Allergies and intolerance – testing to protect

by Phil Goodwin, Diagnostics Innovations Ltd.

Allergies and intolerances of all kinds are increasing in prevalence in the developed world and in the UK are now said to affect 30% of adults and 40% of children.

The prevalence of food allergy in particular is increasing, the age at onset is decreasing and the condition can have severe effects, including breathing difficulties, anaphylaxis and in rare instances, death.

Accordingly, legislation has been put in place in many countries to ensure that allergy sufferers are informed of the intentional inclusion of key food allergens.

In the EC, Directives 2003/89 and 2005/26 have been fully implemented and more allergens added. In the USA, the Food Allergen Labelling and Consumer Protection Act (FALCPA) came into force early in 2006; both mandate the clear labelling of particular food allergens.

Listed ingredients

In the EC the intentional presence of the following foods must be listed in the ingredients: celery/celeriac; cereals (*wheat*, rye, barley, oats, spelt, kamut or their hybridised strains); *crustacea, eggs*; *fish*; lupin, molluscs (gastropods, bivalves, cephalopods), mustard; *peanuts*; sesame; *soya and tree nuts (almond, hazelnut, walnut, cashew, pecan, Brazil, pistachio, macadamia/ Queensland)*. In the USA the list is similar but includes only those foods in italics above.

Food producers are being advised by food protection agencies such as the UK's Food Standards Agency (FSA) and the US FDA to restrict the use of so-called 'may contain' labelling to products whose manufacturing processes have been assessed using HACCP and for which the presence of undeclared allergens is both significant and unavoidable.

Accurate labelling

Gluten intolerance and food allergies are not curable and the only way of improving the condition is avoidance of gluten or the allergen(s) concerned. Because there are no cures for these conditions and avoidance is mandated, legislation has been enacted in many countries (EU; USA; Japan; Australia etc) to ensure that allergy and intolerance sufferers are informed of the intentional inclusion of allergens via accurate food labelling. In addition, specialist food producers must adopt either regulatory or retailer guidance documents to restrict the use of 'may contain' labels.

Finally, for various reasons perhaps related to undiagnosed food intolerances, a significant proportion of consumers in the developed world are choosing to avoid some foods, particularly those including wheat/ cereal and dairy based ingredients.

This phenomenon has led to a rise in the availability of premium-priced 'free from' foods. In the UK, one retailer alone has over 150 gluten free products in their 'free from' range.

Codex Standard 118 and recent EC Regulations define 'gluten free' foods for PARticular NUTritional use (PARNUTS) as containing less than 20 parts per million (ppm or mg/kg). PARNUTS foods above 20ppm but below 100ppm must be labelled 'very low gluten'.

The presence of allergens through ingredient or labelling mistakes or cross contamination has become a significant cause of expensive food recalls and withdrawals over the past few years

For these reasons, environmental monitoring and laboratory testing to detect 'key allergens' and other food contaminants is increasingly included in allergen and gluten control plans and retailer codes of practice within Hazard Analysis Critical Control Point programmes in the food industry. Swabbing techniques can help validate and verify clean-down routines of food raw materials suppliers, food manufacturing facilities, catering establishments and analytical laboratories. In all of these locations the possibility of cross-contamination of one commodity, product or laboratory sample with another is very real and cleaning protocols must be effective.

The FlowThrough (FT) device has been developed for the rapid detection of glutens and food allergens in the manufacturing environment.

It has the advantage of being extremely simple to prepare, making both the test development phase and subsequent manufacturing easier and quicker. In addition a relatively large amount (1-2mls), of swabbing solution can be tested. This is a much higher volume than

The Imutest FlowThrough unit and AllerSnap.

can be tested using, for example, lateral flow test strips, making FT tests more sensitive for the detection of allergens/gluten at very low concentrations in swab solutions.

Swabbing system

FT cards are combined with a swabbing system and a tube of vivid pink, specific (allergen/gluten) detection reagent. Imutest FT kits have been specially designed to make the process of swabbing and testing simple, fast, effective and completely safe. Tests for the presence of gluten and other allergens such as milk proteins are available. Pre-wetted swabs are used to swab a representative area of surface and hard-to-reach locations such as within complex equipment and air sampling devices.

When the swab is mixed with the Imutest swabbing solution any protein present is dissolved and stabilised and can be used to carry out a FlowThrough test immediately to check for the presence of gluten or other allergens. The process takes a few minutes and the test results are shown as a visible pink colouration (see Fig. 1).

The test spot on the left of the test area indicates the presence of gluten at a level of about 0.5mg/kg or above in the swabbing solution; the darker the test spot the more allergen/gluten is present in the

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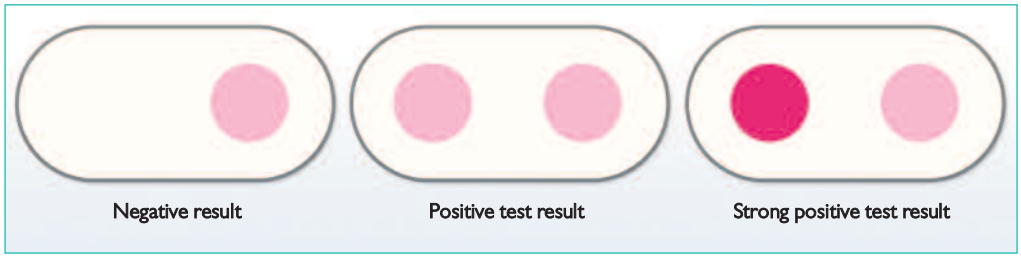
Fig. 1. Results of ImuTest FlowThrough test.



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swabbing solution. A pink control spot of medium intensity should always appear on the right hand side of the test area; this indicates that the sample is suitable, the test has been performed correctly and all reagents are active.

ATP bioluminescence has long provided an established direct and objective test of cleaning effectiveness. Recent improvements in detection capabilities and sensitivity mean that it is now capable of detecting food residues below the limit of detection of specific allergen tests.

The new EnSURE instrument and SuperSnap reagent swab from Hygiena provide additional sensitivity with low background noise and low variation for precise accurate and consistent results.

This means that the system is 10x more sensitive than Hygiena SystemSURE Plus with UltraSnap swabs and is claimed to be 100x more sensitive than other ATP systems. The results are quantitative and give a linear response to increasing amounts of food residue.

Food allergens are mainly proteins and can be detected by a simple colourimetric test such as is used in AllerSnap swabs. However, this non-specific protein test cannot differentiate non-allergen protein from true allergens.

This protein test can detect allergenic foodstuffs but for maximum sensitivity (1-3µg protein) the test needs to be run at elevated time and temperature combinations such as 37°C for 30 minutes. The results are semi-quantitative and the scope and sensitivity of the protein test is limited to 10-100ppm for certain allergenic foods.

Effective monitoring tool

Factory trials have shown that combined high sensitivity ATP and protein tests provide an effective monitoring tool as part of an allergen management program. Before cleaning all test results were positive and after cleaning most test results were negative.

The ATP test detected residues below that of protein tests and specific allergens were not detected, thus confirming the highest level of cleaning had been achieved and that allergens were absent.

A combination of three high sensitivity detection methods (ATP, protein and specific allergen tests) provide more comprehensive, sensitive and rapid results that deliver a timely, cost effective solution.

The regular use of these tests enable high standards of cleaning to be maintained that can be supplemented with specific allergen tests only as required during cleaning verification. ■

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Gluten-free – how can you prove it?

by Elisabeth Hammer, Product Manager, Romer Labs Division Holding GmbH.

Over the past decade the demand for gluten-free food has soared and, therefore, more and more of these products can be found in the stores. The spectrum of consumers with difficulties in digesting gluten has grown to around 10%. These individuals show varying degrees of sensitivity towards gluten, but their situation generally improves when following a gluten-free diet. Furthermore, there is a growing perception amongst increasing numbers of consumers that a gluten-free diet is better for you. But what is gluten? Why can it be toxic? And how can gluten be detected in food?

What is gluten?

The name 'gluten' is derived from the Latin word for glue and it refers to the composite of the proteins called prolamins and glutelins found in wheat, barley rye, oats and their crossbred varieties. Prolamins are defined as the fraction that can be extracted using 40-70% of ethanol and this fraction is called gliadin, hordein, secalin or avenin respectively, depending on the grain variety. In general, it can be estimated that prolamins and glutelins occur in the same ratio in gluten.

Worldwide, gluten is an important source of nutritional protein, both in foods prepared directly from sources containing it, and as an additive to foods otherwise low in protein. Gluten contributes texture and form to food products, due to its physicochemical properties.

Together with water and when kneaded, gluten forms a viscoelastic dough with a special protein network which is responsible for the shape of bakery products.

Coeliac disease

Following the transition from the hunter-gatherer lifestyle to the beginning of agriculture 10,000 years ago, cereals have been a main pillar of the human diet, which raises the question as to why gluten is causing increasing levels of health problems nowadays.

Approximately 1% of the world's population is affected by coeliac disease – an immune-mediated enteropathy caused by the ingestion of gluten. Interestingly, it is more frequent in women than in men.

Symptoms are diverse and not confined to the gastrointestinal tract. Examples are not only diarrhoea, abdominal pain, flatulence, indigestion or weight loss, but also irritability, depression and anxiety.

However, all these symptoms are considered to be unreliable as an indicator for the disease. Coeliac disease can be diagnosed by a screening for certain antibodies in the serum. Another recommended diagnosis is a biopsy of the mucosa and the small intestine to affirm damage, as the disease leads to the destruction of microscopic, finger-like projections in the small intestine that are called villi.

As intestinal villi are responsible for the absorption of nutrients, malnourishment is a problem that – on a long term basis – may lead to development delays, osteoporosis or nutrient deficiencies, amongst other problems.

Coeliac disease is a genetically predisposed auto-immune disorder, in which the immune system responds inappropriately to dietary gluten. The enzyme called tissue transglutaminase modifies gluten peptides by deamidation in a way that T-cell epitopes are formed.

This stimulates the immune system and cross-reacts with the small intestine tissue, causing an inflammatory reaction that leads to the truncation of the villi. The majority of proteins responsible for such an immune reaction are prolamins.

The strongest response is directed towards an α2-gliadin fragment that is 33 amino acids long and a principal contributor to gluten immunotoxicity. This so-called 33-mer is highly resistant to breakdown by digestive enzymes and is, therefore, a suitable molecule for use as an analytical marker. Homologues have been found in food grains that are toxic for coeliac patients, but are absent in non-toxic grains.

The only effective treatment for coeliac disease up to now has been a lifelong gluten-free diet. This is challenging to maintain as gluten is very common in food.

'Hidden' gluten that is used as a protein filler can be found in unexpected products such as pharmaceuticals, sausages, sauces and desserts. In addition, gluten-free products may contain gluten due to cross contamination during milling, storage and production. Gluten-free food is usually based on rice, maize or buckwheat, as well as purified starch that still contains low levels of gluten. It is very difficult to set limits because sensitivity varies from individual to individual. According to scientific studies, the ingestion of gluten should be maintained at below 50mg per day.

Legislation and standards

The Codex Alimentarius Committee started to discuss recommendations for limits in the late 1970s, cumulating in the 2008 Codex Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten (Codex Stan 118 – 1979).

This recommendation was taken into European legislation through Commission Regulation (EC) No 41/2009 of 20th January 2009, concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten.

In contrast to other food allergens, thresholds have been defined. Food labelled as gluten-free must not exceed 20ppm, whereas food containing low levels of gluten has to be lower than 100ppm. A proposed rule for gluten-free labelling of foods is in preparation in the US.

Gluten analysis

As there are regulations in place, there is a need for appropriate detection methods for gluten in food. Several technologies such as specific antibody based tests, for example enzyme linked immunosorbent assays (ELISA) or lateral flow assays, polymerase chain reaction (PCR) methods and newer concepts like mass spectrometry are available – all with varying degrees of commercialisation – giving both qualitative and quantitative results.

An analytical test system should preferably be able to detect epitopes that are involved in coeliac disease. The fact that gluten is a complex mixture of proteins and that it occurs in a wide range of unprocessed as well as processed matrices creates a huge challenge in terms of correct quantification and makes it difficult to find a suitable reference material.

In 1985, the Prolamin Working

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Group (PWG) was founded in Europe. One of its tasks was to establish a recognised gluten – respectively gliadin – standard. By extracting gliadins from a selection of the most common wheat varieties, they managed to get a reference material.

The IRMM (Institute for Reference Material and Measurements) initially accepted the PWG gliadin as a certified reference material, but later withdrew its acceptance. However, it is still the only reference that has some acceptance and has been widely used for calibration of test systems.

Enzyme linked immunosorbent assays (ELISA) are the recommended method for the detection of gluten in food and a large number of test kits are available commercially. In immunological methods, antibodies are applied that have been raised against different prolamin fractions or specific sequences that are harmful.

Different test kits do not necessarily give similar results for several reasons. These include different specificities of the polyclonal and monoclonal antibodies used, different extraction methods, and different materials for the calibration of the assays.

Numerous monoclonal and polyclonal antibodies have been developed for gluten testing, but only some of them are accepted on a broader basis. In the late 1980s, the Skerritt antibody was developed.

This monoclonal antibody was raised against wheat gliadin from an Australian wheat variety and recognizes HMW (high molecular weight) glutenin subunits and the heat stable subfraction called ω-gliadins, which makes the Skerritt antibody suitable for gluten analysis in processed foods. Even so, as the quantitation is based on the amount of ω-gliadins, which differ among cereals species, this can cause considerable differences in results. Moreover, the Skerritt antibody only has a weak response to hordein.

Another monoclonal antibody for the detection of gluten is the R5 antibody, which was developed by Professor Mendez in Spain. The R5 antibody was raised against rye secalin, but showed strong cross reactivity to wheat gliadin. However, it also detects proteins from soy and lupin that are not harmful prolamins.

The change in direction to detecting immunotoxic peptides that play a role in the pathogenesis of coeliac disease from the detection of prolamins, led to the development of a next generation of antibodies.

The G12 antibody employed in the AgraQuant Gluten G12 ELISA and AgraStrip Gluten G12 Lateral Flow Test belongs to this next generation.

The G12 antibody specifically recognises the 33-mer of the gliadin protein present in gluten. This toxic fragment was identified by the University of Stanford and published in 2002 in a paper in Science.

The G12 antibody was raised against this 33-mer peptide using knowledge gained from this publication, and recognises the hexapeptide sequence QPQLPY and similar peptides found in barley, rye and oats.

In contrast, the R5 antibody was raised against a secalin extract and later the epitope it reacts with was identified as the QQPFP pentapeptide.

The distinction between the two antibodies relates to the fact that the G12 antibody specifically targets the toxic fragment that triggers the auto-immune reaction in coeliac patients, rather than a peptide sequence unrelated to clinical outcomes. It was confirmed during validation studies that G12 does not give any false positive signals with soy and is, therefore, suitable for measuring gluten in products containing soy. There is also no cross reactivity to maize or rice.

There is an on-going debate whether oats are safe. Several publications conclude that certain oat varieties may cause an auto-immune response in coeliac patients.

During the validation of AgraQuant Gluten G12 ELISA test and AgraStrip Gluten G12 Lateral Flow Test, positive and negative responses to oat varieties were observed. The positive results appear to be a specific reaction of the antibody with the toxic fragment, rather than a non-specific response. Therefore, the G12 antibody may shed new light on this debate by recognising oat varieties that trigger a response in coeliac patients. The Spanish Coeliac Association has recently awarded the 6th National Prize for Research on coeliac disease to a scientific team that used the G12 antibody to identify oat varieties containing low levels of gluten, in this regard.

Conclusion

Coeliac patients depend on the correct labelling of gluten-free food in order to maintain their health. Ensuring the safety of food is a demanding task and, therefore, new developments in the field of detection methods for gluten are ongoing. The results obtained from new immunochemical test systems based on the G12 antibody should be considered to be closer to the ideal of a food safety test as they establish the important link between coeliac disease and detection of the immunotoxic peptides. ■

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