

Food Allergen Detection Methods: A Coordinated Approach

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The levels (1–2%) and increasing severity of allergic responses to food in the adult population are well documented, as is the phenomenon of even higher (3–8%) and apparently increasing incidence in children, albeit that susceptibility decreases with age. Problematic foods include peanut, milk, eggs, tree nuts, and sesame, but the list is growing as awareness continues to rise. The amounts of such foods that can cause allergic reactions is difficult to gauge; however, the general consensus is that ingestion of low parts per million is sufficient to cause severe reactions in badly affected individuals. Symptoms can rapidly—within minutes—progress from minor discomfort to severe, even life-threatening anaphylactic shock in those worst affected. Given the combination of high incidence of atopy, potential severity of response, and apparently widespread instances of “hidden” allergens in the food supply, it is not surprising that this issue is increasingly subject to legislative and regulatory scrutiny. In order to assist in the control of allergen levels in foods to acceptable levels, analysts require a combination of test methods, each designed to produce accurate, timely, and cost-effective analytical information. Such information contributes significantly to Hazard Analysis Critical Control Point programs to determine food manufacturers’ risk and improves the accuracy of monitoring and surveillance by food industry, commercial, and enforcement laboratories. Analysis thereby facilitates improvements in compliance with labeling laws with concomitant reductions in risks to atopic consumers. This article describes a combination of analytical approaches to fulfill the various needs of these 3 analytical communities.

asthma, hay fever, urticaria, and anaphylaxis, produced upon exposure to a particular allergen without inoculation. The incidence of food-related atopy (1–6) is generally agreed to be 1–2% in adults and 5–8% in children, meaning that the condition directly affects many millions of people worldwide. There is also evidence that the condition may be increasing in prevalence (7), and that individuals’ perception that they are allergic to particular foods is as high as 20–30% (8); such perceptions often lead to avoidance of foods thought to be problematic. Given the incurable nature of the true atopic condition and its potentially life-threatening consequences (9), the management of food allergy concentrates on educating the patient to avoid ingestion of the food(s) concerned and to initiate immediate therapy should ingestion occur (10).

In 1995, a Technical Consultation by the Food and Agriculture Organization (11) drafted a list of the main food allergens, which was subsequently proposed by the Codex Alimentarius Commission for food labeling purposes (12). This list is broadly reflected in recent U.S. legislation (13) and widened in a European directive (14) and Canadian recommendations (15). The major causative foods in each case are listed in Table 1, but other candidate allergens, e.g., kiwi fruit, are being identified and still more will surely be added in the future. For all of these high-risk foods, the consideration of threshold levels, above which the risk to food allergy sufferers is considered to be too high, is an area of increasingly intense debate (16, 17). It is generally considered that ingestion of milligram quantities of an allergenic food can be problematic, although smaller quantities may have resulted in severe, even fatal reactions (18–21). In practice, this means that levels of allergens in food for consumption by allergic individuals must, at most, be in the low parts per million (ppm; mg/kg) range.

Given the long and growing list of problematic foods, current thinking on threshold levels, and the increasing complexity of food ingredients and manufacturing, it is perhaps not surprising that the occurrence of significant quantities of unlabeled or “hidden” allergens in the food supply can be high (22–24). Both legislators and the food industry are therefore increasingly concerning themselves with the need to improve food allergen labeling compliance. As far as enforcement of legislation is concerned, inspections of manufacturing premises, including analysis of samples of food being produced, as well as the surveillance of products on sale in retail outlets, are important. For the food industry, the use of Hazard Analysis Critical Control Point (HACCP)-type assessments of manufacturing

Human atopy is an incurable, possibly hereditary, allergic condition characterized by symptoms such as

Guest edited as a special report on "Methodological Aspects of Food Allergens" by Kristina Williams.

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Table 1. Identified food allergens—United States, European Community, and Canada

Food allergen	USA	EC ^a	Canada ^b
Celeriac		✓	
Crustacea	✓	✓	✓
Shellfish			✓
Egg	✓	✓	✓
Fish	✓	✓	✓
Wheat	✓	✓	✓
Milk	✓	✓	✓
Mustard		✓	
Peanuts	✓	✓	✓
Sesame		✓	✓
Soya	✓	✓	✓
Tree nuts	✓	✓	✓

^a 2003/89 EC allergen labeling directive.^b Recommended; tree nuts include pine nuts.

processes (25–27), combined with supportive analysis of allergen content, are highly effective in reducing or even effectively eliminating risks for consumers.

In both cases, access to robust, validated, flexible, and cost-effective analytical techniques is vital to aid effective decision-making. Historically, a wide range of methods has been used for establishing the presence of allergens in foods (28, 29). More recently, 2 particular techniques, immunoassays for major proteins contained in allergenic foods and polymerase chain reaction (PCR)-based detection of DNA from the allergen species of interest, have increased in importance. The former method usually detects a specific protein or protein mixture from the allergenic food concerned; however, the indirect nature of the assay response (development of color or visible line intensity) cannot be absolutely guaranteed to be specific. PCR-based testing, although highly specific and direct (if required, PCR products can be sequenced for absolute confirmation), is merely an indicator of the presence of a particular species, rather than the potential allergenicity of the food itself.

DNA is generally, but not always, more resistant to processing than protein; therefore, the analytical technique of choice may vary. In the real-time manufacturing environment, time is of the essence, yet analytical ability may be compromised by the conditions. Testing of raw materials during incoming inspection, surface swabs, partially processed or fully finished foodstuffs can be performed using simple yes/no or semiquantitative lateral flow-type immunoassay tests, similar to those used in home pregnancy testing. To support allergen control within HACCP programs, for example, environmental testing of surfaces or factory air filtrates, more accurate testing of raw materials or process samples, or for labeling compliance surveillance testing, laboratory immunoassays enable relatively high throughputs

with lower limits of detection (LOD) and acceptable accuracy. Where confirmatory analysis is required to back up unexpected positive results or to confirm results above action limits with a different and more direct analytical technique, the high level of specificity and direct read-out conferred by PCR-based techniques are especially useful.

Sample Preparation

The variable nature of food allergens necessitates a clear understanding of the sampling process and of sample preparation and extraction in order to produce meaningful analytical results and interpret them effectively. Allergens such as milk, eggs, and gluten are reasonably homogeneous, well spread throughout foodstuffs, and relatively easily solubilized; fish and crustacean tissues are also quite easily dispersed. For these allergens, as long as the sampling technique adequately reflects the low levels of allergen to be detected, dispersal of the sample to form a homogeneous test portion, for example, by stomaching or blending, is relatively simple. In the case of tree nuts, peanuts, sesame, and soya, however, the durable nature of the commodity increases sampling difficulties and necessitates the use of a much more rigorous process to break up the commodity and release allergenic protein so that it can be detected.

The analytical matrix can also have a confounding effect on allergen extraction owing to the propensity of allergenic proteins to bind to carbohydrates and other proteins (30). Complex but relatively low protein matrixes such as plain chocolate and absorbent materials such as powders and flours with high carbohydrate and/or protein contents may require a complex extraction solution, usually containing high concentrations of additional, nonreactive protein. Finally, problems of analytical sample and laboratory cleanliness must be carefully considered if accurate results in the low ppm range are to be consistently achieved. In our laboratories, it has proved exceedingly difficult to obtain food matrixes that are truly free of a particular allergen, and keep them that way, when carrying out analyses or experiments to show a lack of cross-reactivity and interference. For such unrelated commodities to be of use, they must truly be 100% pure; even contamination levels of fractions of a ppm can present major problems in developing or maintaining test methods with particularly low LODs. Proteins and DNA from allergenic foods can be notoriously difficult to clean from surfaces in the laboratory, e.g., on blenders and other processing equipment, benches, glassware, laboratory coats, etc., which therefore present significant cross-contamination risks. Even the analyst's diet during the working day presents a threat that cannot be ignored. It is by no means an easy task to ensure that all foods being analyzed are effectively sampled, made as homogeneous as possible, and remain uncontaminated prior to and during testing; scrupulous attention to such issues is rarely misplaced.

Analytical Methodologies

Immunoassay

Immunoassay techniques are distinguished by their use of antibody-based detection reagents. All higher animals, as a defense against infectious or parasitic agents and other harmful entities, produce antibodies that react to antigens on the foreign material. This reaction can be harnessed to produce analytical reagents of high sensitivity and specificity. These latter properties are conferred by the intricate “lock and key” nature of the antibody-antigen reaction. Given the immunological nature of the atopic response, it is not surprising that specific antibodies that react strongly to any food allergen of interest can be readily obtained. A favored immunogen is a known allergenic protein of high purity—and preferably one that is relatively robust to food processing challenges such as heat, pressure, acidity, and salinity. Antibodies raised to these more defined allergens, rather than those produced by using crude protein extracts from allergenic commodities, will generally return analytical data that is easier to interpret. Such antibodies may be either monoclonal (reacting to a single antigenic epitope or binding site; usually obtained from cell culture) or polyclonal (reacting to multiple epitopes; obtained from individual, immunized animals and therefore more variable) in nature. Antibodies can easily be purified either by using the antibody’s immunoglobulin structure (e.g., IgG antibodies purified with Protein-A or Protein-G) or by specific affinity purification against the allergenic protein of interest, producing highly specific antibodies to that protein. These purified antibodies can then be used, for example, to coat surfaces such as nitrocellulose membranes or polystyrene microwells (as capture reagents) or, after modification to attach reactive molecules such as enzymes or biotin, or highly colored latex particles to their surface, as detection reagents.

Rapid Lateral Flow Immunoassays

Lateral flow tests are truly rapid immunoassays. In their most familiar form, they are used in tests, designed for home

use, which can confirm pregnancy virtually in seconds and at an early stage by detecting minute quantities of hormones in urine. Such rapid tests are now increasingly being used in food testing applications because of their speed and simplicity. We have developed such a test for detecting wheat gluten down to low ppm levels in a wide variety of food matrixes. Initially, a sample of the food being tested is extracted by being agitated for a short time in a buffer solution, which solubilizes gluten in the food sample. This extract is measured and consistently presented to the test strip by a sample “comb” (Figure 1). The test strip itself, which is held within a protective plastic housing, Figure 2 is constructed by laminating various layers onto clear, adhesive plastic backing tape (Figure 3). At the front end of the strip, a filter pad is used to separate out particulates from the food extract. In the next (glass fiber) pad, the extract solubilizes bright blue polystyrene latex particles held in the pad, to which gluten antibody has been attached. Any gluten in the sample extract binds to antibodies on the latex particles, which then flow onto the next nitrocellulose layer of the strip. On the nitrocellulose layer, 3 test lines have been deposited with a sophisticated dispensing device, which operates much like an ink-jet type of printer. The 3 lines function as follows (Figure 4):

Test Line ONE.—The active reagent is gluten antibody. If no gluten is present, no line is formed at this position (Figure 4A). In low to moderately high gluten level samples, antibodies on this line capture gluten bound to antibodies on the blue latex particles, resulting in a blue line of varying intensity if gluten is present (Figure 4, B and C).

Hook Line TWO.—The active reagent is gluten protein. Any latex particles with antibody that has not bound to gluten in the sample will attach to the gluten antigen forming a second blue line, which we term the “hook” line—see below.

Control Line THREE.—Captures any unreacted particles, thus acting as a check that the sample extract has mobilized the latex particles successfully, flowed across the strip, and the reagent lines on the test strip have remained functional, e.g., during extended room temperature storage. At the furthest end of the test strip, a “sink” pad absorbs the liquid extract as it



Figure 1. Rapid lateral flow-type test components; sample application comb at top right; test device at bottom right.

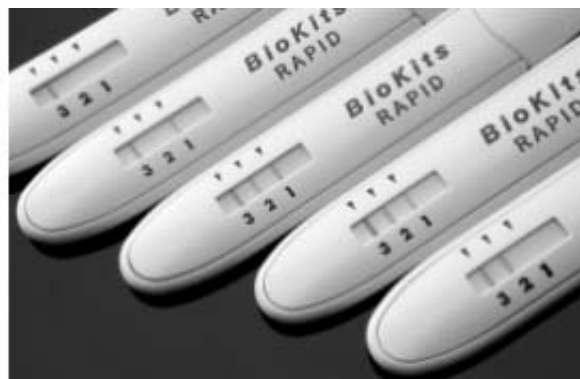


Figure 2. From right to left: Rapid test results for negative; positive; high positive (line 2 fading); higher positive (line 2 absent); very high positive (lines 1 and 2 absent) samples.

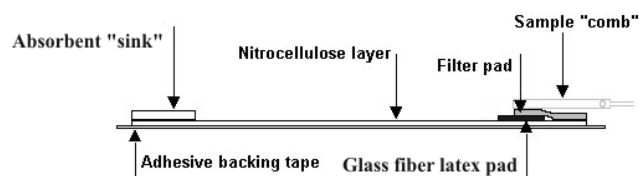


Figure 3. Schematic of Rapid lateral flow test construction.

wicks across the nitrocellulose layer to ensure that all of the extract migrates across the test window.

The necessity of a 3-line system, rather than simply using Test and Control lines, is born of the need for such rapid tests to work more or less anywhere, with any sample, no equipment, and a simple extraction technique. Under such circumstances, there is a real possibility that high levels of the protein being detected will be introduced into the test, e.g., in the case of a wheat gluten test, if a wheat flour or gluten powder sample were to be unwittingly tested. Should this be the case, high levels of gluten in the extract will exhaust the antibody binding capacity on both the latex particles and Test Line One so that no line is formed at this position, giving a problematic false-negative response. This paradoxical reduction in test response is a feature of homogeneous immunoassays and is sometimes referred to as the "prozone" or "high dose hook" effect. In our test format, such false-negative interpretations are prevented by Hook Line Two. Clearly, if the entire gluten antibody binding capacity on the latex particles is exhausted by gluten in the extract, Line Two will also disappear, but Line Three will always appear (Figure 4D). The 3-line format, therefore, even enables a crude quantitative estimate of gluten content (from no gluten to low, moderate, or very high gluten; Figure 2) to be made rapidly, with little or no training and in virtually any location.

Rapid Gluten Test Performance

We tested 114 samples in parallel using the Rapid test and a high-sensitivity gluten enzyme-linked immunosorbent assay (ELISA). A level of 50 ppm gluten by ELISA was arbitrarily set as the cut-off for positivity; 61 samples were classified as positive and 53 samples as negative by ELISA. Sensitivity and specificity for the Rapid gluten test were calculated as follows:

$$\text{Sensitivity} = \frac{\text{true positive} \times 100}{(\text{true positive} + \text{false negative})} = \frac{53 \times 100}{(53 + 0)} = 100\%$$

$$\text{Specificity} = \frac{\text{true negative} \times 100}{(\text{true negative} + \text{false positive})} = \frac{61 \times 100}{(61 + 6)} = 91\%$$

The 6 samples interpreted as false positives by the Rapid test contained low levels of gluten (between 10 and 39 ppm by ELISA), indicating a LOD for the Rapid test in the low ppm range. A further 60 samples with undetectable gluten content

by ELISA showed positive results in the Rapid test when spiked with low levels of gluten. The Rapid test also showed good reproducibility and repeatability and proved to be robust to changes in sample weight, extraction buffer volume, extraction, and result reading times.

ELISAs

We found a modification of the simple double antibody sandwich ELISA format to be useful in providing relatively fast but very robust allergen detection. For the detection of peanut content in foods at very low ppm levels, the assay must detect the allergen component after extraction and dilution steps, necessitating LOD in the low or sub-nanogram per milliliter range in the extract. We used polyclonal antibodies, originally developed at the Institute of Food Research (Norwich, UK; 31), to a preparation of conarachin (designated allergen nomenclature Ara h 1), an allergenic storage protein found in high abundance in peanuts. This ELISA utilizes a detector antibody conjugated to biotin, which has proved to be far more stable than antibodies directly linked to the detector enzyme. The advantage of biotin, which has a low molecular weight, as compared to direct conjugation to enzyme, is that multiple biotin molecules can be attached to each antibody. Subsequent reaction of such antibodies with a streptavidin-enzyme reagent can enhance assay response several fold.

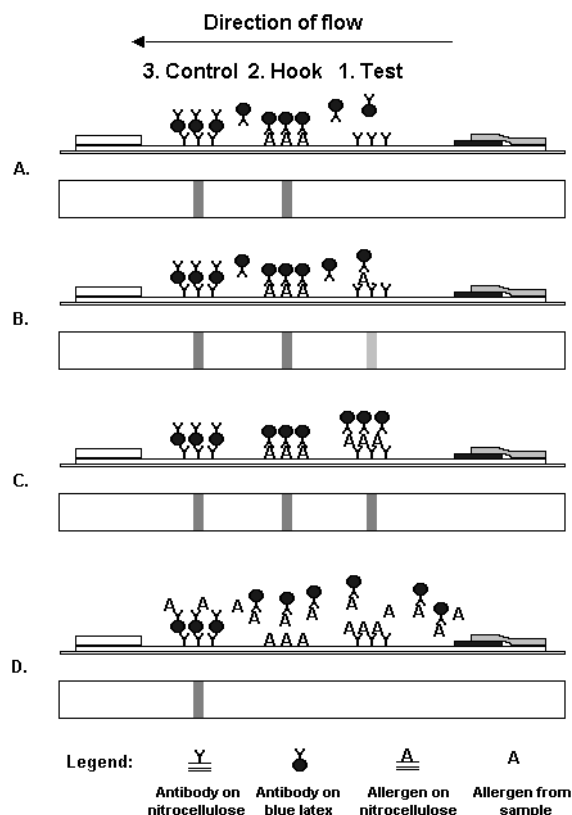


Figure 4. Schematic of Rapid lateral flow test chemistry. (A) No allergen/gluten present; (B) low allergen/gluten level; (C) moderate allergen/gluten level; (D) very high "hooked" allergen/gluten level.

In this assay, samples were extracted with a high-salt, protein-containing buffer. Diluted extract was then added to 96-well polystyrene plates, in which each well is coated with purified anti-conarachin (Figure 5A). The more peanut allergen in the extract, the more conarachin will bind to antibodies attached to the well (Figure 5B). After incubation, unbound material is removed by washing. Conarachin bound to the antibody-coated well is detected by reaction initially with a second, biotinylated anti-conarachin (Figure 5C) and then with a streptavidin-peroxidase conjugate (Figure 5D). The streptavidin reagent binds strongly and virtually irreversibly with biotin on the second antibody. After each incubation, excess reagent is removed by washing. Finally, bound peroxidase enzyme activity is determined by reacting with tetramethylbenzidine (TMB) substrate. A blue color, proportional to the amount of conarachin in the extract, develops in the presence of peroxidase (Figure 5E). Determination of peanut content (qualitative or quantitative) can be made by spectrophotometric absorbance data, e.g., from a microwell plate reader (Figure 6). Because of the convenient microwell format, 96-well ELISAs can be a cost-effective, high-throughput screening technique that can be performed even in relatively simple laboratory facilities.

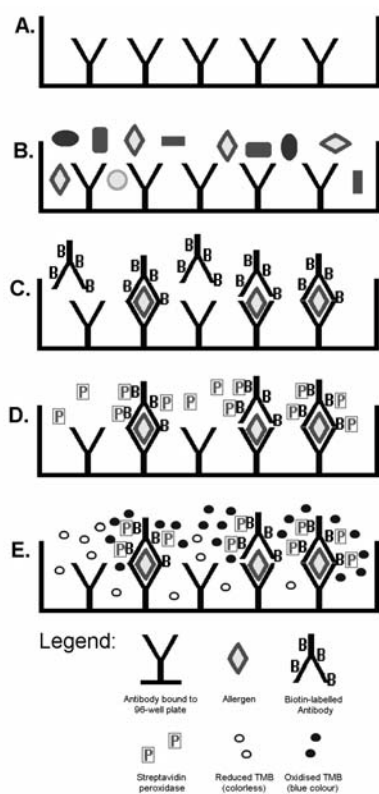


Figure 5. Schematic of enhanced, capture ELISA chemistry. (A) Anti-allergen antibody coated 96-well plate; (B) allergen capture; (C) biotinylated anti-allergen antibody binding; (D) streptavidin peroxidase binding; (E) TMB oxidation to blue-colored product.

Peanut ELISA Test Performance

The peanut ELISA described above has been validated by AOAC's Research Institute under its *Performance Tested Methods*SM program (Certificate No. 030402). Three independent laboratories, in conjunction with our own testing, produced the following results:

Sensitivity and selectivity.—Six high-risk food matrixes were selected for analysis (breakfast cereal, cookies, ice cream, milled nuts, plain and milk chocolate). The 60 replicate extracts of each blank matrix and 60 of each matrix spiked with 5 ppm peanut butter were all correctly identified by the assay.

Robustness.—Estimated (32) separately for the sample extraction and peanut ELISA processes. Seven variations of each process were compared with the normal protocol. The assay proved to be robust to changes in sample weight, extraction buffer composition, and volume changes, extraction, incubation, and result reading times.

Cross-reactivity.—Thirty-two potentially cross-reacting food commodities (almond, barley, beef gelatin, brazil, buck wheat, cashew, chestnut, chick pea, cocoa, coconut, hazelnut, lecithin, lima bean, macadamia, maize, oat, pea, pecan, pine kernel, pistachio, poppy seed, pork gelatin, pumpkin seed, rice, rye, sesame, skim milk, soy, sunflower seed, walnut, wheat, and wheat gluten) were tested to assess their response in the assay. All returned negative results in the assay.

Interference.—Three replicates of each of the same 32 cross-reaction commodities, spiked with 5 ppm peanut, were also tested. All gave positive results in the assay, although there were differences in the apparent recovery of peanut content [recovery range <25% (wheat gluten and poppy seed) to >115% (buckwheat, lecithin, and lima/butter bean)].

PCR Detection of DNA

PCR methodology is commonly used to establish the presence or absence of DNA sequences, such as those found

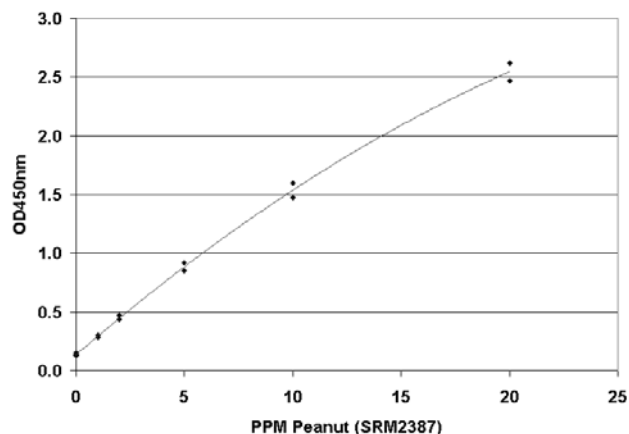


Figure 6. BioKits Peanut ELISA (AOAC PTM No. 030402) standard curve.

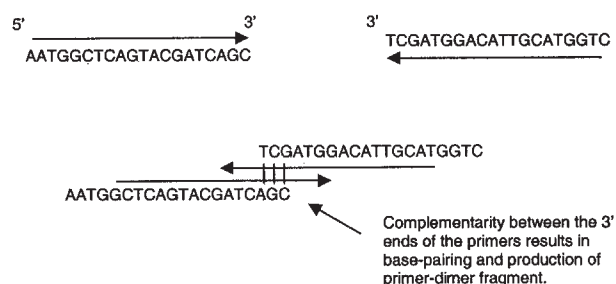


Figure 7. Example of a bad primer pair with complementary 3' ends.

in genetically modified organisms (GMOs), or from particular animal species. This technique is now being applied to the amplification of DNA targets from a variety of common allergens, such as peanut, soya, and milk, and can be suitable for use with raw or processed foods. PCR relies on the convenient peculiarity of the DNA helix to split into its 2 halves at high temperature, and the thermostability of an enzyme, called *Taq* Polymerase, which has a remarkable ability to copy DNA fragments. Using multiple cycles of heat and cooling, a single fragment of DNA can be reproduced several million-fold in only a few hours.

Oligonucleotide Design

The first step in designing a PCR-based test for a food allergen species is to decide on a particular protein of interest. The protein structures and amino acid sequences of many food allergens are known, and therefore it is relatively simple to determine the gene sequence on the DNA molecule which determines—or codes for—the protein of interest. Once this sequence is known, much of the art of constructing a robust PCR test is in the design of an oligonucleotide (or primer) to facilitate the PCR process. One of the important aspects for oligonucleotide design in the field of food testing is to ensure that the size of the amplified fragment (amplicon) is kept to less than approximately 400 base pairs (bp). Because the type of samples sent for analysis can be anything from raw ingredients to highly processed canned products, the state of the DNA from such samples will also vary. The DNA extracted from a canned product, or a highly heat-treated foodstuff, will tend to be of a poor quality and degraded into small fragments. By designing a small amplicon (in the region of 100–200 bp), the chances of achieving successful PCR tests from processed sample DNA extracts are greatly increased without compromising the specificity that the structure of DNA confers.

Oligonucleotide (or primer) pairs must be matched in terms of their so-called melting temperature. This will affect the conditions under which the PCR test is run. The melting temperature of each oligonucleotide is dependent on its DNA sequence. The greater the number of guanine (G) and cytosine (C) residues in each oligonucleotide, the higher the melting temperature. Conversely, adenine (A) and thymine (T)-rich oligonucleotides will have much reduced melting

temperatures. The ideal mix is a 50:50 ratio of A, T, G, and C residues, resulting in a melting temperature (which translates to a PCR annealing temperature) of between 55° and 65°C.

Furthermore, the oligonucleotide pair must be designed in such a way to avoid any complementarity, particularly around the extending (3') end. If there are any significant bp matches between the oligonucleotides, the efficiency of amplification of the desired fragment can become greatly reduced due to production of primer-dimer amplicons (Figure 7).

Inefficient PCR reactions can also be caused by oligonucleotides containing internal secondary structure.

In this instance, the oligonucleotide's interaction with itself will compete with the binding of the primer to the target DNA (Figure 8). Therefore, oligonucleotides with runs of complementary, repetitive bases should be avoided.

Having said all of the above, there are a number of computer programs available for primer design, both commercially and free on the Internet. Such programs, given the gene sequence of interest, will find primers of suitable sequence and melting temperature for a given amplicon size. We have developed a variety of specific primers that offer high levels of specificity to the allergen species being detected as well as ppm LOD of milk (bovine content), peanut, soya, fish, and crustacea in raw or processed foods.

PCR Laboratory Set-Up

In addition to the problems of allergen cleanliness mentioned earlier, the power of the PCR technique necessitates that the procedure is performed in 3 separate areas: (1) *DNA-free area*.—This area should not contain any double-stranded DNA. Only PCR mastermixes and *Taq* DNA Polymerase should be stored and used in this area. (2) *Template DNA area*.—This area is used for extraction of sample DNA and addition of template and control DNA to the PCR mastermix. DNA extracts should also be stored in this area. (3) *Post-PCR analysis area*.—This area houses the PCR thermocycling machines, gel electrophoresis, and gel imaging equipment.

Each area should ideally have dedicated laboratory coats, gloves, and micropipets. A one-way workflow is advisable between the 3 areas, i.e., from DNA-free to template to post-PCR, to reduce or prevent the possibility of cross-contamination.

Test samples are first treated to solubilize and/or remove proteins; then target or “template” DNA is extracted by using magnetic beads that have a high affinity for DNA in ethanolic solutions but release it in a simple water wash. In order to

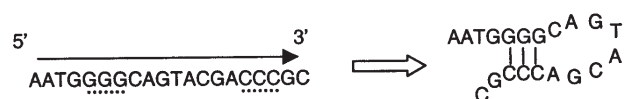


Figure 8. Example of a bad primer with complementary internal structure causing unwanted self-interaction.

make the PCR analyses more consistent and simple to set up, species-specific mastermixes are prepared containing everything required for PCR, with the exception of template (target) DNA and *Taq* polymerase enzyme. Template DNA and *Taq* polymerase enzyme are added to the mastermix solution prior to thermocycling. After 40–50 cycles of heating and cooling, template DNA is copied, or amplified, many millions of times and the high concentration of DNA produced can be analyzed on an agarose electrophoresis gel. Comparison of the migration of the bands of DNA produced during PCR with those of a 50 or 100 bp DNA marker “ladder” control on the agarose gel enables the qualitative identification of the allergen’s specific band, because its size is determined by the original primers (Figure 9). In many instances, quality control of the PCR process can be enhanced by the inclusion of a housekeeping gene fragment of known size within the mastermix. This is known as multiplexing. During PCR, the housekeeping gene will also be replicated and can be visualized on the agarose gel as a second band of known size. Presence of the housekeeping band, with or without the species-specific band, is an indication that the PCR process was successful and that the sample is positive or negative, respectively, for the species of interest. Although this technique may simplify the quality control of PCR testing, in our allergen DNA detection systems, it proved to be insufficiently sensitive.

Apart from electrophoretic detection of the specific PCR products, there are 2 other main methods of analysis: PCR-ELISA and real-time PCR. The PCR products from the former method are biotinylated during PCR and subsequently captured by a streptavidin-coated microwell. After denaturation to remove the complementary DNA strand, a second, fluorescein-labeled probe is added. This then binds to enzyme-labeled anti-fluorescein antibody; after washing, colorimetric substrate is added to facilitate color development proportional to the original allergen content. In real-time PCR, both amplification and detection of fluorescent detector molecules are performed simultaneously during thermocycling in a detector instrument. Although real-time PCR, in theory, offers tantalizing possibilities of multiple

allergen detection and quantitation, to date, these have both proved to be difficult to realize because of the complexity of processed food matrixes.

Allergen DNA Test Performance

The single PCR methods described above for peanut, soya, and milk/bovine content have been validated in our laboratories and found to be both sensitive (e.g., LODs <1 ppm for raw peanut flour, and <5 ppm for roasted peanut butter), highly specific, and robust. No cross-reactivities or interferences were observed with a wide range of food commodities and types.

Results and Discussion

The application of these 3 very different technologies in robust commercial formats will undoubtedly improve the quality of analysis of allergens in food, and thereby reduce the threat from hidden allergenic proteins in the food supply over the coming years. Given the increasing numbers of foods identified as high-risk, however, it will be some time before Rapid, ELISA, and DNA confirmatory testing methods will become available for all of them, but methods for the most problematic foods (peanuts, milk, eggs) will no doubt lead the way. Finally, however, with the introduction of such a variety of new methods will come new problems related to their validation and effective use, especially in ISO 17025-accredited laboratories. At the moment, the availability of certified reference materials, vital to allow calibration of different methods with reference to well-defined sources, and proficiency testing schemes that assist laboratories in their day-to-day maintenance of analytical quality, are virtually nonexistent. Until reference materials are specifically designed for, and proficiency schemes such as the United Kingdom’s Food Analysis Performance Assessment Scheme (FAPAS) become more comprehensive with respect to trace allergen analysis, the widespread dissemination of high quality analyses will be made all the harder.

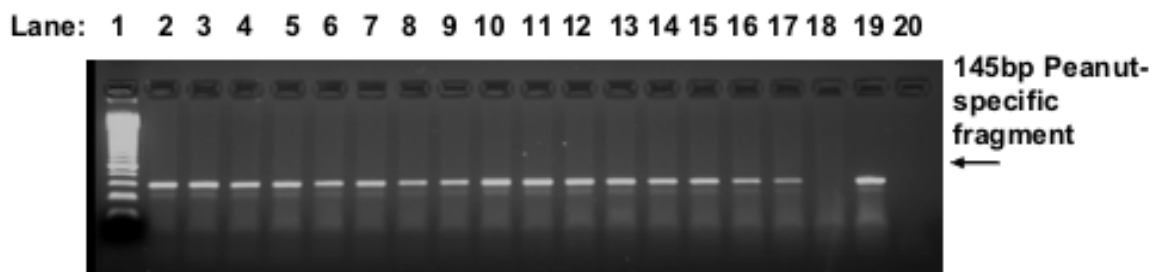


Figure 9. Peanut detection: single PCR visualized on agarose gel. Lane 1: ladder; lanes 2 and 3: 1000 ppm ground peanut; lanes 4 and 5: 100 ppm ground peanut; lanes 6 and 7: 10 ppm ground peanut; lanes 8 and 9: 1 ppm ground peanut; lanes 10 and 11: 1000 ppm defatted peanut meal; lanes 12 and 13: 100 ppm defatted peanut meal; lanes 14 and 15: 10 ppm defatted peanut meal; lanes 16 and 17: 1 ppm defatted peanut meal; lane 18: 0 ppm peanut (ground biscuit); lane 19: positive control; lane 20: water blank.

Acknowledgments

I would like to thank Jacqueline Coutts, Anne Cattell, Denise Nicholls, and their DNA, ELISA, and Rapid testing technical teams for creating the techniques and data given herein.

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