hygiena™

GlutenTox® SticksPlus

Gluten detection kit for foods, drinks and working surfaces.

REF KIT3005 (KT-5340)

GlutenTox® SticksPlus

Contents

1. Intended Purpose

GlutenTox Sticks Plus is a rapid immunochromatographic test for the detection of gluten*, which is harmful for celiac disease sufferers, in food and on surfaces. This kit is recommended for consumers, commercial kitchens and industry.

* not for hydrolyzed sources of gluten.

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 “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 is an immunochromatographic (lateral low) test for the semi-quantitative determination 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].

GlutenTox Sticks Plus can also be used for surface testing, to conirm that these surfaces are suited to produce gluten-free products. 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, beer, cocoa and derivatives, soy, berries, 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 in the sample reacts irst with the anti-gliadin G12 antibody [ref. 3] conjugated to red colored particles, previously placed in 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 result zone of the stick. 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 region where, if the test was properly performed, a BLUE line will appear, due to the accumulation of blue colored particles also included in the stick.

The presence of this BLUE 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 BLUE line does not appear, the test should be considered invalid.

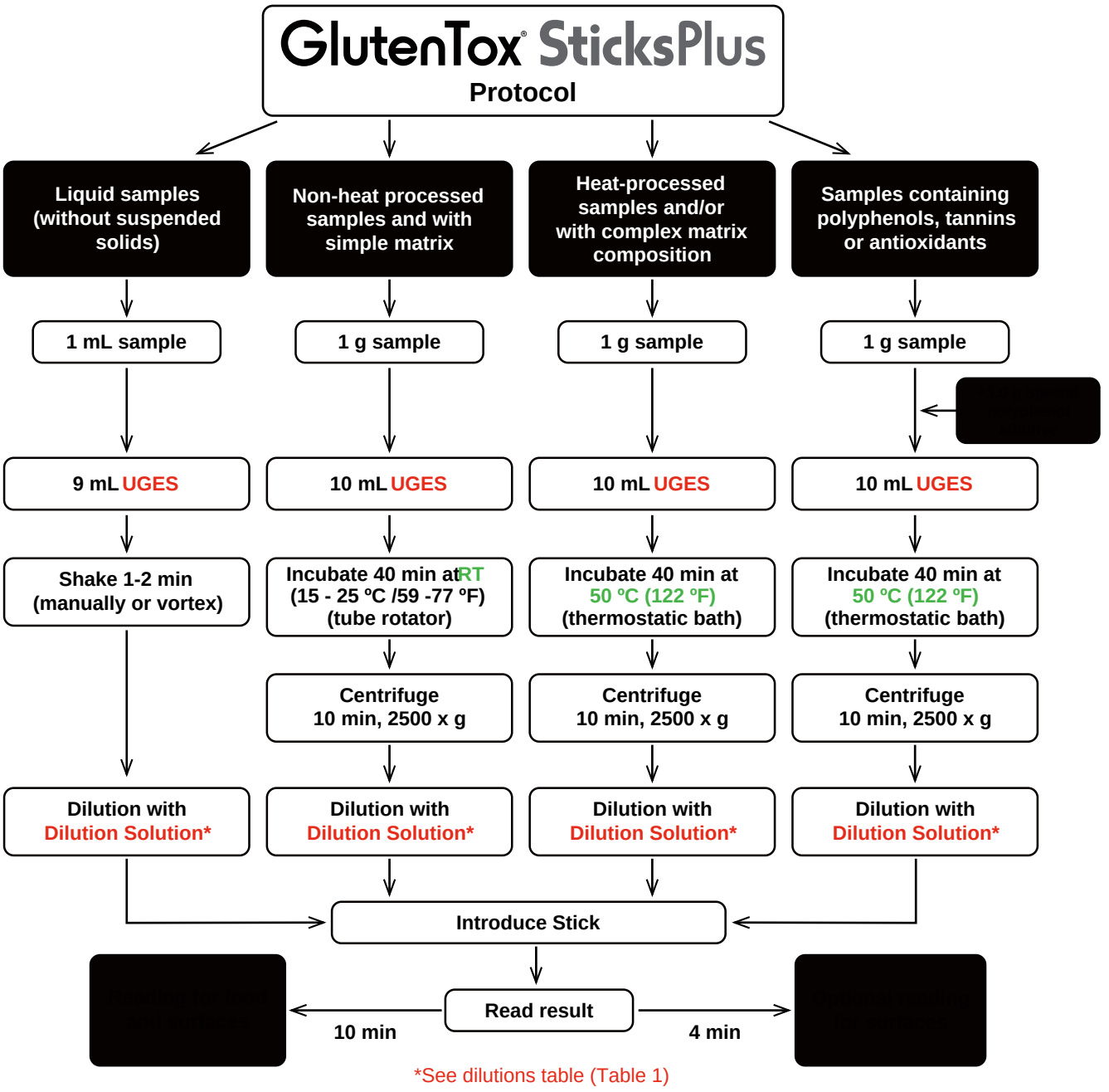


Figure 1. Scheme of use for GlutenTox Sticks Plus

4. Supplied materials

- GlutenTox Sticks (25 sticks in a tube)
- Universal Gluten Extraction Solution (UGES) (250 mL)
- Dilution Solution (30 mL)
- Microtiter plate strips (4 strips x 8 wells)
- 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)
- Tube rotator
- Watch/chronometer

For testing **food containing polyphenols (including tannins) and cosmetics containing antioxidants** please acquire the **Polyphenol Pack** (KT-5320/KIT3008)*, available from Hygiena™. 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 or lentils, etc.

IMPORTANT NOTE!

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

* For more information contact your supplier.

6. Storage conditions and stability

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

WARNING: The tube with the sticks should not be opened until the time of use. Once the seal is broken, keep the tube with the sticks tightly closed at room temperature (15 - 25 ºC /59 - 77 ºF). To avoid water condensation, do not refrigerate the tube after opening. Never freeze it.

7. Precautions

- Only for testing food, beverages, other consumer products and surfaces.
- Do not ingest any of the solutions (liquids) and/or additive of the kit.
- Do not use after the expiration date.
- The use of non-powdered disposable gloves is recommended.
- Manipulate the sticks with gloves or washed hands and do not touch the white end.
- 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). For provided positive and negative controls, perform the same procedure.

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 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 or samples containing polyphenols, tannins or antioxidants:

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).

6. Transfer the clariied supernatant to a clean tube.

IMPORTANT NOTE!

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

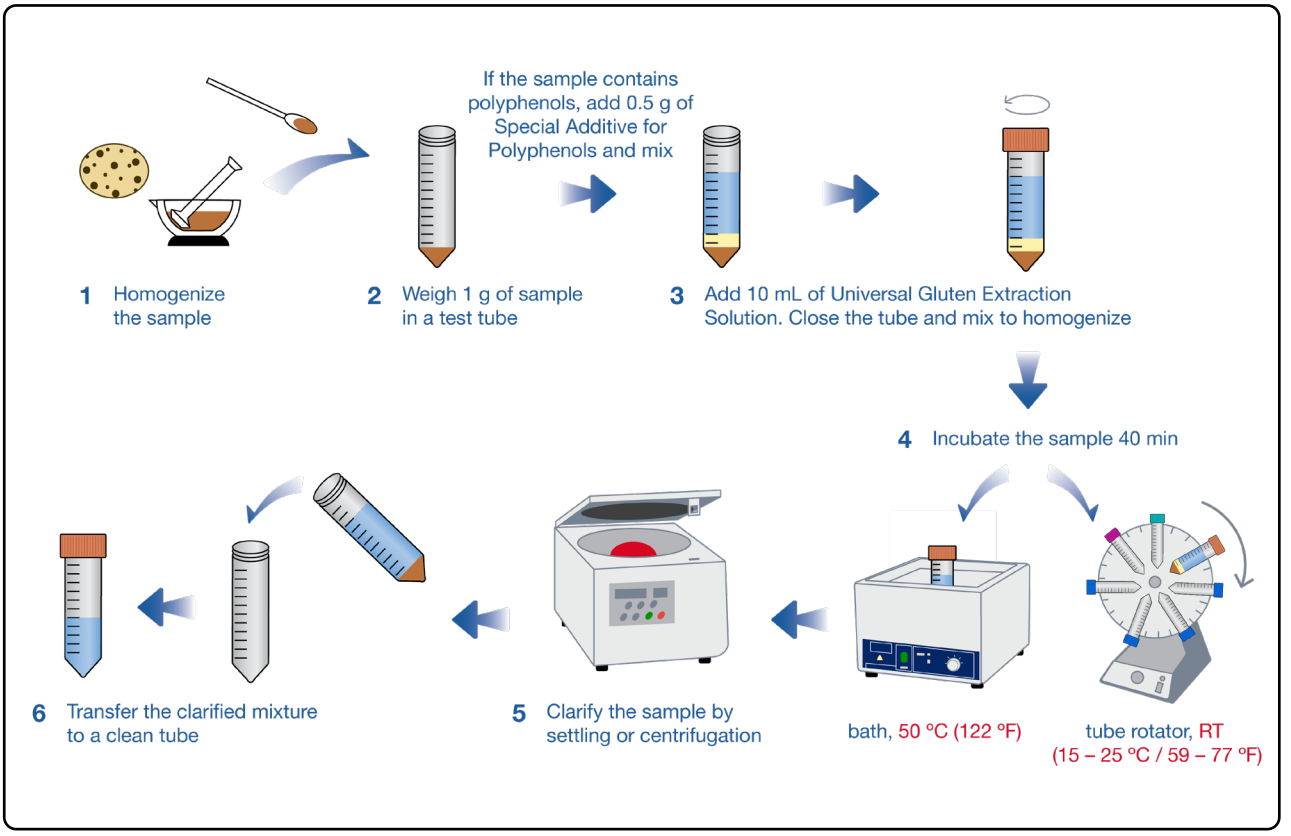


Figure 2. Scheme of the extraction procedure of the solid samples

8.2. Liquid samples

IMPORTANT NOTE!

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

Liquid samples such as milk, juices, soft drinks, organic drinks (soy, rice, oat, spelt drinks), beers and broths do not require intensive extraction, 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). Close the tube and mix to homogenize (for example, using a vortex mixer).

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, Dilution Solution and the tube with the GlutenTox Sticks to room temperature (15 - 25 ºC / 59 - 77 ºF).

2. Dilute the sample with the Dilution Solution in test tubes or vials. A inal volume of 900-1000 µL is suficient to perform the test.

Table 1. Dilutions table

Dilution	Rounded gluten detection limit (ppm)	Real gluten detection limit (ppm)	Volume of extracted sample (µl)	Volume of Dilution Solution (µl)
1:25	3	3.5	40	960
1:75	10	10.5	13.3	986.7
1:150	20	21	6.6	993.4
1:250	30	35	4	996
1:750	100	105	1.3	998.7

If the expected amount of gluten in the sample is unknown, we recommend testing with the lowest dilution (i.e., dilution 1:25) allowing maximum sensitivity. In case of a positive result (appearance of RED line), the test can be repeated with a greater dilution for a semi-quantitative estimation of the gluten concentration in the sample (see section 14 "Analytical Features").

IMPORTANT NOTE!

- **In samples with polyphenols, tannins or antioxidants, in which the special polyphenol additive is added in the extraction step, the minimum dilution admitted is 1:75. Never perform at lower dilutions (i.e., dilution 1:25), since the presence of the special polyphenol additive affects the proper performance of the stick if the dilution is less than 1:75.**

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. Place 100 µL of the diluted sample in a well of the microtiter strip supplied in the kit.

4. Open the tube with GlutenTox Sticks, take out the number of sticks necessary and close the tube immediately.

5. Introduce the white end of the stick vertically into the well with the diluted sample.

6. **Wait 10 minutes** and read the result on the stick.

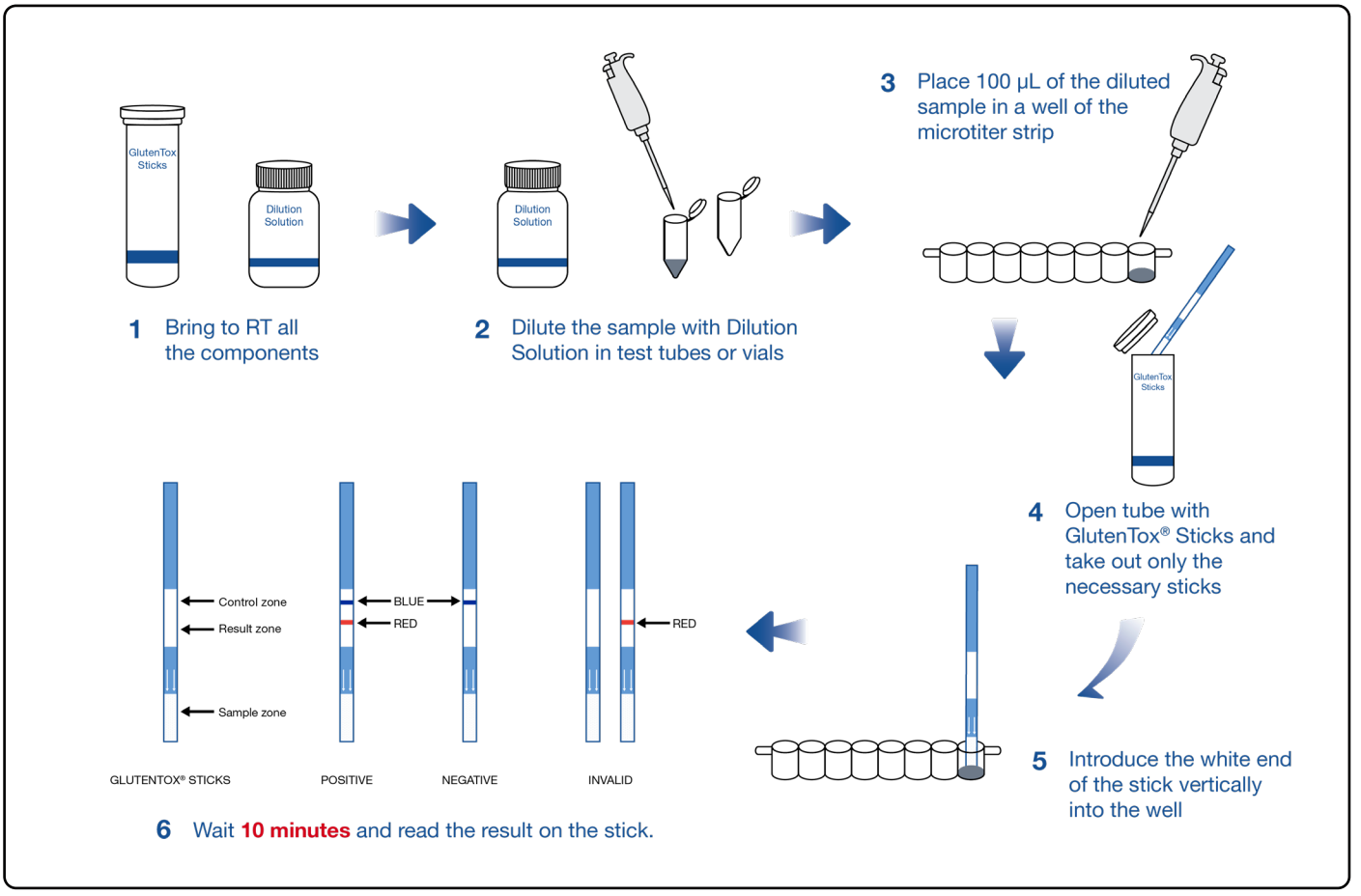


Figure 3. Procedure to follow for the sample analysis

10. Interpretation of results for food samples

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. See Table 2 to read the result depending on the selected Detection Limit.

IMPORTANT NOTE!

- **The intensity of the red line in the result zone will vary depending on the gluten concentration in the sample.**

INVALID: The control line (BLUE) does not appear, whether or not the result line (RED) appears. The most common causes of an invalid result are using an insuficient (< 100 µL) volume of sample in the microtiter well, 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 test. If the problem persists, please contact your supplier.

11. Surface Analysis

1. Bring the Dilution Solution and the tube with the GlutenTox Sticks to room temperature (15 - 25 ºC / 59 - 77 ºF).
2. Place 100 µL of the Dilution Solution in a well of the microtiter strip supplied in the kit.
3. Rub the cotton wool side of the white end of the stick against ive areas of 1cm^2 or along a line of 1 x 5 cm (see Figure 4).
4. After the stick has been rubbed on the surface to be analyzed, introduce its white end into the well.
5. **Wait 10 minutes** and read the result on the stick.

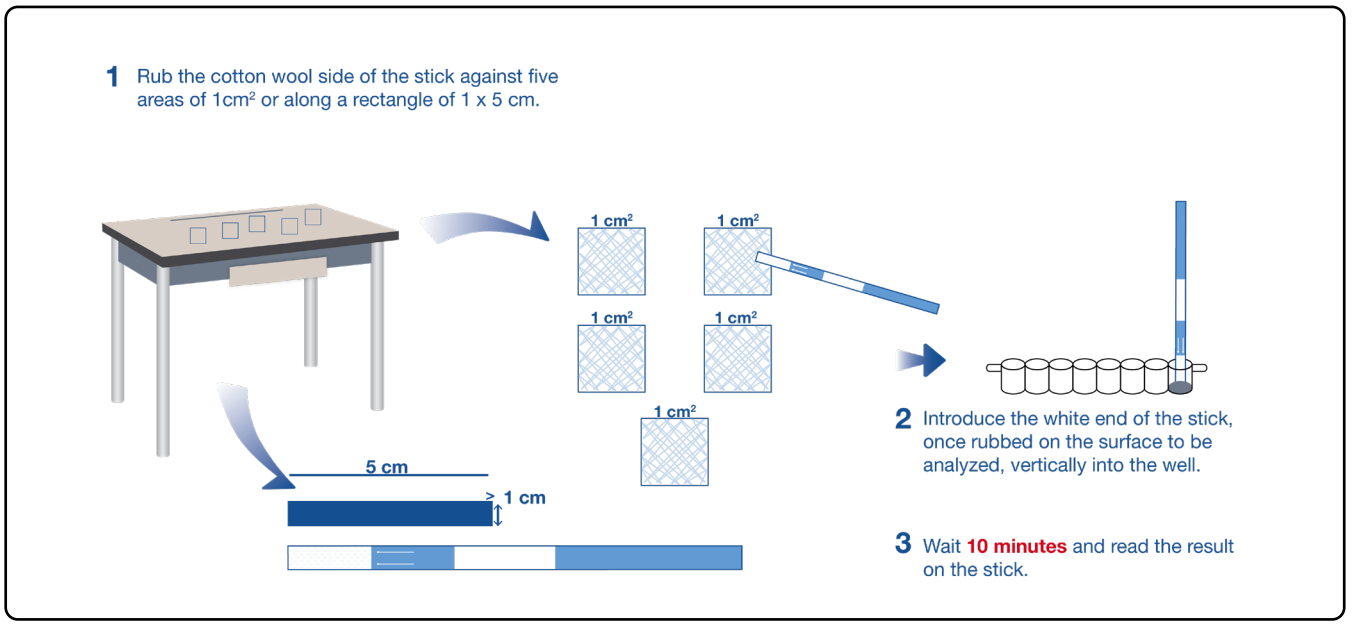
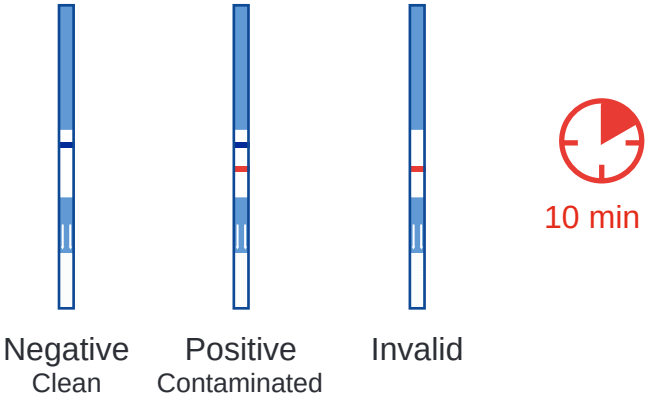


Figure 4. Procedure for surface analysis

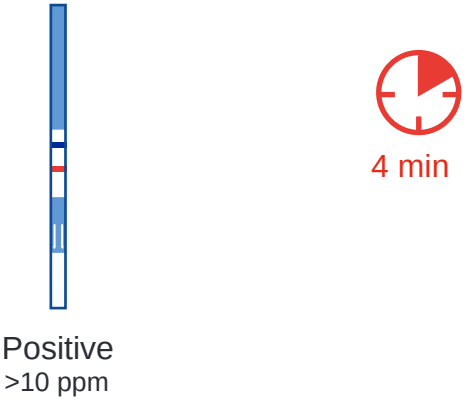
12. Interpretation of results for surface analysis

Final reading of the stick at 10 minutes



Initial reading of the stick at 4 minutes

Optionally, an initial reading of the stick after 4 minutes can be done to obtain a semiquantitative measurement. If a red line appears already after 4 minutes, the surface area tested had a gluten content equivalent to > 10 ppm.



13. Quality control

Internal procedural quality control is included in the test. The blue line in the control zone 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, and at any tested dilutions if the test procedure was conducted properly.

14. Analytical features

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

Sensitivity

The detection limit of GlutenTox Sticks Plus is 3 ppm of gluten. This value was obtained using different solutions of known concentrations of gliadin (one of the proteins in gluten) in the Dilution Solution.

For food, beverages and other consumer products, the detection limit of the test, in ppm of gluten, will depend on the dilution factor applied to the sample, once extracted.

Table 2 outlines the dilution to be carried out according to the level of gluten to detect.

Table 2. Detection limit in ppm of gluten in the sample before extraction.

		Detection Limit / ppm Gluten				
		Dilutions				
		1:25	1:75	1:150	1:250	1:750
Test Result	Positive	>3 ppm	>10 ppm	>20 ppm	>30 ppm	>100 ppm
	Negative	<3 ppm	<10 ppm	<20 ppm	<30 ppm	<100 ppm

Sensitivity in surface analysis

When testing surfaces, the result obtained with the test indicates the presence or absence of gluten on the analyzed surface; it cannot be directly extrapolated into any value of gluten in ppm.

By analyzing ive areas of 1 cm^2 or a rectangle of 1 x 5 cm a minimum of 40 ng/cm^2 is detected. If the test result is negative, it can be "estimated" that for an analyzed working surface of 1000 cm^2 (40 cm x 25 cm), working with a food mass of 1 Kg, the inal product will have less than 0.04 ppm (0.04 mg gluten/Kg of food). This quantity is about 500 times less than the quantity recommended by the European norms established at 20 ppm (20 mg gluten/Kg of food).

This means that the method has a large safety margin and that its use provides a guarantee to clients, celiac associations and food safety inspectors [ref. 4].

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, as well as with common working surface materials, a broad range of commercial products has been tested. After analyzing the samples with GlutenTox Sticks in all types of matrices tested (see Tables 3 and 4) the results were satisfactory and consistent with the gluten contents determined with the approved method of Codex Alimentarius. Results also showed the applicability of the test to common working surface materials (Table 5).

Table 3. 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 4. 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)

Table 5. Surfaces validated for GlutenTox Sticks Plus

Group	Tested samples
Surfaces	Stainless steel, rubber, painted wood

15. Intellectual Property

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

16. 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.

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

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