Food Allergen Testing Facts vs. Fiction

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As food allergens represent an ever-present risk for individuals with allergies, it is crucial for food producers to conduct routine tests for potential allergen contamination in their products. This sounds simpler than it often is. Food products can range widely from straight raw materials, such as cereals, to highly processed ready-to-eat products. Therefore, the composition of food products varies according to the amount of protein, fat, salt and other compounds present. Test methods are expected to analyse all food sample types for allergens with equally reliable results. This however is often far from achievable in reality. In this series, we will shed some light on common misconceptions in allergen testing.

Does a test kit from the shelf work with any food matrix?

Taking a test kit from the shelf and starting to test right away does sound tempting and would be the quickest way to get to results. However, are these results reliable? In reality, food products are highly diverse and certain test methods may work better for certain food samples. The extent of processing adds further complexity to this equation. The best testing method must be determined in a food matrix validation study. Here we explain some prior considerations and how to perform these studies on-site.

● Handling new food types

When we receive or encounter a new food type that has not been tested before, we undertake a spike recovery validation to ensure it works as expected with our test kits. We will spike three different levels of allergen in to the sample – low, medium and high – to cover the detection range of the assay. The low allergen spike will either be close to the Lower Limit of Quantitation (LLOQ) of a quantitative test kit such as ELISA (in this case the lowest value calibrator above 0ppm), or close to the Limit of Detection (LOD) of a qualitative lateral flow device (LFD). The medium spike will be in the middle of the ELISA calibration curve, and the high spike will be at or near the Upper Limit of Quantitation (ULOQ) (the highest ppm value calibrator).

● What can influence my spike?

If we spike for example almond into chocolate, we would expect to see a recovery of 40% or less which could be boosted to 60% or above when using extraction additives. From experience, chocolate is one of the most challenging food matrices to test – it is full of tannins and other polyphenols which can bind to any allergenic protein that may be present and form insoluble complexes which are difficult to extract.

● Improving the extraction of your spike

Such difficulties can be overcome by adding extra protein to the extraction buffer. The excess protein binds to the polyphenols and makes the allergens available for extraction. One protein of choice is fish gelatin. Other proteins such as milk powder can be used to improve the extraction efficiency from high polyphenol containing foods. If you are using milk powder, be careful not to contaminate your laboratory space, especially if you are carrying out milk allergen testing.

● The result...

During implementation of an allergen control plan, it is highly recommended that the selected allergen test method is fully validated on the food producer's specific food matrices. By following the described validation rules, a reliable and accurate result is guaranteed for almost every food product.

In our next issue of "Food Allergen Testing – Facts vs. Fiction" we will give insight into the common misconception that "May contain..." statements can solve all our problems. Stay tuned.

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Food allergens not only represent a serious health risk for individuals with allergies, they are also a topic of great discussion when it comes to their analysis and proper labelling. Food allergen labelling intends to make the lives of people with allergies easier and safer, but it often causes confusion in the food industry as most laws fail to state the levels above which an allergen must be labelled. To avoid the problem of undeclared allergens in a food preparation, producers often use 'may contain…" statements. However, there are more perspectives to consider. This second part of our series sheds some light on allergen testing and labelling misconceptions.

'May contain…' statements can solve all our problems

Most labelling regulations only apply to allergenic ingredients that are deliberately added to the product. However, increasing concern from consumers about unintended allergen contamination has caused uncertainty among food producers as to whether they need to label potential allergens if their presence cannot be excluded. Consequently, food manufacturers often display precautionary (also called advisory) allergen statements, such as 'may contain…' on packaging. It is of upmost importance to remember that such statements are only voluntary. They make the consumer aware of the possibility that the product may contain a certain allergen, and they prevent the producer having to make potential allergen-related product recalls.

Are we on the safe side if we use 'may contain'?

In some cases, precautionary allergen labelling makes sense. For example, if the producer regularly tests for a certain allergen and although most samples show a negative test result, low levels of the allergen can be detected in a few batches, a precautionary statement would be displayed. Depending on the particular country's national law, the statement 'may contain' is unlikely to meet legal requirements, if the relevant substance is present as an ingredient of a compound ingredient, a component of a food additive, or a processing aid. In addition, advisory statements never substitute for good manufacturing practice, such as allergen identification and control, cleaning of shared equipment, and segregation during processing. If there is evidence of allergen presence, or even only a possibility for the presence of the allergen to be in the final product, the 'may contain…' statement is not appropriate.

Considering the consumer's perspective

The overuse of precautionary labels can lead to risk-taking behaviour in consumers. With a significantly reduced variety of food products that are suitable for allergic individuals on offer, some may risk consuming products which display advisory statements. However, studies showed that up to 9% of products with advisory labels in fact contained detectable levels of allergens. Therefore, there is a real risk of allergen contamination in products that only display a precautionary statement. As there are varying reasons why manufacturers include such statements, consumers will find it increasingly difficult to interpret them.

The consequence…

Consumers with allergies should avoid products with precautionary labels, as the risk is not assessable. In return, food producers should avoid using a 'may contain…' statement without reasonable suspicion. Adding such a statement is not always a safe and simple way for protecting the company legally. Careful usage of these statements will ensure a broader selection of products is available to affected people.

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Food Allergen Testing Facts vs. Fiction

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Undeclared food allergens represent a serious health issue for consumers with allergies. To comply with legal requirements, food manufacturers need to implement allergen management plans, in which critical control points and the required testing method should be defined. As well as ELISA test kits, commercially available PCR (polymerase chain reaction) kits utilise a more modern technology. But can we assume that they are the better choice?

This third part in our series on food allergen testing sheds light on this specific allergen testing method and the related misconceptions.

PCR is more reliable than immunological tests

PCR is a complex method in which DNA is extracted from a sample. Specially designed primers anneal to a target fragment of the DNA that is specific for a certain species. Then the polymerase chain reaction takes place, whereby the target DNA is multiplied by a factor of 100 million to 1 billion. Upon addition of a dye, the target DNA fragment can then easily be detected.

A method offering many advantages...

This amplification process makes the method extremely sensitive and therefore attractive for use in cases of low level allergen contaminations. Furthermore, DNA is a remarkably stable molecule that remains unaffected by most of the common food processing methods. PCR is a highly specific method, meaning that it is able to overcome any cross-reactivity issue where other methods might fail.

Celery is an example of the need for specificity. To date, no antibodies have been developed that can reliably detect celery without also giving a signal to related species, such as fennel, carrot or parsley. Hence, celery detection with an immunological test is currently not possible.

... but also disadvantages

Performing a PCR is difficult, compared to an ELISA. Sample preparation and result interpretation are complex and require specially trained personnel.

The analytical target also possesses one fundamental drawback: the DNA molecule itself is not responsible for the allergic reaction. So the presence of the DNA is only an indicator of the allergenic potential of the sample. In addition, there are commodities that contain a lot of proteins, but very little to almost no DNA. And in some instances, DNA and proteins are separated as a consequence of food processing. Furthermore, it is not possible to distinguish between allergens (milk and egg) and tissue (beef or chicken meat) as a PCR test will only detect the presence of cow or chicken DNA.

So, are immunological tests the better choice after all?

It is hard to conclude which method is 'better'. ELISA or lateral flow methods are well-established, while PCR kits for food allergen detection are relatively new.

Immunological rapid tests are still the gold standard and should be preferred in most cases as they directly detect food allergens. However, in some cases, PCR is a great alternative.

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Food allergen testing sounds quite simple at first, but there are many difficulties associated with it, which can transform accurate analysis into a rather complex topic. In recent years, researchers focused on mass spectrometry for allergen detection. The scientific literature is full of promising reports on the performance and it is commonly believed that mass spectrometry can revolutionise food allergen testing.

This fourth part of our series sheds some light on the misconception that mass spectrometry will soon replace rapid tests as the future gold-standard of allergen testing.

Will mass spectrometry soon replace allergen rapid tests?

Mass spectrometry (MS) is a high-end technology that is already used in several fields for routine analysis. Today, researchers all over the world are trying to adapt this method for the detection of food allergens. The goal is clear: MS should not only improve the accuracy of analytical results for single allergens, but it should also be capable of multi-allergen analyses from one single sample.

How allergen MS measurements work

Any MS analysis follows the same basic principle: a molecule – in our case the allergenic protein – is broken down into small pieces (peptides) and their mass is subsequently determined. This fragmentation process always results in the same protein fragments. The obtained peptide pattern allows the identification of allergens at high levels of accuracy and sensitivity.

Could this be the future allergen reference method?

MS is a very promising method for allergen analysis. However, it is still in its infancy and is currently restricted to research applications. As a result, we do not know how MS will perform in routine analysis. Multi-methods are feasible and would greatly facilitate the work of service laboratories. However, considering multi-methods as a reference method is not realistic because an accurate definition of marker peptides for each allergen would be required and food processing affects the fragmentation process of proteins, resulting in varying peptide patterns.

MS does not yet deliver the highest level of accuracy

The fragmentation process may not only be affected by food processing. The tryptic digestion also causes varying results because the reproducibility of this approach is very limited. Another bottleneck of MS technology is the extraction efficiency. A method can only detect what has previously been extracted from the sample. Recent studies have shown that recovery rates of ELISA and MS measurements are at least comparable and in some cases better for ELISA kits.

Will MS replace rapid tests?

Without a doubt, MS technology will continue to develop and improve in the future. It might become a valuable tool for multi-allergen analyses in service laboratories, in the same way that it is commonly used for mycotoxins already. However, accurate results from MS requires highly trained personnel and extremely expensive equipment. There will always be a demand for fast and inexpensive in-house testing, making it rather unlikely that rapid tests such as ELISAs will be replaced.

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Almost everyone working in the field of food allergen testing will have seen a single food sample produce remarkably different test results when it is analysed with test kits from various manufacturers. And the analyst would of course like to know which of the results is correct. Unfortunately, as allergen testing lacks reference methods and materials, it is difficult to claim that one kit is more accurate than another.

This fifth part of our series explains why results from different test kits are not always comparable.

The myth: all test kits on the market detect the same

It is highly improbable, if not impossible, that the performance of all commercially available test kits is the same. This is due to the lack of rules about which analyte an allergen test kit must detect. For each food allergen, there is a variety of different allergenic proteins with varying known conformations, but there is no recognised standard defining which of them must be detected. Therefore, we cannot assume that all test kits detect the same and consequently give comparable results.

Reasons for variability

Allergen test kits only have one thing in common: the overall analytical target (peanut). The rest of the kit could not pose more diversity. Commercially available test kits use different extraction buffers and procedures, which generate individual patterns of extracted allergenic proteins. Another crucial factor is the antibody used for the detection of the extracted proteins. Each antibody targets one specific epitope of the allergen, which is subject to modifications depending on the level of food processing. Different calibrators, individual cross-reactivities or matrix interferences, special reagents and the whole assay design are just a few more sources of inconsistent test kit results.

Coping with test kit diversity

Although not obvious at first sight, it may also be a benefit that most test kits on the market do not detect the same proteins. An antibody raised against the native allergen will give accurate results when no extensive processing steps have been applied to the food preparation, but it will have difficulties if heat-treated products are to be analysed or vice-versa. When choosing your test kit, a close discussion with the kit manufacturer is highly recommended as they can provide information about the test kit's performance specifications.

Which is the best test kit for me?

In a food factory, analysts should carefully review and summarise all the processing steps that are applied to a food product to assess which kit is most suitable for their individual application.

Service laboratories dealing with a wide variety of mostly unknown samples face challenges of kit diversity and comparability of test results. Often, a compromise between the performance with native and processed samples is most desirable. However, it is important to prove that results are consistent within each type of food.

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This sixth and final issue of our series sheds some light on the common misconception that allergen reference materials are available.

Currently available allergen 'reference materials' improve testing reliability

Although there are some materials on the market that claim to be an 'allergen reference material', none of them are really accepted as such. People often believe that this has mainly political reasons, but in fact, producing this kind of material in large quantities is a very difficult task and many aspects need to be considered.

The controversy about allergen references

In other fields of food safety, producing reference materials requires high-end technology, but the procedures for doing so are well-established. If we take mycotoxins for example, we have one defined molecule, allowing accurate calculations of the final concentration. Unfortunately, with food allergens there is not just one specific molecule. An allergenic commodity consists of a mixture of different proteins. To date, several allergenic proteins have been identified, but the majority of them remain unknown. Furthermore, the protein pattern varies between different cultivars of the same species. And to make the situation even worse, proteins can change their conformations, which may lead to a change in their allergenic potential.

Allergen 'reference materials' on the market

Of course, it is possible and also common practice to produce mixtures of allergenic food commodities in certain matrices and label them as reference materials. But even if all calculations and handling steps are completed with the greatest of care, we never know if the material is really representative. Is the matrix a relevant one? How much difference is there to cultivars that are used in other parts of the world? Does the material mimic the same situation that allergic individuals face? Have altered protein conformations been considered? What about homogeneity, stability?

So, should I forget about these materials?

It must be remembered that these materials may not be real reference materials. They are not able to tell if a test result is correct or not. Nevertheless, if used with care and considering all known limitations, they can be very beneficial for regular checking test performance. The result should never be correlated with the result of an unknown sample, but it is a useful tool to confirm the consistency of test results. For example, they can be used to reveal handling errors or deficits of the test kit. Materials that are produced in-house using the matrix in question result in even more significant evaluations of test performance, and represent the best possible alternative we currently have until standardisation bodies define specifications for actual reference materials.

That was the final part of our series highlighting the facts around allergen testing. We hope you found these articles useful.
For more information about allergen testing in your facility, please contact us at: allergens@romerlabs.com
An archive of these articles can be found at https://www.romerlabs.com/en/knowledge-center/knowledge-library/

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