Analytical tools to detect gluten immunotoxic fractions in food based on monoclonal antibodies raised against the gliadin 33-mer peptide

Catherine Torgler[1], Miguel Angel Síglez[1], Felipe Vilchez[1], Angel Cebolla[1], Carolina Sousa[2]

[1] *Biomedal S. L., Sevilla, Spain*
[2] *Universidad de Sevilla, Departamento de Microbiología y Parasitología, Facultad de Farmacia, Sevilla, Spain*

Introduction

Immunotoxic gluten peptides that are recalcitrant to degradation of digestive enzymes appear to trigger coeliac disease (CD). A 33-mer peptide from α-2 gliadin has been identified as a principal contributor to gluten immunotoxicity [1]. A gluten-free diet is the unique current therapy for CD patients; therefore, the characterization and quantification of the toxic portion of gluten in foodstuffs is crucial to avoid coeliac damage. Our aim was to develop immunological assays as novel food analysis tools to measure cereal fractions that are immunotoxic to CD patients.

Two monoclonal antibodies (mAb), G12 and A1, were developed against a highly immunotoxic gliadin 33-mer peptide [2]. In comparison to other ELISAs, those based on these antibodies showed a wider specificity for prolamins that are toxic to CD patients, along with a higher degree of sensitivity, accuracy, and reproducibility, than did the other ELISAs. Analyses of the available prolamin sequences revealed the potential epitopes in the immunotoxic prolamins of rye, wheat and barley [3]. Although G12 affinity for the 33-mer was superior to A1, the sensitivity for gluten detection was higher for A1. This observation correlated to the higher number of A1 epitopes found in prolamins than G12 epitopes. Both antibodies have been evaluated as analytical tools to develop different analytical techniques, including ELISA (competitive and sandwich) and immunochromatographic sticks. To satisfy the increasing demand from CD patients or their relatives and other potential non-specialized food-related professionals, we also developed a user-friendly immunochromatographic "sticks" version, called GlutenTox Home, with G12 mAbs showing consistent results compared to laboratory techniques for a broad range of food products.

Material and methods

All methods were used according to the manufacturer's instruction manual (Biomedal S.L. - GlutenTox ELISA Competitive [ref. KT-4758], GlutenTox ELISA Sandwich [ref. KT-5196], GlutenTox Sticks [ref. KT-4711]; Ingenasa S.L. - Ingezim Gluten Assay I-30.GLU.K2; R-Biopharm - RidaQuick Gliadin R7003). For the user-friendly gluten detection method (GlutenTox Home), the protocol is a simplified version of the GlutenTox Sticks instructions.

Food sample were ground with a clean food grinder, knife or hammer. With a graduated plastic spoon (1 mL), two level spoons of ground food was added to a bottle containing 10 mL of extraction solution (60% EtOH). For liquid samples, only one spoon (1 mL) was sufficient. For gluten extraction, the tube containing the sample was shaken vigorously for a total of 1min, then settled for 5 min to allow the solid rest to sink to the bottom of the tube. Using a platic pipette, a few drops were taken out from the upper extracted solution. Eight drops to detect 20 ppm were added to a tube containing 2 mL of dilution solution (1x PBT) The tube was mixed softly and 5 to 6 drops were added to a well at the tip of the immunochromatographic stick encased in a plastic cassette. After 10 min, the result was read. When a blue control line and a pink line appeared, the result was positive and above the chosen determined threshold (20 ppm, Codex Alimentarius norms). When a single blue line appeared, the result was negative and below 20 ppm and suitable for consumption by CD patients. The results were then compared with the results from an ELISA Sandwich G12.

Results and discussion

Comparison of R5 and G12 analytical techniques

Several hundreds food analyses were performed to compare G12-based analytical tools (ELISA Competitive as well as immunochromatographic sticks) to R5 antibody-related techniques. Our results showed concordance in the detection of gluten free food (< 20 ppm) in > 85% of the analyzed food from external analytical services as well as spiked samples (data not shown). However, certain discrepancies were found, and some of them are shown in Table 1. The main discrepancies were found in beer, probably because the ELISA Sandwich R5 could underestimate immunotoxic gluten peptides due to the abundance of single epitopes, which cannot be detected by a sandwich ELISA, although this is feasible by means of the ELISA competitive methods [4].

We detected two food samples containing soybean with no gluten-containing cereals in the ingredients list, that gave noticeable signals with R5 (Table 1). We also demonstrated, via different spiked samples, that the immunochromatographic sticks could consistently estimate gliadin content with different matrices when the dilution of extracted samples was adjusted (see examples in Table 1).

TABLE 1. *Gluten content analysis of food samples by means of methods based on G12 and R5 antibodies*

Spiked samples and analytical standard food	Sandwich ELISA R5 [ppm]	GlutenTox ELISA Competitive G12 [ppm]	GlutenTox Sticks G12 [ppm]
Chocolate cake mix (172 ppm)	231	254	> 100
Spiked maize bread (200 ppm)	171	140	> 100
Baby food (15 ppm)	12	11	> 10, < 20
Baby food (50 ppm)	39	42	> 20, < 100
Beer (160 ppm)	76	144	> 100
Beer (40 ppm)	12	31	> 20, < 100
Soybean sauce	11.5	< 3	< 3
Soybean flour	27.4	< 3	< 3

We also tested the capacity of different immunochromatographic sticks with either G12 or A1 antibodies to detect gliadin as well as the main immunotoxic peptide, the gliadin 33-mer. The immunochromatographic sticks with R5, A1 and G12 showed equivalent sensitivity in detecting gliadin (Table 2). However, R5 showed poor sensitivity in detecting 33-mer epitopes, since the detection limit was from 62 to 60,000-fold less sensitive than the A1 and G12 sticks, respectively. Equivalent differences were found by using ELISA methods (data not shown). These observations may be of particular relevance for hydrolyzed gliadin since the R5 may underestimate the presence of immunotoxic peptides.

TABLE 2. *Sensitivity of immunochromatographic dip sticks to PWG gliadin and to synthetic wheat gliadin 33-mer peptide*

Immunochromatographic dip sticks (lateral flow)	Detection limits (mg/Kg gliadin)	Detection limits (ng/mL gliadin 33-mer)
G12 mAb (GlutenTox Sticks G12)	1.5	0.01
A1 mAb (GlutenTox Sticks A1)	1	9.7
R5 mAb (R-Biopharm)	2.5	625

TABLE 3. *Analysis of gluten content of different commercial comestible products and food ingredients by ELISA Sandwich R5, ELISA Sandwich G12/G12 and GlutenTox Home*

Matrices	Results ELISA Sandwich R5	Results ELISA Sandwich G12/G12	Results GlutenTox Home
Corn starch	21.5 ppm	16.3 ppm	< 20 ppm
Sugar+milk	220 ppm	191 ppm	> 20 ppm
BBQ spices	19.2 ppm	23.3 ppm	> 20 ppm
Paprika	< 3 ppm	< 3 ppm	< 20 ppm
Wheat starch	203 ppm	166 ppm	> 20 ppm
Strawberry flavour	14.6 ppm	12 ppm	< 20 ppm
Pudding	28.6 ppm	36 ppm	> 20 ppm
Ham flavour	< 3 ppm	< 3 ppm	< 20 ppm
Glucose syrops	244 ppm	256 ppm	> 20 ppm
Rice milk	68.2 ppm	80 ppm	> 20 ppm
Sausage 1	98.2 ppm	113 ppm	> 20 ppm
Sausage 2	155 ppm	> 100 ppm	> 20 ppm
Cured loin of pork	< 3 ppm	< 3 ppm	< 20 ppm
Hamburgers	89 ppm	96 ppm	> 20 ppm
Cake (gluten free)	< 3 ppm	< 3 ppm	< 20 ppm
Aperitive snacks	< 3 ppm	< 3 ppm	< 20 ppm
Baby food	105 ppm	96 ppm	> 20 ppm
Biscuit (gluten free)	< 3 ppm	< 3 ppm	< 20 ppm
Bread (gluten-free)	< 3 ppm	< 3 ppm	< 20 ppm
Chocolate	3.2 ppm	< 3 ppm	< 20 ppm
Ice cream	< 3 ppm	< 3 ppm	< 20 ppm
Cream	< 3 ppm	< 3ppm	< 20 ppm

The robustness and the sensitivity of the immunochromatographic sticks encouraged us to develop a user-friendly kit for gluten detection in food (GlutenTox Home) without laboratory equipment. Various food samples with different types of matrices were selected for this study to assess whether a shorter and user-friendly method was satisfactory for estimating gluten content. Most of the results of this study revealed that despite the simplicity of the method, the consistency was high, with no discrepancies in a variety of food matrices (Table 3).

Conclusions

Our study suggests that mAb G12 and A1-based immunotechniques are robust and sensitive methods to evaluate the potential immunotoxicity of gluten in all types of food matrices that were tested. The R5-based products showed that they were at least two orders of magnitude less sensitive to the gliadin 33-mer peptide than G12 or A1-based methods. The user-friendly lateral flow test (GlutenTox Home), using the anti-gliadin 33-mer antibody, demonstrated that it could be useful for reliable gluten content estimations in a variety of food samples despite the simplicity and rapidity of the protocol.

References

1. Shan L, Molberg Ø, Parrot I, *et al.* Structural basis for gluten intolerance in celiac sprue. *Science* 2002; **297**: 2275-2279.
2. Morón B, Cebolla A, Manyani H, *et al.* Sensitive detection of cereal fractions that are toxic to celiac disease patients by using monoclonal antibodies to a main immunogenic wheat peptide. *Am J Clin Nutr* 2008a; **87**: 405-414.
3. Morón B, Bethune M, Comino I, *et al.* Toward the assessment of food toxicity for celiac patients: characterization of monoclonal antibodies to a main immunogenic gluten peptide. *PloS One* 2008b; **3**: e2294.
4. Dostalek P, Hochel I, Mendez E, *et al.* Immunochemical determination of gluten in malts and beers. *Food Addit Contam* 2006; **23**: 1074-1078.