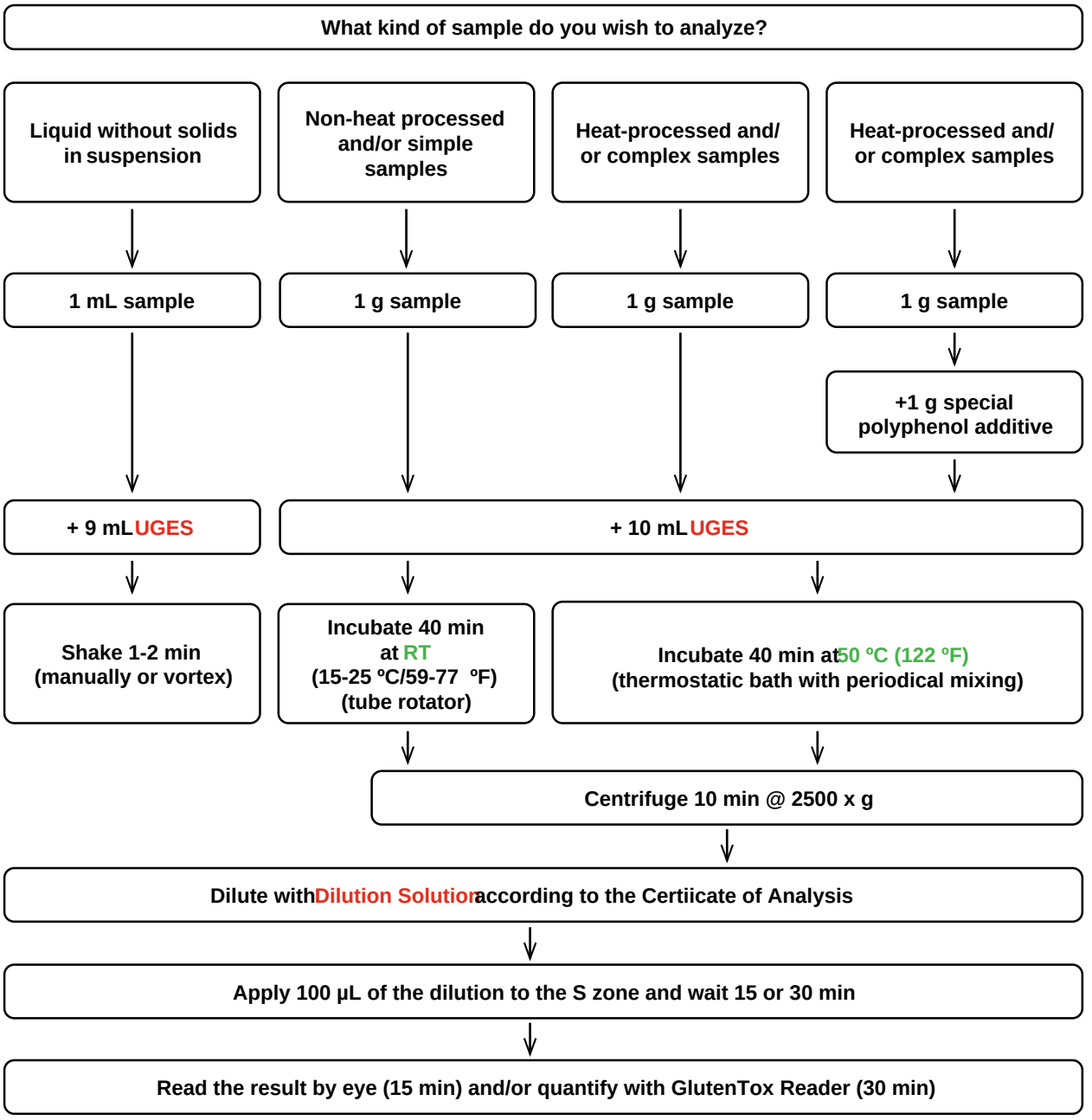

Figure 1. Scheme of use for GlutenTox Sticks Plus for Reader

This test allows two options for the reading of the results: a visual reading and a digital scanning with the GlutenTox Reader. GlutenTox Reader combines a highly sensitive optical detector with an integrated electronic system for eficient data processing. GlutenTox Reader is CE labeled and produced according to ISO 9001 and ISO 13485.

4. Supplied materials

- GlutenTox cassettes (12 cassettes)
- Universal Gluten Extraction Solution (UGES) (125 mL)
- Dilution Solution (15 mL)
- Positive Control (wholegrain oat lour contaminated with gluten, 10 g)
- Negative Control (corn lour, 10 g)
- Instructions for use

5. Necessary materials not supplied

- Analytical scale (accurate to 0.1 g)
- Thermostatic bath (not necessary for non-heat- processed samples with simple matrix composition)
- Capped centrifuge test tubes (>10 mL)
- Test vials (1.5 – 2 mL)
- Centrifuge (optional)
- Pipettes and disposable tips
- Disposable gloves
- Vortex mixer (optional)
- Watch/chronometer
- Tube rotator

For testing food containing polyphenols (including tannins) and cosmetic containing antioxidants, please acquire the Polyphenol Pack (KT-5320/KIT3008)* available from Hygiena TM. This pack contains:

- Special polyphenol additive (25 g).
- Positive Control containing polyphenols (cocoa powder with gluten, 10 g).
- Negative Control containing polyphenols (gluten-free cocoa powder, 10 g).

IMPORTANT NOTE!

- Foods rich in polyphenols or tannins are: chocolate, tea, coffee, wine, purple corn and corn iber, soy, berries, legumes like chickpeas and lentils, etc.

IMPORTANT NOTE!

- The most common antioxidants in cosmetic products are vitamins A, C and E, caarotenes, carotenoids, etc.

*For more information contact your supplier.

6. Storage conditions and stability

For optimal test performance, GlutenTox Sticks Plus for Reader must be stored in its original packaging, between 15 °C - 25 °C (59 °F - 77 °F) and used before the expiration date printed on the label.

WARNING: The envelopes with the cassettes should not be opened until the time of use. Never freeze.

7. Precautions

- Only for testing food, beverages and other consumer products.
- Do not ingest the solutions (liquids) of the kit.
- Do not use after the expiration date.
- The use of non-powdered disposable gloves is recommended.
- Manipulate the cassettes with gloves or washed hands and do not touch the Sample Zone (S) within the cassette, to avoid accidental contamination of the test with gluten traces.
- If a sample is heterogeneous (e.g. a salad), make sure to take a representative part of each ingredient, and mix them to make a homogenous sample. If the gluten in the sample is unevenly distributed and you do not do this, a false negative could be obtained.

8. Sample preparation (food, beverages and other consumer products)

8.1. Solid samples

1. Homogenize, mill and/or triturate the sample.

2. Weigh 1 g of sample and add it to a test tube.

IMPORTANT NOTE!

- If the sample, solid or liquid, contains polyphenols, tannins (e.g. chocolate) or antioxidants, weigh and add 1 g of special polyphenol additive (KT-5320/KIT3008), to the sample tube and mix it vigorously to achieve complete homogenization of the mixture.

3. Add 10 mL of Universal Gluten Extraction Solution (UGES). Close the tube and mix to homogenize (for example, using a vortex mixer).

4. Depending on the complexity of the sample matrix and whether the food sample has been processed by heat or not, follow one of the 2 options below (see Figure 2):

a) Non-heat processed samples with simple matrix composition:

Incubate the sample at room temperature (15 - 25 ºC / 59 - 77 ºF) for 40 minutes with a tube rotator.

b) Heat-processed samples and/or with complex matrix composition:

Incubate the sample at 50 ºC (122 ºF) in a water bath for 40 minutes, shaking the tube periodically by tipping it over or using a vortex mixer.

IMPORTANT NOTE!

- If the type of sample is dificult to determine, we recommend heating at 50 ºC (122 ºF) (option b) to facilitate the extraction.

5. Allow separation of solids by settling or centrifugation (10 min at 2500 x g). Solid parts can alter the results.

6. Transfer the clariied supernatant to a clean tube.

IMPORTANT NOTE!

- Once extracted, the samples must be analyzed as quickly as possible.

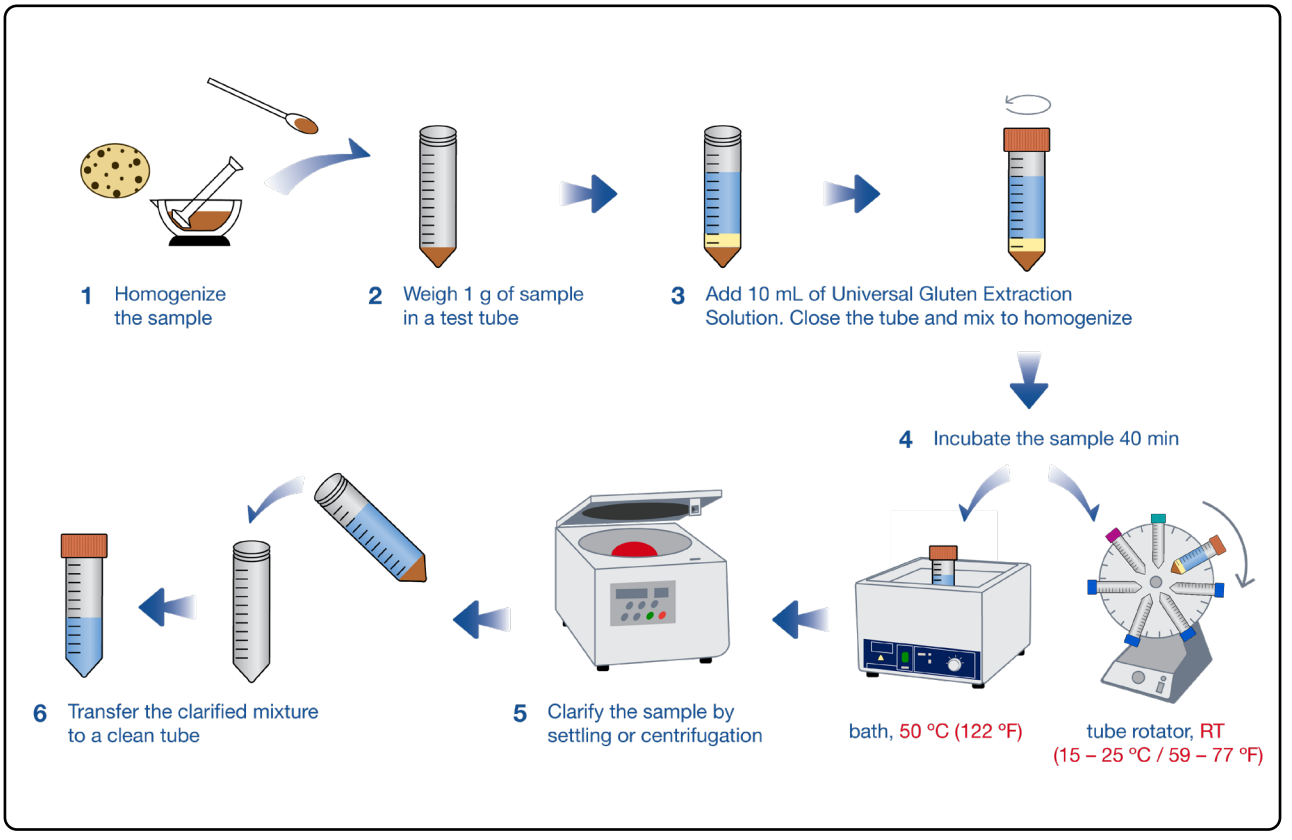

Figure 2. Scheme of the extraction procedure of the solid samples

8.2. Liquid samples

Liquid samples such as milk, juices, soft drinks, organic drinks (soy, rice, oat, spelt drinks), beers and broths do not require intensive extraction. For this reason, manually shaking for 1 or 2 minutes is suficient and the extracts do not require a centrifugation or settling step.

1. Add 1 mL of sample to a test tube.

2. Add 9 mL of Universal Gluten Extraction Solution (UGES) and close the tube cap tightly.

IMPORTANT NOTE!

- Liquid samples with polyphenols, tannins or antioxidants must be extracted according to the point 8.1 Solid Samples.

3. Shake the sample for 1-2 minutes, manually or using a vortex mixer.

IMPORTANT NOTE!

- Once extracted, the samples must be analyzed as soon as possible.

9. Test implementation for extracted samples

1. Bring the extracted samples, controls, the Dilution Solution, and the cassettes to room temperature (15 - 25 ºC / 59 - 77 ºF).

2. Dilute the extracted samples with Dilution Solution in test tubes or vials.The appropriate dilution corresponding to the batch of GlutenTox cassettes can be found in the Certiicate of Analysis included in each kit. A inal volume of 900-1000 L is suficient to perform the test.

IMPORTANT NOTE!

- In samples with high levels of fat, avoid taking the upper layer that contains the fat.

IMPORTANT NOTE!

- The diluted samples must be analyzed as quickly as possible and the remaining material should be discarded.

3. Open the envelope of the cassette. If you wish to perform positive and negative controls, you will need one cassette for each control.

4. Add 100 L of the diluted sample or control into the Sample Zone (S) of the cassette.

5. Wait 15 minutes and read the result on the cassette (see section 11).

6. To quantify the gluten in the sample, wait at least 30 minutes (and not longer than 2 hours) before introducing the cassette into the GlutenTox Reader.

10. Use of the GlutenTox Reader

The Reader is designed exclusively for the quantiication of gluten content with GlutenTox Sticks cassettes. The quantiication potential of the kit ranges from 1 ppm to 40 ppm gluten. To quantify your sample, follow these steps:

1. At least 30 minutes after adding the sample to the cassette, insert the cassette into the cassette holder and in the Reader.

2. When using the Reader in stand-alone mode, enter the identiication data of the sample in the device and press "measure" to start the reading. The result will appear on the screen as “ppm of gluten”.
3. When using the GlutenTox Reader while plugged into a computer, use the provided LF Software to identify the sample and start the reading.

IMPORTANT NOTE!

- **Before performing the test and the quantiication of the results, make sure the method installed in your GlutenTox Reader is the right one for the lot number of the cassettes that you plan to use. Methods are available at www.hygiena.com.**

11. Visual interpretation of results

For quantiication with GlutenTox Reader, see section 10. For visual interpretation of the results, it is enough to wait 15 min after adding the sample to the cassette. A result can be:

NEGATIVE: A single GREEN line (control line) appears in the Control Zone (C) of the cassette. The sample contains less than 1 ppm of gluten.

POSITIVE: In addition to the control line (GREEN), a RED line appears in the Test Zone (T). Sample contains more than 1 ppm of gluten and is quantiiable with GlutenTox Reader.

INVALID: The control line (GREEN) does not appear, whether or not the test line (RED) appears. The most common causes for the appearance of an invalid result are adding an insuficient (<100 L) volume of sample to the cassette, performing an incorrect procedure, or deterioration of the kit reagents. In the case of an invalid result, it is necessary to revise the procedure and repeat the experiment with a new cassette. If the problem persists, please contact your supplier.

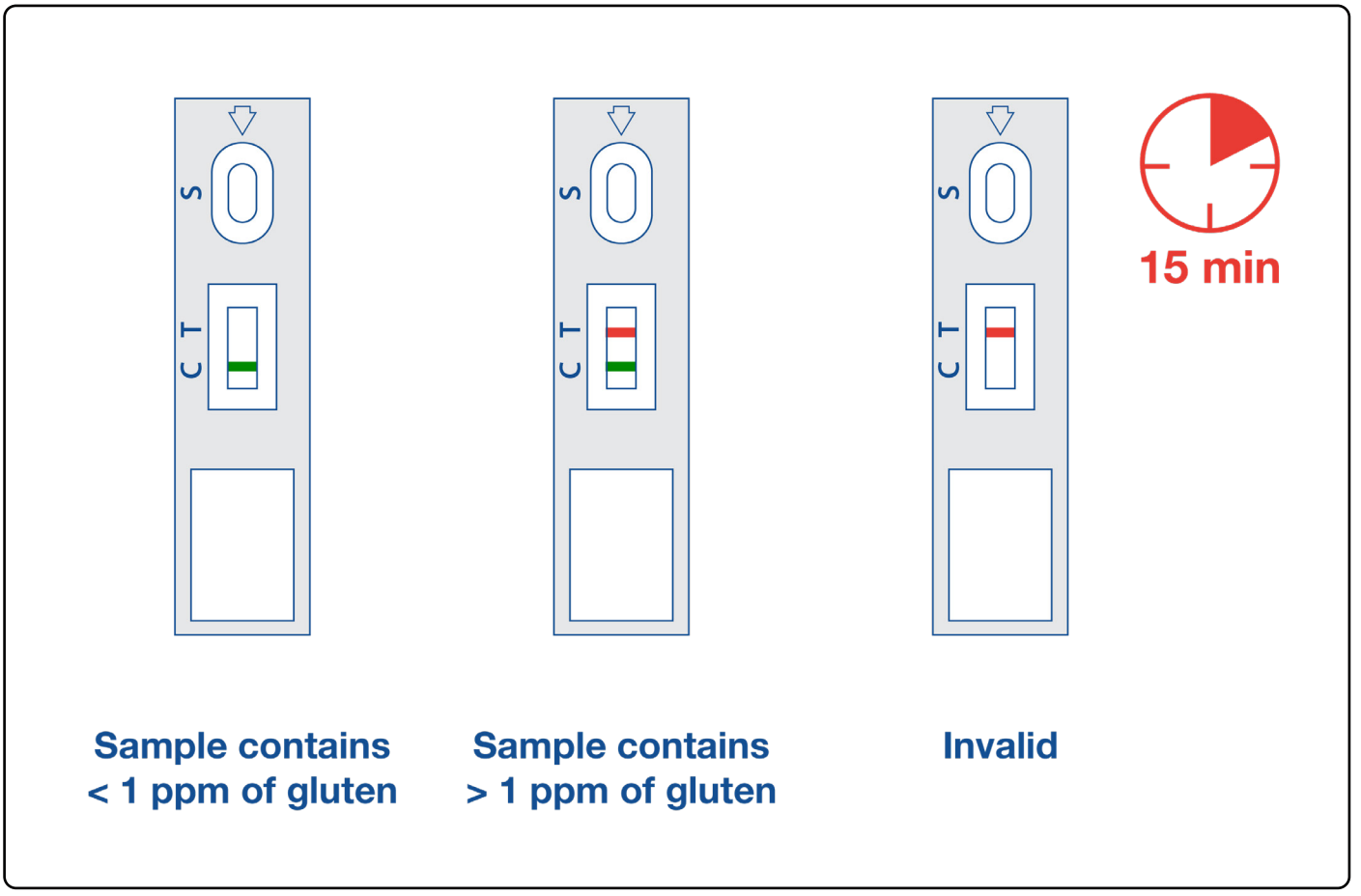

Figure 3. Visual interpretation of results

12. Quality control

Internal procedural quality control is included in the test. The green line in the Control Zone (C) is a built-in feature that indicates both a suficient volume and a correct low of the sample, with proper release of the conjugate particles. In addition, the kit includes Positive and Negative Controls that can be used, according to the instructions in point 8.1.2 and onwards, to conirm a correct test performance; these control materials must provide clear positive and negative results, respectively, if the test procedure was conducted properly.

13. Analytical features

Different assays have been carried out to characterize the main analytical parameters of the test: sensitivity and speciicity.

Sensitivity

The detection limit of GlutenTox Sticks Plus for Reader is 1 ppm gluten. This value was obtained using different solutions of known concentrations of gliadin (one of the proteins of gluten) in the Dilution Solution.

Speciicity

This test can speciically detect the presence of the toxic fraction of the prolamins of wheat (gliadin), rye (secalin), barley (hordein) and some varieties of immunogenic oats (avenin) that can therefore be harmful for celiac patients [ref. 2]. However, when the samples contain celiac-safe foods like rice, corn, soy, buckwheat, sesame, millet, teff, quinoa and amaranth, no positive signal is observed.

Internal Validation

To ensure the test's performance with all types of food and other materials such as cosmetics and personal care products, a broad range of commercial products have been tested. After analyzing the samples with GlutenTox Sticks in all types of matrices tested (see Table 1 and 2) the results were satisfactory and consistent with the gluten contents determined with the approved method of Codex Alimentarius.

Table 1. Food samples tested for validation of GlutenTox Sticks Plus

Group	Tested samples
Flour and semolina	Corn lour, precooked corn lour, corn semolina, rice lour, wheat lour, buckwheat lour
Milk products	Cow milk, milk with soluble iber, milk with cereals, lavoured or natural yogurt, cheese spread, shredded cheese blend
Baked and cereal products	Toast, bread stick, biscuits (Rich tea), chocolate cookies, Madeleine, cake, cornlakes, pastas, corn pancakes, rice cakes, spelt cake, snacks
Meat products	Minced turkey, minced chicken, turkey sausage, chicken nuggets, pork sausages, chorizo, pork liver pâté
Fishery products	Cod and Hake
Vegetables	Lettuce mix, fried vegetables
Broth, soups, creams and dry mixes	Vegetable broth, chicken rice soup, dehydrated vegetable soup, stock cubes, vegetable soup, peanut butter
Sauces, dressing, spices and condiments	Yogurt salad dressing, ketchup, soy sauce, salad dressing, garlic powder, paprika powder, cooking cream
Sugars	Glucose syrup, powdered sugar
Prepared meals and dishes	Meatballs in sauce with peas, Meat Ravioli in Egg Dough, bean stew
Fatty foods	Olive oil, sunlower oil, butter, margarine, cream
Acidic foods	Tomate sauce, wine vinegar, apple cider vinegar, lemon juice
Beverages	Water, milk, fruit juices, beer, soy drinks, rice drinks, oat drinks, soft drinks

Table 2. Non-food samples tested for validation of GlutenTox Sticks Plus

Group	Tested samples
Personal care products	Bath gel, shampoo, deodorant, toothpaste, mouthwash
Cosmetics	Creams (face, body and hands), cleanser, lip balm
Others	Pet food (dry food, wet food), cleaning products, drugs (tablets, capsules and syrups)

14. Intellectual property

The immunoreagents used in this kit are commercialized under the exclusive license for biological material from the Spanish National Research Council (CSIC).

15. References

1. SHAN L., et al.,"Structural basis for gluten intolerance in celiac sprue"; Science; 2002; 297: 2275-2279.

2. COMINO I. et al.,"Diversity in oat potential immunogenicity: basis for the selection of oat varieties with no toxicity in coeliac disease."; Gut; 2011; 60:915-922.

3. MORÓN B., et al.,"Sensitive detection of cereal fractions that are toxic to celiac disease patients by using monoclonal antibodies to a main immunogenic wheat peptide", 2008;87:405-414.

4. SÍGLEZ M.A., et al.,"Método de detección de gluten en supericies"; Alimentaria; 2010; 411:67-70.

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