Comprehensive Reviews in Food Science and Food Safety

Peanut Allergy, Peanut Allergens, and Methods for the Detection of Peanut Contamination in Food Products

Hsiao-Wei Wen, Wlodzimierz Borejsza-Wysocki, Thomas R. DeCory, and Richard A. Durst

ABSTRACT: Attention to peanut allergy has been rising rapidly for the last 5 y, because it accounts for the majority of severe food-related anaphylaxis, it tends to appear early in life, and it usually is not resolved. Low milligram amounts of peanut allergens can induce severe allergic reactions in highly sensitized individuals, and no cure is available for peanut allergy. This review presents updated information on peanut allergy, peanut allergens (Ara h1 to h8), and available methods for detecting peanuts in foods. These methods are based on the detection of either peanut proteins or a specific DNA fragment of peanut allergens. A summary of published methods for detecting peanut in foods is given with a comparison of assay formats, target analyte, and assay sensitivity. Moreover, a summary of the current availability of commercial peanut allergen kits is presented with information about assay format, target analyte, sensitivity, testing time, company/kit name, and AOAC validation.

Introduction

Peanut allergy affects approximately 0.8% of young children and 0.6% of adults in the United States (Sampson 2004). Its increasing prevalence has been observed in both the United States and Europe (Sampson 1996; Grundy and others 2002; Sicherer and others 2003) and has become a major health issue in most developed countries (Bannon and others 2000). Diagnosis of peanut allergy generally begins with a medical history and a physical examination, followed by a prick skin test, a fluoroenzyme immunoassay, or oral food challenges (Scurlock and Burks 2004). No cure has been found for peanut allergy, and strict avoidance of peanuts is the only way to prevent severe symptoms (Sampson and others 2003). Although no cure has been successfully developed, several future treatments are currently under investigation (Sampson 2004). Thus, to prevent exposure, reliable detection and quantification methods of allergenic foods in food products are urgently required (Poms and others 2005).

MS 20060634 Submitted 11/17/06, Accepted 11/19/06. Author Wen is with Dept. of Food Science, Natl. I-Lan Univ., 1 Section 1 Shen-Lung Rd., I-Lan, Taiwan 260, ROC. Authors Borejsza-Wysocki and DeCory are with Dept. of Food Science and Technology, Cornell Univ., 630 West North St., Geneva, NY 14456. Author Durst is with Dept. of Biological and Environmental Engineering, Riley-Robb Hall, Cornell Univ., Ithaca, NY 14853. Direct inquiries to author Durst (E-mail: rad2@cornell.edu).

Peanut allergens are naturally occurring peanut proteins in peanut kernels (Taylor and others 1981; Bush and others 1989). Until now, 8 peanut allergens (Ara h1 to h8) have been identified and characterized (Kleber-Janke and others 1999; Bannon and others 2000; Flicker and others 2000). To detect peanut contamination in foods, highly sensitive and specific methods have been developed. They can be classified into 2 groups: protein-based or DNA-based assays. Protein-based assays detect either a specific peanut allergen (Ara h1 or Ara h2) or total soluble peanut proteins (Poms and others 2004). DNA-based techniques detect the presence of allergens by amplifying a specific DNA fragment of a peanut allergen gene through polymerase chain reaction (PCR) (Goodwin 2004).

Arachis hypogea

Peanuts are also called groundnuts, earthnuts, and monkey nuts. Even though their names imply nuts, the peanut plant (*Arachis hypogea*) belongs to the botanical family of Leguminosae, which includes green peas, lentils, soybeans, and kidney beans (Bewley and Black 1994). Peanuts are annual self-pollinating plants and peanut pods are harvested by pulling the pods out of the ground. Typically a pod contains 2 to 3 oval-shaped seeds, and each seed contains 2 lobes. Peanuts originated in South America about 4000 to 5000 y ago, were ex-

© 2007 Institute of Food Technologists

ported to Africa, and then moved to North America during the beginning of slave trade (Saavedra-Delgado 1989). In the United States, the most common peanut cultivars are Virginia, Spanish, Valencia, and Runner (Koppelman and others 2001), which are grown in Georgia, Texas, Alabama, North Carolina, Florida, Virginia, and Oklahoma. Georgia has the largest production, accounting for more than 40% of all peanuts (American Peanut Council 2005). Americans annually consume more than 1 million tons of peanuts; about 50% is consumed as peanut butter (Peanut Inst. 2005). In the world, the United States ranks 3rd behind China and India in peanut production (Sampson 2002).

Regardless of their popularity, peanuts have 2 major safety issues. One is contamination by aflatoxin, which is produced by the molds *Aspergillus flavus* and *A. parasiticus*. Aflatoxin is a carcinogen and is also linked to mental retardation (Yu and others 2003). Fortunately, proper storage and handling methods can completely eliminate the risk of aflatoxin contamination (Boutrif 1995). The other problem is that peanut is one of the most allergenic foods in the world. Peanut-induced anaphylaxis is an IgE-mediated food allergy reaction, in which IgE reacts with peanut allergens to release messengers such as histamine. It is estimated that peanut-induced anaphylaxis affects 1.5 million people and causes 50 to 100 deaths per year in the United States (Tariq and others 1996; Kanny and others 2001; Leung and others 2003).

Food Allergy Compared with Food Intolerance

Adverse food reactions can be classified into immune-related and nonimmune-related. The American Academy of Allergy and Immunology (AAAI) Committee on Adverse Reactions to Foods defines food allergy as "an immunologic reaction resulting from the ingestion of a food or food additive," and food intolerance as "a general term describing an abnormal physiological response to an ingested food or food additive that is not proven to be immunogenic" (Bindslev-Jensen and others 1994).

Food allergy, a type of external hypersensitivity, is an abnormal immune response to food (Bruijnzeel-Koomen and others 1995). In this type of reaction, the immune system is inappropriately triggered by an allergen, which is a food protein or a non-amino acid portion of a protein, such as carbohydrate (Ferguson 1992). In addition, food allergens are naturally produced in foods and are generally resistant to high temperature, low pH in the stomach, and enzyme digestion in the gastrointestinal tract (Hefle and others 1996). However, a recent study found that there was not a clear relationship between digestibility in vitro and protein allergenicity (Fu and others 2002). Food allergy is more common in children than in adults. In the United States, about 3.7% of adults and up to 6% of young children are allergic to different foods (Sampson 2004; Sicherer and others 2004). In the United States, cow milk (2.5%), egg (1.3%), and peanut (0.8%) account for the majority of food allergic reactions in children, whereas crustacean shellfish (2%), peanut (0.6%), tree nuts (0.5%), and fish (0.4%) are responsible for most of the food allergic reactions in adults (Sampson 2004; Sichere and Sampson 2006). Cow's milk, eggs, soy, wheat, peanuts, tree nuts, fish, and crustacean shellfish cause approximately 90% of the food allergic reactions, and they are also the primary foods causing anaphylaxis (Sicherer and Sampson 2000). Early food allergies such as egg and milk allergy are often outgrown by the age of 5 y (approximately 80%), but certain allergies, especially peanuts, tree nuts, fish, and crustacean shellfish, have low outgrowth rates (Kitts and others 1997; Wood 2003). Most food allergic reactions are induced immediately after exposure to the allergenic food, except celiac disease, which is caused by a deaminated gluten peptide and is a delayed-type food allergic reaction (Type IV reaction).

Food intolerance can be the result of a genetic deficiency, drug-induced metabolic disorder, or anaphylactoid reactions. Most symptoms of food intolerance are similar to food allergy (Sampson 2004). However, food intolerance reactions can be delayed up to 48 h or more. An example of food intolerance caused by genetic deficiency is lactose intolerance, which is present in up to 15% of people of northern European descent, up to 80% of Blacks and Latinos, and up to 100% of American Indians and Asians (Swagerty and others 2002). The cause for lactose intolerance is deficiency of the intestinal enzyme lactase (McNeish 1984). Taking monoamine oxidase inhibitors (MAOI) can cause a drug-induced metabolic disorder because MAOI can interfere with the clearance of tyramine. A too high concentration of tyramine in blood can induce severe headaches and increase heartbeat and blood pressure, so people taking MAOI are instructed not to ingest fermented foods such as cheese, olives, pickles, sausages, wine, and beer (Jansen and others 2003). Anaphylactoid reactions are similar to anaphylaxis since both are immediate systemic reactions. However, anaphylaxis is caused by IgE-mediated immune release of mediators, whereas anaphylactoid reactions are not IgE-mediated but are caused by exposure to provoking agents, such as radiocontrast agents. They are presumed to induce anaphylactoid reactions in susceptible persons through direct mediator release from basophiles and mast cells (Anonymous 1998).

Prevalence, Threshold Dose, and Outgrowth Rate of Peanut Allergy

Peanut allergy is characterized by more severe symptoms than other food allergies due to its potentially fatal anaphylactic reactions and the high rate of reactions on minimal contact (Hourihane and others 1997; Sicherer 2002). Annually in the United States, there are about 30000 emergency-room visits for food anaphylaxis (Sampson 2000) and approximately one third of these visits are due to exposure to peanuts (Sampson and Ho 1997; Burks 2003). In an analysis of 32 fatal cases due to food anaphylactic reactions, peanuts and tree nuts were responsible for more than 90% of the fatalities (Bock and others 2001).

A study of 4374 households in the United States, representing 12032 individuals, reported that peanut allergy occurred in 0.4% of children and 0.7% of adults (Sicherer and others 1999). In a study with American children, the prevalence of allergic reactions to peanuts between 1990 and 1994 was nearly twice as high as that evaluated between 1980 and 1984 (Sampson 1996). In France, the food allergy population is estimated at 3.24%, and the peanut allergic population is estimated at about 1% (Kanny and others 2001). In the United Kingdom, the peanut allergic prevalence was reported in about 0.5% of the general population and the prevalence of sensitization to peanuts increased from 1.3% to 3.2% from 1989 to 1995, based on a population-based study of 3-y-olds (Emmett and others 1999). Taken together, these studies indicated that approximately 1 in 150 to 200 individuals has a peanut allergy in these westernized countries (Sicherer 2002).

To protect peanut allergic individuals, many studies have focused on finding the threshold dose for peanut allergic reactions by the use of a double-blind placebo-controlled food challenge (DBPCFC). The challenge result can be regarded as positive either when objective symptoms (wheezing, vomiting, diarrhea, swelling in the oral cavity, and hoarseness) occur or when subjective symptoms (itching of the skin or in the oral cavity, nausea, abdominal pain, and dyspnea) occur repeatedly after active doses (Sampson 1988a). Threshold dose can be defined as the lowest dose of peanut or peanut protein eliciting a positive reaction (Wensing and others 2002). Therefore, the threshold dose actually lies somewhere between the nonobserved adverse effect level (NOAEL) and the lowest observed adverse effect level

(LOAEL) (Madsen 2001). In a series of studies based on food challenge tests with 306 individuals, the lowest provoking dose was 1 mg of peanuts (Taylor and others 2002). However, in a DBPCFC study with 26 patients, threshold doses for subjective responses ranged from 0.1 mg up to 1000 mg of peanut proteins. In this study, they also observed that patients with severe reactions react to lower doses than patients with mild symptoms (Wensing and others 2002). However, more typical doses that trigger reactions in patients with the DBPCFC test for objective responses are generally in the range of 100 to 1000 mg of peanuts (Moneret-Vautrin and others 1998; Sicherer and others 2000). From a summary of published papers, the LOAEL for peanut allergens is 0.25 to 10 mg of peanut proteins (USFDA 2005). One peanut kernel weighs approximately 800 mg (Sicherer 2002) and contains about 200 mg of proteins (Goldman 1998). Therefore, peanut allergic reactions can be induced by less than 1 peanut kernel (USFDA 2005). Besides accidental ingestion, peanut allergic reaction can also be elicited by touching or kissing. Swelling of lips and tongue was reported in a card player because the cards were contaminated by peanuts eaten by fellow players (Lepp and others 2002). Occasionally, peanut allergen can be introduced by kissing (Wuthrich and others 2001; Hallett and others 2002). It was reported that 2 persons out of 1139 food-allergic patients who answered a questionnaire had peanut allergic symptoms after kissing (Eriksson and others 2003).

The outgrowth rate is the percentage of children who stop having allergic reactions as they grow older. Unlike egg or milk allergies, peanut allergy has a low outgrowth rate. A study reported that an evaluation of 32 patients that had a positive DBPCFC and positive skin tests to peanuts were followed for 2 to 14 y with no resolution of the allergy (Bock and Atkins 1989). However, more recent studies have shown that peanut allergy can be outgrown. A study with 223 patients reported that peanut allergy was outgrown in 21.5% of individuals, especially individuals that had low levels of peanut-specific serum IgE antibodies in their infancy (Skolnick and others 2001). Moreover, patients with milder reactions had a better chance of developing tolerance than the individuals whose 1st reaction was anaphylaxis (Spergel and Fiedler 2001). Therefore, children with low levels of peanut-specific IgE or mild responses to peanuts should be reevaluated periodically to determine if they have developed tolerance to peanuts (Sampson 2002). Although some individuals can outgrow peanut allergy, others did experience a recurrence of the allergy. In a study with 80 individuals, 2 had experienced subsequent reactions to peanut after passing their challenge. Thus, a recurrence of peanut allergy may occur but appears to be uncommon (Fleischer and others 2003).

Mechanism and Development of Peanut Allergic Reactions

IgE and mast cells are 2 major features in peanut allergic reactions. IgE is 1 type of immunoglobulin circulating in blood vessels with the shortest half-life of all of the classes of immunoglobulins (IgG, IgA, IgM, IgD, and IgE) and the lowest concentration in serum (Harlow and Lane 1999). IgE has an extra domain in the Fc portion that has high affinity for the receptors of mast cells and basophils. Basophils are found in the blood while mast cells are generally found in areas that are typical sites of allergic reactions, including the blood vessels in connective tissue, in the lining of the gastrointestinal tract and the lungs, and in skin (Benjamini and Leskowitz 1991).

There are 5 steps contributing to peanut allergic reactions: antigen presentation, IgE production, mast cell activation, mediator release, and symptom exhibition. The sequence of peanut allergic sensitization is shown in Figure 1. After ingestion of peanuts, peanut allergens cross the intestinal mucosal membrane. Antigen presenting cells (APCs) such as macrophages, dendritic cells, or B-lymphocytes take these allergens in by endocytosis or phagocytosis. Once these allergens are internalized, they are degraded into oligopeptides and bind to the major histocompatability complex class II (MHC class II) to form MHC class II-peptide complexes. The complexes are transferred to the plasma membrane, where the peptides are presented to $CD4^+$ T-helper-2 (Th-2) cells. This stage is called antigen presentation. The interaction between MHC class II-peptide complexes and T-cell receptors (TCR) on the surface of Th-2 cells activates T-helper cells and stimulates the production of lymphokines such as interleukin-4 (IL-4) to activate B-cells. The activated B-cells begin proliferation, differentiation, and IgE synthesis. Newly synthesized IgE antibodies are secreted into the blood stream and then bind to receptors on mast cells or basophils (Wraith and others 1989; De Vries and others 1991; Romagnani 1991). After a peanut allergic person is re-exposed to peanut, peanut allergens bind to the surface of mast cells through the prebound IgE molecules. Because each allergen has more than 1 epitope (the site that is recognized by antibodies), 1 allergen can bind several different IgE antibodies, thereby cross-linking IgE antibodies and instantly triggering degranulation inside mast cells. This step is called the mast cell activation. The degranulation reaction releases mediators and thus starts the peanut allergic symptoms (Lehrer and others 1996; Taylor and Lehrer 1996). The released mediators can be classified into 2 groups. One group is the preformed mediators such as histamine, which is formed by decarboxylation of histidine and can cause smooth muscle constriction or an increase in the vascular permeability. The other group of mediators is newly synthesized mediators such as prostaglandins and leukotrienes, and they are chemotactic for neutrophils, eosinophils, basophils, and monocytes. The production of this group is based on the conversion of phospholipids of cellular membranes. Leukotrienes can cause prolonged constriction of smooth muscle; while prostaglandins can cause bronchoconstriction (Lehrer and others 1996; Taylor and Lehrer 1996).

Peanut can induce allergic reaction immediately with a variety of symptoms (Sicherer and others 2001b; Sampson 2002). In a study with 662 self-reported peanut allergic cases, peanut allergic reactions can be provoked within 5 min for 76% of individuals and within 30 min for 93% of individuals (Hourihane and others 1997). The allergic symptoms occur throughout the body, from the minor symptoms such as itching tongue or abdominal pain to severe systematic reactions (anaphylaxis), including tightening of the airways, a drop of blood pressure (hypotension), loss of consciousness, or even death (Benjamini and Leskowitz 1991). In a study with 4685 peanut allergic individuals, it was observed that the median age of a 1st reaction was 14 mo with the following distribution of organ systems affected: skin 89%, respiratory 42%, gastrointestinal 26%, and cardiovascular 4%. About half the individuals have allergic reactions in 1 target-organ system, 30% have symptoms in 2 systems, 10% to 15% in 3 systems, and 1% in 4 systems (Sicherer and others 2001b).

Two possible early life origins of peanut allergy are in utero exposure and breast milk (Frank and others 1999). Most peanut allergic individuals react to their 1st exposure to peanuts (Hourihane and others 1997), implying sensitization may have occurred by in utero exposure to peanut. Therefore, the avoidance of peanuts during pregnancy is recommended. In a study with 23 mothers, a low median concentration of 200 ng/mL of peanut protein was detected in the breast milk of 11 of 23 mothers who consumed 50 g of peanuts (Vadas and others 2001). Since infants may get peanut allergens through breast milk, maternal dietary restriction

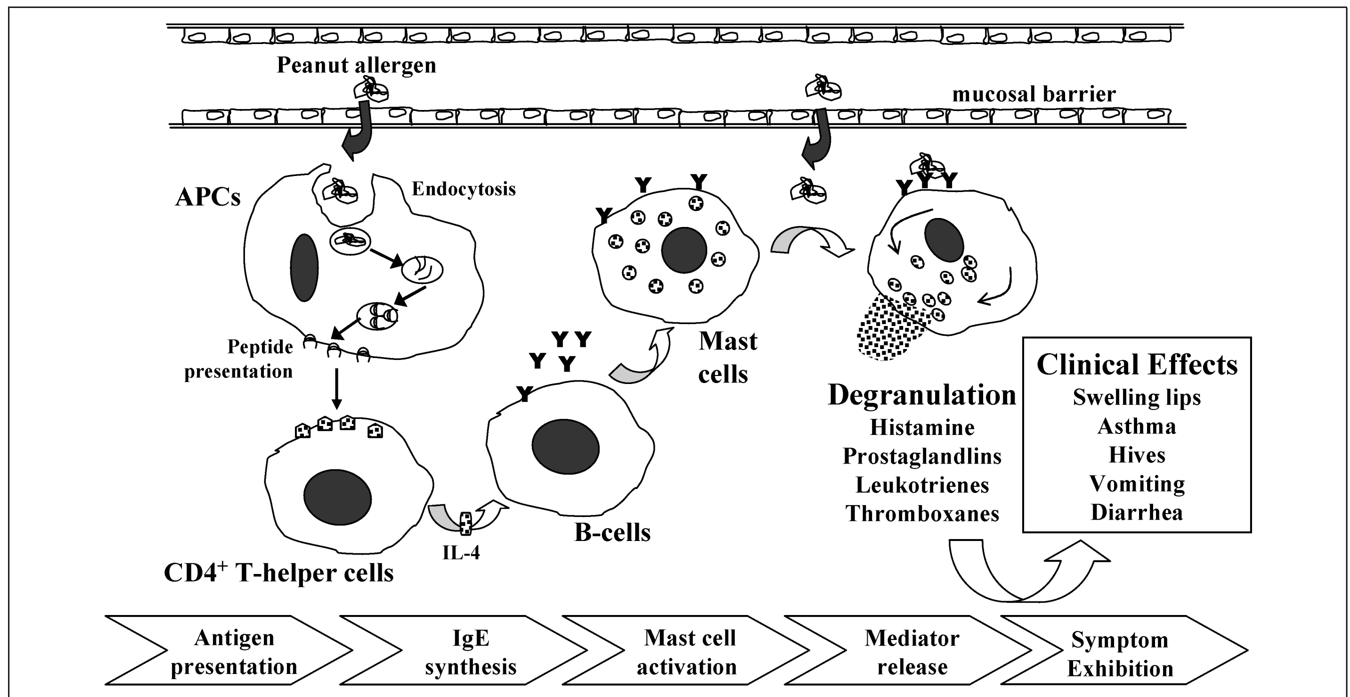

Figure 1 — The 1st 4 steps in initiating a peanut allergic reaction (Benjamini and Leskowitz 1991; Kay 2001; Lehrer and others 2002; Merz 2003; Bohle 2004)

to peanuts during breastfeeding is highly recommended. In addition, because of the immaturity of the immune system for fetuses and infants, food allergies are likely to develop in the 1st few years of life (Sampson 1999a). Moreover, a lower incidence of peanuts allergy was observed in countries such as Denmark and Norway, where peanut butter is rarely eaten (Sampson 2002). Therefore, to prevent the development of peanut allergy, the American Academy of Pediatrics recommends not adding peanuts to the diet until a child is 3 y old (Zeiger 2003).

In addition to the exposure to peanuts, the development of peanut allergy is also dependent on the way peanuts are prepared (Beyer and others 2001). Peanut allergy normally is implied as a disease of developed countries where people usually consume roasted peanut (Hourihane 2002). In contrast, peanut allergy is less prevalent in China, where people consume similar volumes of peanuts, because peanuts are boiled or fried, and not roasted. Roasting could increase the levels of advanced glycation end (AGE) products, which are more resistant to acid degradation and have a positive association with increased allergenicity of roasted peanuts (Chung and Champagne 2001; Mondoulet and others 2005). On the other hand, boiling caused a two-fold decrease in allergenicity of peanuts possibly due to a removal of low-molecular-weight allergens from peanuts into the water during cooking (Mondoulet and others 2005).

Diagnosis of Peanut Allergy

The clinical expression of peanut allergy is fairly predictable since it has a tendency to be severe, although the severity may be different with individuals and doses of ingestion (Hourihane and others 1997; Al-Muhsen and others 2003). The methods for diagnosing peanut allergy include differential diagnosis, laboratory tests, and oral food challenges (Sampson 1999b; Sicherer 1999). Differential diagnosis, the 1st approach, analyzes a patient's medical history and involves a physical examination. It distinguishes food allergy from food intolerance or other illnesses based on the suspected mechanisms determined by the symptoms, the timing of reactions, and the food suspected of causing the reactions (Sampson 1999b). To confirm the result of differential diagnosis, a laboratory examination or a clinical procedure would be the next step.

For peanut allergy diagnosis, the prick skin test (PST) is a common clinical procedure, while a fluoroenzyme immunoassay (Pharmacia ImmunoCAP-FEIA) is a frequently used laboratory examination. In the PST, peanut extracts, positive control (histamine), and negative control (saline) are applied by the prick technique (Bock and others 1978). When the elicited wheals are at least 3 mm larger than those induced by negative controls, they are considered positive. The accuracy of positive PST results is less than 50%, but the accuracy of negative PST results is greater than 95% (Sampson and Albergo 1984; Sampson 1988b). The 2nd examination, ImmunoCAP-FEIA, measures peanut-specific IgE in the patient's serum (Al-Muhsen and others 2003). Peanut allergens are coated onto a solid matrix and then exposed to a serum sample. If a patient's serum has IgE against peanut allergens, they bind to the immobilized peanut allergens and can be detected by a labeled secondary antibody specific to human IgE. Even though ImmunoCAP-FEIA is generally less sensitive than PST, the accuracy of positive ImmunoCAP-FEIA is around 100%. Patients with peanut-specific IgE levels of at least 15 kUA/L have a probability of developing allergic reactions of 95% or greater if they ingest peanuts (Sampson 2001a, 2002). If patients have acute allergic responses after exposure to peanuts and positive results from the PST or ImmunoCAP-FEIA test, they do not need to undergo an oral peanut challenge to establish the diagnosis.

In the case where a conclusive diagnosis is impossible, the oral peanut challenge is required.

Oral food challenges can be classified into 3 types: openly performed (patient and health professional know the challenge content), single-blind (only the health professional knows the challenge content), or double-blind and placebo-controlled food challenges (health professional and patient do not know the challenge content). DBPCFC is the "gold standard" for diagnosis of food allergies since it is the most rigorous and least subjective method (May 1976; Bock and others 1988). If the response to DBPCFC is positive, peanut is confirmed as an allergic food and should be avoided. If the DBPCFC response is negative, the patient has tolerance to peanuts and peanuts can be reintroduced (Al-Muhsen and others 2003). Because there is a risk of inducing severe anaphylactic reactions, the oral challenge must be performed in a hospital with appropriately trained professionals and access to emergency care and medications (Hamilos and others 1993). If the patient has a history for very severe reactions to peanuts, the challenge tests might not always be performed.

Management: Avoidance and Treatments

The management of peanut allergy includes strict avoidance of peanut allergens, recognition of the signs of allergic reactions, and emergency treatments (Al-Muhsen and others 2003). The most important step for avoidance is to check food labels for the presence of peanuts, and it is the most common treatment approach. Allergic individuals also need to avoid high-risk situations when eating outside of the home, such as school, restaurants, and relatives' or friends' homes. In schools, 25% of the reactions were severe and most reactions were caused by food sharing or parties/special occasions (Sicherer and others 2001a). Among restaurants and food establishments, allergic reactions more commonly occur in bakeries, ice cream shops, and Asian restaurants (Furlong and others 2001). Normally peanut was a hidden ingredient in sauces, stews, dressing, or unlabeled/unspecific food ingredients such as spices or natural flavorings (Sicherer 2002). Minute amounts of peanut in food can result from contaminated cooking utensils, direct or indirect contact between foods, and inhaled fumes during cooking (Kemp and Lockey 1996). Several foods have been related to causing anaphylaxis in peanut allergic patients: candy, chocolate products, cookies, chili, dry soup mixtures, pasta, sandwiches, Vietnamese dishes (Hefle and others 1994b; McKenna and Klontz 1997; Sampson and others 2002). Moreover, in most food factories different products are manufactured with various ingredients and sometimes run on the same production line. Therefore, it is hard to eliminate the risk of cross-contamination completely among different products (Kiening and others 2005). Other reasons for cross-contamination of peanut-free products include contaminated raw materials, common transport containers for peanuts and other foods, unsafe rework-management, and insufficient sanitation (Schappi and others 2001). It is estimated that fewer than 15% of peanut allergic persons are reactive to other members of the legume family, such as soybean, pea, and chickpea. However, 25% to 35% of peanut allergic individuals can react to the tree nut family, such as almonds, walnuts, brazil nuts, cashews, hazel nuts, and pistachios. Thus, individuals normally allergic to peanuts are advised to also completely avoid tree nuts (Sicherer and others 1998; de Leon and others 2003). The basis for the cross-reactivity is poorly understood.

Potential factors for developing peanut allergy include atopy, family history of peanut allergy, and parental maternal consumption of peanuts (Hourihane and others 1996). Therefore, pregnant women or nursing mothers from families with a history of atopy should avoid eating peanuts. In addition, infants should avoid consumption of peanuts and peanut oils (Moneret-Vautrin and others 1994), use of maternal breast creams containing peanut oil (Lever 1996), and consumption of soy milk or formula (Zimmerman and others 1989). However, not all of these risk factors have been consistently reproduced in the development of peanut allergy (Tariq and others 1996; Klemola and others 2005).

Severe allergic reactions sometimes are hard to avoid. Once the symptoms of anaphylaxis start, the best treatment is an immediate injection of epinephrine, followed by professional medical treatment (Stark and Sullivan 1986; Sampson 2003). Epinephrine is a synthetic form of adrenaline. It is effective for the cardiovascular and respiratory systems by rapidly constricting the blood vessels, relaxing the smooth muscles in the airway and lungs to improve breathing, and stimulating the heartbeat. Since the sooner a person receives epinephrine the better the chance for survival, individuals with an anaphylaxis history should always carry an epinephrine auto-injector (Genasi 2001; Ellis and Day 2003). Persons who have peanut allergic reactions that require the use of epinephrine should always go to a local emergency room in case they have persistent biphasic response (a recurrence of anaphylactic reactions) (Sampson 2002). Around 25% of patients will experience a biphasic response and 90% of biphasic responses occur within 4 h after the 1st symptom (Sampson and others 1992). For reducing mild allergic symptoms, individuals can take antihistamine, which is the most common medication for suppressing symptoms by preventing the release of histamine (King 2000).

Unlike immunotherapy for seasonal allergies, also known as spring allergies or rose fever, injection of peanut extracts has an unacceptable risk of inducing life-threatening anaphylaxis. Because of recent advances in understanding the mechanisms of allergic reactions and the characteristics of allergens, 6 different potential therapies have been under investigation (Burks and others 2004; Nowak-Wegrzyn and Sampson 2004; Li 2005). The first, anti-IgE immunotherapy, is based on blocking the recognition between human IgE antibody and mast cells or basophils by monoclonal antihuman peanut-specific IgE antibody. This blocking inhibits allergic reactions in the early stages and can efficiently stop allergic symptoms before they start (Sampson 2001b). In a recent study with 84 peanut allergic patients, a significant number of patients receiving the highest dose of anti-IgE antibody for 4 mo had a significant decrease in their clinical symptoms on peanut challenges (Leung and others 2003). It is the only therapy that has progressed to human trials. The second, mutated protein immunotherapy, uses engineered major allergens, in which the IgE binding sites have been changed by altering the primary amino acid sequence. For example, mutant Ara h2 presented a less allergenic reaction to peanut specific IgE serum, compared to native Ara h2 (Stanley and others 1997). In animal studies with peanut allergic mice, engineered recombinant peanut allergens (Ara h1, Ara h2, and Ara h3) reduced the peanut-specific IgE level and their clinical symptoms on peanut challenge (Li and others 2003). The third, DNA immunization, is a method of therapy that introduces DNA-encoding peanut protein. This approach results in endogenous production of allergenic proteins, which may result in intolerance instead of allergenicity (Spiegelberg and others 1997). In a study with peanut allergic mice, oral allergen-gene immunization using DNA nanoparticles synthesized by complexing Ara h2 plasmid DNA with chitosan reduced clinical symptoms on peanut challenge, levels of IgE, plasma histamine, and vascular leakage (Roy and others 1999). The fourth, immunization with immunostimulatory sequences (ISS), is based on the capability of ISS to stimulate APCs and natural killer cells to secrete IFN-r and IL-12, promoting immune deviation from the allergic Th2 phenotype to the tolerant Th1 phenotype (Chu and

others 1997). The fifth, peptide immunotherapy, is an approach that uses pepsin-digested peanut extract. The results of this study demonstrated that pepsin-digested extract contained T-cell epitopes instead of IgE-binding epitopes, since IgE reactivity was eliminated but T-cell reactivity was maintained (Hong and others 1999). The 6th and last therapy, traditional Chinese medicine (TCM), has been applied to treat peanut allergic mice with 2 food allergy herbal formulas (FAHF). FAHF-1 contains a mixture of 11 herbs (according to the package, Ganoderma Lucidum, Radix Lateralis Aconiti Carmichaeli Praeparata, Fructus Pruni Mume, Pericarpium Zanthoxyli Bu, Herba Cum Radice Asari, Rhizoma Coptidis, Cortex Phellodendri, Rhizoma Zingiberis Officinalis, Ramulus Cinnamomi Cassiae, Radix Ginseng, and Corpus Radix Angelicae Sinensis) and protected peanut-sensitized mice from anaphylactic reactions (Li and others 2001). FAHF-2, a modified formula of FAHF-1, from which 2 herbs (Radix Lateralis Aconiti Carmichaeli Praeparata and Herba Cum Radice Asari) were eliminated, could completely eliminate anaphylaxis in peanut allergic mice on peanut challenges for as long as 5 wk posttherapy (Srivastava and others 2005). Since it is hard for total avoidance of exposure to peanuts, these future immunotherapies bring real hope to the treatment of IgE-mediated allergies (Nowak-Wegrzyn and Sampson 2004).

Peanut Allergens: Ara h1 to h8

Food allergens can be grouped into major and minor allergens. Major allergens are proteins that can interact with specific IgE of more than 50% of the allergic patients studied (Fu and others 2002). Generally, many major food allergens belong to major food proteins. This suggests that a high dose of a particular food protein increases the chance of inducing an allergic reaction (Lehrer and others 2002). This observation can be applied to peanut allergy since 2 major peanut allergens, Ara h1 and Ara h2, are also major peanut proteins and comprise 12% to 16% and 5.9% to 9.3% of total peanut protein content, respectively (Koppelman and others 2001). Both major peanut allergens have a highly stable nature (Maleki and others 2000b; Sen and others 2002; Mondoulet and others 2005) and more than 95% of peanut allergic individuals had specific IgE to Ara h1 or Ara h2 (Scurlock and Burks 2004). Other peanut allergens (Ara h3 to h8) are considered as minor allergens due to their lower sensitizing rate in peanut allergic individuals. Eight different peanut allergens have been identified, cloned, and expressed, and their basic characteristics are listed in Table 1 (Bannon and others 2000). On the basis of biological functions, most of the peanut allergens, besides Ara h5 and Ara h8, are classified as seed-storage proteins. The Ara h5 allergen is classified into the profilins, which are 12-kDa to 15-kDa monomeric actin-binding proteins regulating the actin cytoskeleton in plant cells (Breiteneder and Ebner 2000). The Ara h8 allergen has not been classified but its protein sequence alignment presented a 45.9% identity with Bet v 1 (Mittag and others 2004), which is a birch pollen allergen and a member of pathogenesis-related protein 10 (PR-10) (Breiteneder and Ebner 2000).

Ara h1 is a glycoprotein with a molecular weight of 63.5 kDa and an isoelectric point (pI) of 4.55 (Burks and others 1991). Ara h1 has a signal peptide and a single glycosylation addition site (NAS: asparagine, alanine, serine) at amino acid position 521 to 523. By performing the DNA sequence analysis, it was found that Ara h1 has significant homology with the vicilin seed storage protein family (Burks and others 1995), which belongs to the cupin superfamily possessing a common β-barrel structure (Mills and others 2002). In addition, Ara h1 is classified as a conarachin since it was purified from the 40% to 85% fraction of ammonium sulfate saturation (Helm 2001). Moreover, Ara h1 interacts with itself to form a stable trimeric structure (Shin and others 1998). This homo-trimer are able to form in the presence of 1.8 M NaCl, indicating the formation and stability of the Ara h1 trimer are primarily based on hydrophobic interactions (Maleki and others 2000b). The frequency of sensitization by Ara h1 can be up to 100% of peanut allergic patients (Burks and others 1991; Clarke and others 1998; Kleber-Janke and others 1999). Additionally, no difference in IgE-binding ability between Ara h1 from raw and heated peanuts was observed, indicating that heating did not significantly affect the allergenicity of Ara h1 (Koppelman and others 1999). Ara h1 also maintained its allergenicity not only after heating but also after digestion with pepsin, trypsin, or chymotrypsin, since 5 to 8 IgE binding fragments of Ara h1 were detected after the reactions (Maleki and others 2000b). Ara h1 contains 23 linear IgE-binding epitopes. Among them, epitopes 1, 3, 4, and 17 are immunodominant, and a single amino acid change within these peptides had dramatic effects on IgE-binding characteristics (Burks and others 1997). Even though the IgE-binding epitopes had more even distribution in the primary structure, they were located in 2 main regions, which are the overlapping regions between monomers (Shin and others 1998).

Ara h2 is another major peanut allergen and also is a glycoprotein with a molecular weight of 17 kDa and a pI of 5.2 (Burks and others 1992). A clone of Ara h2 with 741 bp was constructed and was capable of encoding a 17.5-kDa protein with homology to the conglutin family of seed storage proteins (Stanley and others 1997). The 3-dimensional models for Ara h2 and other structurally related 2S albumin allergens of dietary nuts demonstrated that an overall 3-dimensional fold was stabilized by disulfide bridges, which were well conserved among all the members of the 2S albumin superfamily. However, Ara h2 showed no structural homology for the IgE binding epitopes with Jug r 1 (walnut), Car i 1 (pecan), or Ber e 1 (Brazil nut) (Barre and others 2005). To study the effect of glycosylation on allergenicity of Ara h2, thermal treatment (about 50 °C) with carbohydrates (fructose, glucose,

Table 1 — Peanut allergens

Allergen	M.W.	pI	Biological function	Protein family	Sensitization (%)[a]
Ara h1	63.5 kDa	4.55	Seed storage protein	Vicilins	65 to 100
Ara h2	17 kDa	5.2	Seed storage protein	Conglutins	71 to 100
Ara h3	57 kDa	5.5	Seed storage protein	Glycinins	44 to 53
Ara h4	35.9 kDa	5.5	Seed storage protein	Glycinins	44 to 53
Ara h5	14 kDa	4.6	Actin-binding protein	Profilins	13
Ara h6	14.5 kDa	5.2	Seed storage protein	Conglutins	38
Ara h7	15.8 kDa	5.6	Seed storage protein	Conglutins	43
Ara h8	16.9 kDa	5.03	NA	NA	NA

NA = not available.
[a]The percent of peanut allergic population.

arabinose, mannose, xylose, galactose, or dextrose) for various times (1 to 5 wk) was performed, and significant increases in the IgE-binding activity was observed for both purified Ara h2 and recombinant Ara h2 (rAra h2). These data indicate that thermal processing may play an important role in enhancing the allergenicity of Ara h2 and rAra h2 (Maleki and others 2000a, 2003; Chung and Champagne 2001; Maleki 2004; Gruber and others 2005). A study of the heat effect on Ara h2 allergenicity indicated that IgE immunoreactivity of purified Ara h2 prepared from roasted peanuts was higher than that of purified Ara h2 from raw and boiled peanuts (Mondoulet and others 2005). The frequency of sensitization by Ara h2 for peanut allergic individuals is up to 100% (Burks and others 1992; Clarke and others 1998; De Jong and others 1998; Kleber-Janke and others 1999). Additionally, 10 IgE-binding epitopes were mapped from the primary amino acid sequence of Ara h2, and among them, 3 immunodominant epitopes (amino acids 27 to 36, 57 to 66, and 65 to 74) were identified. Moreover, mutational analysis of Ara h2 epitopes showed that single amino acid changes result in the loss of IgE-binding (Stanley and others 1997).

Ara h3 was identified as the 3rd peanut allergen with molecular weight of 57 kDa. The amino acid sequence of Ara h3 shows homology to 11S seed-storage proteins (Rabjohn and others 1999), which are typically synthesized as 60-kDa preproglobulins (Staswick and others 1981). After cleavage by endopeptidase, the preproglobulins become an NH_2-terminal acidic chain and a COOH-terminal basic chain, which later are linked by a disulfide bridge and then assembled into hexameric oligomers (Barton and others 1982; Ereken-Tumer and others 1982). Even though the recombinant Ara h3 (rAra h3) had the calculated molecular weight of 57 kDa (Rabjohn and others 1999), Ara h3 appeared as a 14-kDa molecule on immunoblots with IgE serum from peanut-hypersensitive individuals (Eigenmann and others 1996). These molecular weight differences are due to the proteolytic cleavage because the isolated fragments of purified Ara h3 had various molecular weights from 14 to 45 kDa, indicating that Ara h3 is posttranslationally processed (Koppelman and others 2003). The rAra h3 was recognized by about 45% of serum IgE from peanut allergic individuals and 4 IgE-binding epitopes were found with no obvious sequence motif shared (Rabjohn and others 1999). Within these peptides, a single amino acid change could reduce IgE binding to about 35% to 85% in comparison to IgE binding to the wild type. Since the modified Ara h3 retained its ability to stimulate T-cell activation, this recombinant allergen may be applied to the mutant allergen immunotherapy (Rabjohn and others 2002).

Ara h4 was cloned and identified with an estimated molecular weight of 61 kDa and an isoelectric point of 5.2. The recombinant form of Ara h4 was recognized by serum IgE from 53% of peanut allergic individuals (Kleber-Janke and others 1999). Both allergens, Ara h3 (Rabjohn and others 1999) and Ara h4 (Kleber-Janke and others 1999), were identified and cloned independently by 2 different research groups around 1999. A homology search indicates 91.3% amino acid identity between Ara h3 and Ara h4, so Ara h3 and Ara h4 are considered to be the same allergens (Koppelman and others 2003; Boldt and others 2005).

Ara h5 was predicted to be a protein of 131 amino acids with a calculated molecular weight of 14 kDa and a calculated pI of 4.6. It is highly homologous to plant profilins. The frequency of sensitization by Ara h5 to 40 peanut sensitized individuals was detected as only 13%. This low sensitization frequency indicated that profilin may not be an abundant protein within peanut seeds (Kleber-Janke and others 1999). Profilin is a highly conserved 12 to 15 kDa actin-binding protein found in all eukaryotic cells (Mills and Shewry 2004) and is a prominent cross-reactive allergen in birch pollen, grass, and wheat (Valenta and others 1992; Kleber-Janke and others 2001). Ara h5 shows 76% amino acid identity to the *Phleum pratense* (grass) profilin and 83% identity to the soybean profilin. Thus, patients allergic to peanuts may react to profilins from birch or grass pollen (Pastorello and others 1997). The coding region of Ara h6 has 375 nucleotides, a predicted protein of 124 amino acids with a calculated molecular weight of 14.5 kDa and an estimated pI of 5.2. The coding region of Ara h7 has 408 nucleotides and is predicted as a protein of 15.8 kDa with pI of 5.6. Both allergens show similarities to amino acid sequences of proteins from the conglutin family of seed storage proteins. Ara h6 and Ara h7 show 59% and 35% amino acid sequence identity to Ara h2, respectively. However, these 2 allergens reveal only 35% amino acid sequence identity to each other. Therefore, they are not isoforms. The frequency of sensitization of Ara h6 and Ara h7 to 40 peanut-sensitized individuals was detected as 38% and 43%, respectively (Kleber-Janke and others 1999). In addition, the homology of Ara h6 to Ara h2 was located in the middle part and at the C-terminal part of Ara h6 (Koppelman and others 2005). A recent study found that Ara h6 could still be detected after 120 min of pepsin digestion and after 40 min of heating in the presence of sugar, butter, and wheat flour (Suhr and others 2004).

Ara h8 is cloned with a 471-bp open-reading frame coding a protein of 157 amino acid residues with a predicted molecular weight of 16.9 kDa and an estimated pI of 5.03. It has IgE cross-reactivity with Bet v1, which is the major birch (*Betula verrucosa*) pollen antigen and the main cause of IgE-mediated allergies observed in early spring (Flicker and others 2000). These 2 allergens share 45.9% identity in their amino acid sequence and show secondary structure similarities by means of far-UV CD spectroscopy. The IgE cross-reactivity between Ara h8 and Bet v1 suggested that birch pollen allergy may be associated with peanut allergy and the primary sensitization to birch pollen through the respiratory tract may be another way for peanut allergy to develop (Mittag and others 2004).

Detection Methods for the Presence of Peanuts

Due to the heavy use of peanut-containing foods, improper labeling, or peanut contamination in raw materials or in production lines, traces of peanuts may exist in foods supposedly free of peanuts. This fact poses a potential risk for peanut allergic individuals (Koppelman and others 1996; Whitaker and others 2005). Therefore, reliable detection methods for peanut allergens are necessary for ensuring the compliance of food labeling and for improving consumer protection. However, the detection of allergens in food can be very difficult, since allergens are often present in trace amounts and can be masked by the food matrix (Poms and others 2004). There is general agreement that the detection limits for allergens in different foods must be between 1 and 100 mg/kg (Koppelman and others 1996). Besides sensitivity, specificity is another requirement for developing an assay for detecting food allergens (Krska and others 2004).

Several analytical techniques have been applied to the detection of hidden peanut proteins/allergens. They target either the peanut proteins or the DNA fragments encoding peanut allergens. Therefore, these techniques can be divided into 2 categories: protein-based methods and DNA-based methods (Koppelman and others 1996). Protein-based methods can detect either total peanut proteins or a specific peanut allergen. The methods in this category include dot immunoblotting (Blais and Phillippe 2000; Schappi and others 2001; Koch and others 2003), rocket immuno-electrophoresis (RIE) (Malmheden Yman and others 1994; Holzhauser and others 1998), enzyme-linked immunosorbent assay (ELISA) (Hefle and others 1994a; Yeung and Collins 1996; Holzhauser and Vieths 1999; Pomes and others

2003, 2004; Stephan and Vieths 2004; Kiening and others 2005), radioallergosorbent assay (RAST) (Yunginger and others 1983), radioimmunoassay (RIA) (Keating and others 1990), dip stick assay (Mills and others 1997; Stephan and others 2002), lateral-flow immunoassay (LFIA) (Wen and others 2005a, 2005b), and mass spectrometry (LC/MS/MS) (Shefcheck and Musser 2004). Among them, dot immunoblotting, dip stick assay, RIE, and LFIA give only qualitative or semiquantitative results, while ELISA, RAST, RIA, and LC/MS/MS are quantitative methods (Poms 2004). The dipstick format uses 1 to 3 successive steps with an overall assay time of 30 min to 3 h; while LFIA only uses 1 step with an assay time of 5 to 10 min (Krska 2004). RIE requires laborious gel preparation and immuno-staining procedures so it is not widely applied in allergen detection (Besler and others 2002). Immunoanalytical methods are mostly chosen according to the specificity and sensitivity of antibodies. They have been used in food industry and research laboratories and government facilities to detect and quantify the presence of allergens in foods (Poms and others 2004). The DNA-based assays for detecting peanuts are based on the amplification of a specific DNA segment by polymerase chain reaction (PCR). Most of the DNA-based assays have used Ara h2 as the target analyte and real time as the assay format (Hird and others 2003; Stephan and Vieths 2004). Table 2 summarizes the noncommercial protein-based and DNA-based assays from peer-reviewed journals.

Most protein-based assays belong to immunoassays and are based on interactions between the antibody and antigen. Thus, selecting a proper source of antibody is the crucial step. Serum IgE from sensitized patients was the 1st source of antibodies (Keating and others 1990). Even though the IgE molecules from peanut allergic patients can specifically characterize peanut allergens, it also has got several problems. For ethical reasons, it could not be applied to any commercial assays, and it has a high degree of variation due to individuality of patients. Therefore, in commercial products and recent studies, polyclonal antibodies (IgG) from rabbit, sheep, or goat, and monoclonal antibodies from mice have

Table 2—Noncommercial assays for the detection of peanuts

Format	Antibody	Antigen	LOD	References
I. Protein-based assays				
Radioallergosorbent assay	Human IgE	Peanut allergens	0.3% to 3.3% peanut butter in sunflower butter	Yunginger and others (1983)
Radioimmuno inhibition assay	Human IgE	Peanut allergens	87.5 mg/kg peanut	Keating and others (1990)
Rocket immuno-electrophoresis	Antisera	Peanut proteins	(1) 30 mg/kg, (2) 10 mg/kg peanut in chocolate	(1) Malmheden Ymab and others (1994), (2) Holzhauser and others (1998)
Dip stick assay	P-Ab	Ara h1	0.01% peanut in marzipan 0.1% peanut in chocolate	Mills and others (1997)
	Antisera	Peanut proteins	10-ppm peanuts in milk chocolate and semisweet chocolate	Stephan and others (2002)
Immunoaffinity column with ELISA		Peanut proteins	0.1-mg/kg peanut protein in chocolate	Newsome and others (1999)
Dot immunoblotting	Human IgE	Peanut allergens	50 mg/kg to 1% peanut in cereal, cookies, and cakes	Schäppi and others 2001
	Egg IgY	Peanut proteins	0.1-mg/kg peanut protein in cookies, chocolate, ice cream	Blais and others (2003)
Competitive ELISA	Antisera	Peanut proteins	(1) 0.4 mg/kg, (2) 2-mg/kg peanut protein in various food products	(1) Yeung and others (1996), (2) Holzhauser and others (1999)
Sandwich ELISA	P-Ab	Partial purified Ara h1	0.1-mg/kg peanut in processed food	Koppelman and others (1996)
	Antisera	Peanut proteins	10-ppm peanut in milk chocolate and semisweet chocolate	Stephan and others (2004)
	M-Ab	Peanut proteins	40 μg/mL peanut proteins in vanilla ice cream	Hefle and others (1994a)
	M-Ab	Ara h1	0.2% peanut in cookie and pancake mix 16.6% peanut in chocolate	Pomes and others (2003)
	M-Ab	Ara h1	0.16 to 0.33% peanut in chocolate	Pomes and others (2004)
	M-Ab with P-Ab	Peanut proteins	0.2 to 0.8-mg/kg peanut in various food products	Kiening and others (2005)
LC/MS/MS		Ara h1	10-mg/kg peanut protein in vanilla ice cream	Shefcheck and others (2004)
Lateral flow assay	P-Ab	Ara h1	0.45 μg/mL Ara h1 in buffer	Wen and others (2005)
	P-Ab	Ara h1	158-mg/kg peanuts in dark chocolate	Wen and others (2005)
II. DNA-based assays				
Real-time PCR		Ara h2	$<$2-mg/kg roasted peanut powder in a biscuit	Hird and others (2003)
		Ara h2	$<$10-mg/kg peanuts in processed food	Stephan and others (2004)

P-Ab = polyclonal antibody; M-Ab: monoclonal antibody.

replaced IgE for the assay development. On the basis of the types of analytes, the protein-based assays for detecting peanuts can be further divided into 2 groups: protein assay and allergen assay (Krska and others 2004). The allergen assays specifically target peanut allergens, while the protein assays detect entire peanut proteins. Thus, the protein assays can detect the more abundant peanut proteins at low levels. Among the protein-based assays, ELISA is the most frequently used technique due to its high precision, easy handling, and good potential for standardization (Poms and others 2004).

DNA-based methods have been applied to detecting the DNA fragments of a specific peanut allergen. Until now, only Ara h2 has been applied in the DNA-based assays for the detection of peanut allergens. Although DNA-based methods are compromised by extreme fragmentation of DNA and the presence of PCR inhibitors in food matrices, it is a promising detection method due to its low limit of detection (Holzhauser and others 2002). Another advantage of this type of method is that the DNA sequence does not change following geographical and seasonal variations, which may change protein composition (Poms and others 2004). Moreover, the target DNA can be efficiently extracted under harsh denaturing conditions and is less affected than proteins by the extraction from food matrices. However, DNA-based assays detect the gene of allergens instead of the allergens, so results cannot be tied to actual allergenic exposure. Other disadvantages of DNA-based assays are the higher cost for materials and equipment and complicated procedures that make them hard to use inside a processing facility.

Many factors can impair the assay sensitivity in detection and especially in quantification of peanut allergens in food products. The 1st factor is the recovery of peanut from highly processed material (Koch and others 2003; Poms and others 2003). A lower recovery of peanut from foods can result in a lower assay sensitivity (and a possible false negative result). The 2nd factor is food matrix interferences, such as tannins in chocolate. The literature has indicated that the determination of peanut in chocolate can be problematic (Mills and others 1997; Hurst and others 2002). The 3rd factor is the processing condition (such as temperature/time profiles for oil or dry roasting of peanuts). Generally, denatured proteins have lower solubility (Poms and others 2002). Finally, differences in antibody affinity/recognition of peanut proteins from different species and geographical origin may also affect the assay sensitivity (Keck-Gassenmeier and others 1999).

The currently available commercial peanut test kits are summarized in Table 3. The formats of these protein-based assays for peanut test kits can be divided into 2 types: ELISA and lateral-flow assay. Moreover, the targets for commercial kits can be either peanut proteins or one of the major peanut allergens (Ara h1 or Ara h2). The limit of detection (LOD) for ELISA kits is normally less than 5 mg/kg (ppm) and that for lateral-flow assay kits is higher than 5 mg/kg (depending on the food matrix). The testing time after sample preparation for ELISA is around 30 min to 2 h and that for lateral-flow assay is about 10 min. Thus, the ELISA assay is more sensitive than the lateral-flow assay, but it takes longer to perform. Compared with ELISA, lateral flow assays are inexpensive, rapid, and portable without the requirement of instruments. However, lateral-flow assays can only provide yes/no results, while ELISA gives semiquantitative or quantitative results (Krska and others 2004). The DNA-based commercial peanut test kits are based on an amplification of a specific DNA fragment by PCR and then the amplicons of the PCR reaction can be detected by electrophoresis in an agarose gel with a fluorescent dye or by Southern blotting. Superior quantification can be achieved by employing real-time PCR (RT-PCR) or DNA-ELISA (Poms and others 2004). RT-PCR requires more expensive laboratory equipment, but it is extremely accurate and less labor-intensive than other DNA quantification methods (Desjardin and others 1998). DNA-ELISA is a method combining the specificity of the PCR

Table 3—Commercial peanut test kits

Test kit	Target	Format	Screening/ quantification	Sensitivity	Testing time	Company	AOAC validation
I. Protein-based methods — enzyme-linked immunosorbent assay							
Alert	Peanut proteins	Sandwich	Screening	5 ppm	30 min	Neogen	No
Veratox	Peanut proteins	Sandwich	Quantification	2.5 ppm	30 min	Neogen	Yes
RIDASCREEN peanut	Peanut proteins	Sandwich	Quantification	2.5 ppm	90 min	R-Biopharm	No
RIDASCREEN FAST peanut	Peanut proteins	Sandwich	Quantification	1.5 ppm	30 min	R-Biopharm	Yes
BioKits peanut	Ara h1	Sandwich	Quantification	<0.1 ppm	75 min	Tepnel	Yes
Peanut residue	Ara h2	Sandwich	Screening	1 ppm	30 min	Elisa System	No
Peanut visual immunoassay		Sandwich	Quantification	0.5 ppm	120 min	Tecra	No
Single-Aller-Gene © Peanut ELISA	Ara h1	NA	NA	<2.5 ppm	NA	Eurofins Scientific	No
Peanut DiagnoKit	Major allergens	Competitive	Quantification	NA	95 min	Abkem Iberia	No
II. Protein-based methods — lateral flow assay							
Reveal	Peanut proteins	Sandwich	Screening	5 ppm	10 min	Neogen	No
BioKits rapid peanut	Ara h1	Sandwich	Screening	5 ppm	10 min	Tepnel	No
III. DNA-based methods							
Single-Aller-Gene © DNA tests Peanut PCR	NA	NA	NA	12 ppm	NA	Eurofins Scientific	No
Multi-Aller-Gene © Multi-Allergen Screening Test Peanut PCR	NA	NA	Screening	6 ppm	NA	Eurofins Scientific	No
Surefood Peanut PCR-ELISA	NA	PCR-ELISA	NA	10 to 50 ppm	4 to 6 h	R-Biopharm	No
Surefood Peanut Real-Time-PCR	NA	Real-Time-PCR	NA	10 to 50 ppm	60 min	R-Biopharm	No

NA = not available.

method with the signal amplification of the ELISA system. On the market, 2 formats of the DNA-based test (DNA-ELISA and RT-PCR) are available with a limit of detection around 10 to 50 ppm. However, the PCR assay is less commonly used in the food industry than ELISA or LFA since it requires expensive equipment and isolated space to operate. Moreover, PCR does not directly prove the presence or absence of peanut allergens. Three peanut ELISA kits (Neogen Veratox for Peanut, R-Biopharm RIDASCREEN FAST Peanut, and Tepnel BioKits for Peanut Assay) have been certified by the AOAC Research Inst. as Performance Tested Methods for the detection of peanut proteins as low as 5 ppm in a variety of foods, including breakfast cereals, cookies, ice cream, and milk chocolate (Park and others 2005). Validation is the process of verifying correctness (Horwitz 2003). Therefore, assays certified as validated methods can provide reliable results without considering interlaboratory differences.

References

Al-Muhsen S, Clarke AE, Kagan RS. 2003. Peanut allergy: an overview. CMAJ 168(10):1279–85.

American Peanut Council. 2005. About the peanut industry. Available from: http://www.peanutsusa.com. Accessed July 7, 2006.

Anonymous 1998. The diagnosis and management of anaphylaxis. Joint Task Force on Practice Parameters, American Academy of Allergy, Asthma and Immunology, American College of Allergy, Asthma and Immunology, and the Joint Council of Allergy, Asthma and Immunology. J Allergy Clin Immunol 101(6):S465–528.

Bannon GA, Besler M, Hefle SL, Hourihane JOB, Sicherer SH. 2000. Peanut (*Arachis hypogaea*). Internet Symp Food Allergens 2(2):87–122.

Barre A, Borges JP, Culerrier R, Rouge P. 2005. Homology modelling of the major peanut allergen Ara h 2 and surface mapping of IgE-binding epitopes. Immunol Lett 100(2):153–8.

Barton KA, Thompson JF, Madison JT, Rosenthal R, Jarvis NP, Beachy RN. 1982. The biosynthesis and processing of high-molecular-weight precursors of soybean glycinin subunits. J Biol Chem 257(11):6089–95.

Benjamini E, Leskowitz S. 1991. Immunology: a short course. New York: Wiley-Liss.

Besler M, Kasel U, Wichmann G. 2002. Determination of hidden allergens in foods by immunoassays. Internet Symp Food Allergens 4:1–18.

Bewley JD, Black M. 1994. Seeds: physiology of development and germination. New York: Plenum Press.

Beyer K, Morrow E, Li XM, Bardina L, Bannon GA, Burks AW, Sampson HA. 2001. Effects of cooking methods on peanut allergenicity. J Allergy Clin Immunol 107(6):1077–81.

Bindslev-Jensen C, Skov PS, Madsen F, Poulsen LK. 1994. Food allergy and food intolerance—what is the difference? Ann Allergy 72(4):317–20.

Blais BW, Phillippe LM. 2000. A cloth-based enzyme immunoassay for detection of peanut proteins in foods. Food Agric Immunol 12:243–8.

Blais BW, Gaudreault M, Phillippe LM. 2003. Multiplex enzyme immunoassay system for the simultaneous detection of multiple allergens in foods. Food Control 14:43–7.

Bock SA, Atkins FM. 1989. The natural history of peanut allergy. J Allergy Clin Immunol 83(5):900–4.

Bock SA, Lee WY, Remigio L, Holst A, May CD. 1978. Appraisal of skin tests with food extracts for diagnosis of food hypersensitivity. Clin Allergy 8(6):559–64.

Bock SA, Sampson HA, Atkins FM, Zeiger RS, Lehrer S, Sachs M, Bush RK, Metcalfe DD. 1988. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. J Allergy Clin Immunol 82(6):986–97.

Bock SA, Munoz-Furlong A, Sampson HA. 2001. Fatalities due to anaphylactic reactions to foods. J Allergy Clin Immunol 107(1):191–3.

Bohle B. 2004. T lymphocytes and food allergy. Mol Nutr Food Res 48(6):424–33.

Boldt A, Fortunato D, Conti A, Petersen A, Ballmer-Weber B, Lepp U, Reese G, Becker WM. 2005. Analysis of the composition of an immunoglobulin E-reactive high-molecular-weight protein complex of peanut extract containing Ara h 1 and Ara h 3/4. Proteomics 5(3):675–86.

Boutrif E. 1995. FAO programmes for prevention, regulation, and control of mycotoxins in food. Nat Toxins 3(4):322–6.

Breiteneder H, Ebner C. 2000. Molecular and biochemical classification of plant-derived food allergens. J Allergy Clin Immunol 106(1):27–36.

Bruijnzeel-Koomen C, Ortolani C, Aas K, Bindslev-Jensen C, Bjorksten B, Moneret-Vautrin D, Wuthrich B. 1995. Adverse reactions to food. European Academy of Allergology and Clinical Immunology Subcommittee. Allergy 50(8):623–5.

Burks AW, Williams LW, Helm RM, Connaughton C, Cockrell G, O'Brien T. 1991. Identification of a major peanut allergen, Ara h I, in patients with atopic dermatitis and positive peanut challenges. J Allergy Clin Immunol 88(2):172–9.

Burks AW, Williams LW, Connaughton C, Cockrell G, O'Brien TJ, Helm RM. 1992. Identification and characterization of a second major peanut allergen, Ara h II, with use of the sera of patients with atopic dermatitis and positive peanut challenge. J Allergy Clin Immunol 90(6):962–9.

Burks AW, Cockrell G, Stanley JS, Helm RM, Bannon GA. 1995. Recombinant peanut allergen Ara h I expression and IgE binding in patients with peanut hypersensitivity. J Clin Invest 96(4):1715–21.

Burks AW, Shin D, Cockrell G, Stanley JS, Helm RM, Bannon GA. 1997. Mapping and mutational analysis of the IgE-binding epitopes on Ara h 1, a legume vicilin protein and a major allergen in peanut hypersensitivity. Eur J Biochem 245(2):334–9.

Burks W. 2003. Peanut allergy: a growing phenomenon. J Clin Invest 111(7):950–2.

Burks W, Lehrer SB, Bannon GA. 2004. New approaches for treatment of peanut allergy: chances for a cure. Clin Rev Allergy Immunol 27(3):191–6.

Bush RK, Taylor SL, Nordlee JA. 1989. Peanut sensitivity. Allergy Proc 10(4):261–4.

Chu RS, Targoni OS, Krieg AM, Lehmann PV, Harding CV. 1997. CpG oligodeoxynucleotides act as adjuvants that switch on T helper 1 (Th1) immunity. J Exp Med 186(10):1623–31.

Chung SY, Champagne ET. 2001. Association of end-product adducts with increased IgE binding of roasted peanuts. J Agric Food Chem 49(8):3911–6.

Clarke MC, Kilburn SA, Hourihane JO, Dean KR, Warner JO, Dean TP. 1998. Serological characteristics of peanut allergy. Clin Exp Allergy 28(10):1251–7.

De Jong EC, Van Zijverden M, Spanhaak S, Koppelman SJ, Pellegrom H, Penninks AH. 1998. Identification and partial characterization of multiple major allergens in peanut proteins. Clin Exp Allergy 28(6):743–51.

de Leon MP, Glaspole IN, Drew AC, Rolland JM, O'Hehir RE, Suphioglu C. 2003. Immunological analysis of allergenic cross-reactivity between peanut and tree nuts. Clin Exp Allergy 33(9):1273–80.

De Vries JE, Gauchat JF, Aversa GG, Punnonen J, Gascan H, Yssel H. 1991. Regulation of IgE synthesis by cytokines. Curr Opin Immunol 3(6):851–8.

Desjardin LE, Chen Y, Perkins MD, Teixeira L, Cave MD, Eisenach KD. 1998. Comparison of the ABI 7700 system (TaqMan) and competitive PCR for quantification of IS6110 DNA in sputum during treatment of tuberculosis. J Clin Microbiol 36(7):1964–8.

Eigenmann PA, Burks AW, Bannon GA, Sampson HA. 1996. Identification of unique peanut and soy allergens in sera adsorbed with cross-reacting antibodies. J Allergy Clin Immunol 98(5):969–78.

Ellis AK, Day JH. 2003. Diagnosis and management of anaphylaxis. CMAJ 169(4):307–11.

Emmett SE, Angus FJ, Fry JS, Lee PN. 1999. Perceived prevalence of peanut allergy in Great Britain and its association with other atopic conditions and with peanut allergy in other household members. Allergy 54(4):380–5.

Ereken-Tumer N, Richter JD, Nielsen NC. 1982. Structural characterization of the glycinin precursors. J Biol Chem 257(8):4016–8.

Eriksson NE, Moller C, Werner S, Magnusson J, Bengtsson U. 2003. The hazards of kissing when you are food allergic. A survey on the occurrence of kiss-induced allergic reactions among 1139 patients with self-reported food hypersensitivity. J Investig Allergol Clin Immunol 13(3):149–54.

Ferguson A. 1992. Definitions and diagnosis of food intolerance and food allergy: consensus and controversy. J Pediatr 121(5):S7–11.

Fleischer DM, Conover-Walker MK, Christie L, Burks AW, Wood RA. 2003. The natural progression of peanut allergy: resolution and the possibility of recurrence. J Allergy Clin Immunol 112(1):183–9.

Flicker S, Laffer S, Steinberger P, Alhani B, Zhu Y, Laukkanen ML, Keinanen K, Kraft D, Valenta R. 2000. Engineering, purification and applications of His-tagged recombinant antibody fragments with specificity for the major birch pollen allergen, bet v1. Biol Chem 381(1):39–47.

Frank L, Marian A, Visser M, Weinberg E, Potter PC. 1999. Exposure to peanuts in utero and in infancy and the development of sensitization to peanut allergens in young children. Pediatr Allergy Immunol 10(1):27–32.

Fu TJ, Abbott UR, Hatzos C. 2002. Digestibility of food allergens and nonallergenic proteins in stimulated gastric fluid and stimulated intestinal fluid: a comparative study. J Agric Food Chem 50(24):7154–60.

Furlong TJ, Desimone J, Sicherer SH. 2001. Peanut and tree nut allergic reactions in restaurants and other food establishments. J Allergy Clin Immunol 108(5):867–70.

Genasi F. 2001. Anaphylaxis management. Prof Nurse 16(9):1214–5.

Goldman M. 1998. Peanut allergy: how much peanut is too much? Baltimore, Md.: Asthma and Allergy Foundation of America, Maryland-Greater Washington, D.C., Chapter Newsletter, April-May.

Goodwin PR. 2004. Food allergen detection methods: a coordinated approach. J AOAC Int 87(6):1383–90.

Gruber P, Becker WM, Hofmann T. 2005. Influence of the Maillard reaction on the allergenicity of rAra h 2, a recombinant major allergen from peanut (*Arachis hypogaea*), its major epitopes, and peanut agglutinin. J Agric Food Chem 53(6):2289–96.

Grundy J, Matthews S, Bateman B, Dean T, Arshad SH. 2002. Rising prevalence of allergy to peanut in children: data from 2 sequential cohorts. J Allergy Clin Immunol 110(5):784–9.

Hallett R, Haapanen LA, Teuber SS. 2002. Food allergies and kissing. N Engl J Med 346(23):1833–4.

Hamilos DL, Oppenheimer JJ, Nelson HS, Wenzel S, Driscoll S, Lockey RF, Golden DB, Fan L, Sanks RJ, Bock SA. 1993. Suggested approaches for research protocols involving the potential for life-threatening reactions. J Allergy Clin Immunol 91(6):1101–20.

Harlow E, Lane D. 1999. Using antibodies: a laboratory manual. New York: Cold Spring Harbor Laboratory Press.

Hefle SL, Bush RK, Yunginger JW, Chu FS. 1994a. A snadwich enzyme-linked immunosorbent assay (ELISA) for the quantification of selected peanut proteins in foods. J Food Prot 57(5):419–23.

Hefle SL, Lemanske RF, Bush RK. 1994b. Adverse reaction to lupine-fortified pasta. J Allergy Clin Immunol 94(2):167–72.

Hefle SL, Nordlee JA, Taylor SL. 1996. Allergenic foods. Crit Rev Food Sci Nutr 36:S69–89.

Helm RS. 2001. Allergy to plant seed proteins. J New Seeds 3(3):37–60.

Hird H, Lloyd J, Goodier R, Brown J, Reece P. 2003. Detection of peanut using real-time polymerase chain reaction. Eur Food Res Technol 217:265–8.

Holzhauser T, Vieths S. 1999. Indirect competitive ELISA for determination of traces of peanut (*Arachis hypogaea* L.) protein in complex food matrices. J Agric Food Chem 47(2):603–11.

Holzhauser T, Dehne LI, Hoffmann A, Haustein D, Vieths S. 1998. Rocket immunoelectrophoresis (RIE) for determination of potentially allergenic peanut proteins in processed foods as a simple means for quality assurance and food safety. Eur Food Res Technol 206(1):1–8.

Holzhauser T, Stephan O, Vieths S. 2002. Detection of potentially allergenic hazelnut (*Corylus avellana*) residues in food: a comparative study with DNA PCR-ELISA and protein sandwich-ELISA. J Agric Food Chem 50(21):5808–15.

Hong SJ, Michael JG, Fehringer A, Leung DY. 1999. Pepsin-digested peanut contains T-cell epitopes but no IgE epitopes. J Allergy Clin Immunol 104(2):473–8.

Horwitz W. 2003. Validation: an invisible component of measurement. AOAC Intl.. Available from: http://www.aoac.org/dietsupp6/Dietary-Supplement-web-site/HorwitzValid.pdf. Accessed July 5, 2006.
Hourihane JO. 2002. Recent advances in peanut allergy. Curr Opin Allergy Clin Immunol 2(3):227–31.
Hourihane JO, Dean TP, Warner JO.1996. Peanut allergy in relation to heredity, maternal diet, and other atopic diseases: results of a questionnaire survey, skin prick testing, and food challenges. BMJ 313(7056):518–21.
Hourihane JO, Kilburn SA, Dean P, Warner JO. 1997. Clinical characteristics of peanut allergy. Clin Exp Allergy 27(6):634–9.
Hurst WJ, Krout ER, Burks WR. 2002. A comparison of commercially available peanut ELISA test kits on the analysis of samples of dark and milk chocolate. J Immunoassay Immunochem 23(4):451–9.
Jansen SC, Van Dusseldorp M, Bottema KC, Dubois AE. 2003. Intolerance to dietary biogenic amines: a review. Ann Allergy Asthma Immunol 91(3):233–40.
Kanny G, Moneret-Vautrin DA, Flabbee J, Beaudouin E, Morisset M, Thevenin F. 2001. Population study of food allergy in France. J Allergy Clin Immunol 108(1):133–40.
Kay AB. 2001. Allergy and allergic diseases. First of two parts. N Engl J Med 344(1): 30–7.
Keating MU, Jones RT, Worley NJ, Shively CA, Yunginger JW. 1990. Immunoassay of peanut allergens in food-processing materials and finished foods. J Allergy Clin Immunol 86(1): 41–4.
Keck-Gassenmeier B, Benet S, Rosa C, Hischenhuber C. 1999. Determination of peanut traces in food by a commercially-available ELISA test. Food Agric Immuno 11(3):243–50.
Kemp SF, Lockey RF. 1996. Peanut anaphylaxis from food cross-contamination (letter). JAMA 275(21):1636–7.
Kiening M, Niessner R, Drs E, Baumgartner S, Krska R, Bremer M, Tomkies V, Reece P, Danks C, Immer U, Weller MG. 2005. Sandwich immunoassays for the determination of peanut and hazelnut traces in foods. J Agric Food Chem 53(9):3321–7.
King B. 2000. Nut allergy and acute anaphylaxis management. Nurs Times 96(42):41–3.
Kitts D, Yuan Y, Joneja J, Scott F, Szilagyi A, Amiot J, Zarkadas M. 1997. Adverse reactions to food constituents: allergy, intolerance, and autoimmunity. Can J Physiol Pharmacol 75(4):241–54.
Kleber-Janke T, Crameri R, Appenzeller U, Schlaak M, Becker WM. 1999. Selective cloning of peanut allergens, including profilin and 2S albumins, by phage display technology. Int Arch Allergy Immunol 119(4):265–74.
Kleber-Janke T, Crameri R, Scheurer S, Vieths S, Becker WM. 2001. Patient-tailored cloning of allergens by phage display: peanut (*Arachis hypogaea*) profilin, a food allergen derived from a rare mRNA. J Chromatogr B Biomed Sci Appl 756(1–2):295–305.
Klemola T, Kalimo K, Poussa T, Juntunen-Backman K, Korpela R, Valovirta E, Vanto T. 2005. Feeding a soy formula to children with cow's milk allergy: the development of immunoglobulin E-mediated allergy to soy and peanuts. Pediatr Allergy Immunol 16(8):641–6.
Koch P, Schappi GF, Poms RE, Wuthrich B, Anklam E, Battaglia R. 2003. Comparison of commercially available ELISA kits with human sera-based detection methods for peanut allergens in foods. Food Addit Contam 20(9):797–803.
Koppelman SJ, Bleeker-Marcelis H, Duijn G, Hessing M. 1996. Detecting peanut allergens. The development of an immunochemical assay for peanut proteins. World of Ingredients 12:35–8.
Koppelman SJ, Bruijnzeel-Koomen CA, Hessing M, De Jongh HH. 1999. Heat-induced conformational changes of Ara h 1, a major peanut allergen, do not affect its allergenic properties. J Biol Chem 274(8):4770–7.
Koppelman SJ, Vlooswijk RA, Knippels LM, Hessing M, Knol EF, Van Reijsen FC, Bruijnzeel-Koomen CA. 2001. Quantification of major peanut allergens Ara h1 and Ara h2 in the peanut varieties Runner, Spanish, Virginia, and Valencia, bred in different parts of the world. Allergy 56(2):132–7.
Koppelman SJ, Knol EF, Vlooswijk RA, Wensing M, Knulst AC, Hefle SL, Gruppen H, Piersma S. 2003. Peanut allergen Ara h3: isolation from peanuts and biochemical characterization. Allergy 58(11):1144–51.
Koppelman SJ, De Jong GA, Laaper-Ertmann M, Peeters KA, Knulst AC, Hefle SL, Knol EF. 2005. Purification and immunoglobulin E-binding properties of peanut allergen Ara h6: evidence for cross-reactivity with Ara h2. Clin Exp Allergy 35(4):490–7.
Krska R, Welzig E, Baumgartner S. 2004. Immunoanalytical detection of allergenic proteins in food. Anal Bioanal Chem 378(1):63–5.
Lehrer SB, Ayuso R, Reese G. 2002. Current understanding of food allergens. Ann NY Acad Sci 964:69–85.
Lehrer SB, Horner WE, Reese G. 1996. Why are some proteins allergenic? Implications for biotechnology. Crit Rev Food Sci Nutr 36(6):553–64.
Lepp U, Zabel P, Schocker F. 2002. Playing cards as a carrier for peanut allergens. Allergy 57(9):864.
Leung DY, Sampson HA, Yunginger JW, Burks AW Jr, Schneider LC, Wortel CH, Davis FM, Hyun JD, Shanahan WRJr. 2003. Effect of anti-IgE therapy in patients with peanut allergy. N Engl J Med 348(11):986–93.
Lever LR. 1996. Peanut and nut allergy. Creams and ointments containing peanut oil may lead to sensitisation. BMJ 313(7052):299–300.
Li XM. 2005. Beyond allergen avoidance: update on developing therapies for peanut allergy. Curr Opin Allergy Clin Immunol 5(3):287–92.
Li XM, Zhang TF, Huang CK, Srivastava K, Teper AA, Zhang L, Schofield BH, Sampson HA. 2001. Food Allergy Herbal Formula-1 (FAHF-1) blocks peanut-induced anaphylaxis in a murine model. J Allergy Clin Immunol 108(4):639–46.
Li XM, Srivastava K, Huleatt JW, Bottomly K, Burks AW, Sampson HA. 2003. Engineered recombinant peanut protein and heat-killed *Listeria monocytogenes* coadministration protects against peanut-induced anaphylaxis in a murine model. J Immunol 170(6): 3289–95.
Madsen C. 2001. Where are we in risk assessment of food allergens? The regulatory view. Allergy 56:91–3.
Maleki SJ. 2004. Food processing: effects on allergenicity. Curr Opin Allergy Clin Immunol 4(3):241–5.
Maleki SJ, Chung SY, Champagne ET, Raufman JP. 2000a. The effects of roasting on the allergenic properties of peanut proteins. J Allergy Clin Immunol 106(4):763–8.
Maleki SJ, Kopper RA, Shin DS, Park CW, Compadre CM, Sampson H, Burks AW, Bannon GA. 2000b. Structure of the major peanut allergen Ara h1 may protect IgE-binding epitopes from degradation. J Immunol 164(11):5844–9.
Maleki SJ, Viquez O, Jacks T, Dodo H, Champagne ET, Chung SY, Landry SJ. 2003. The major peanut allergen, Ara h2, functions as a trypsin inhibitor, and roasting enhances this function. J Allergy Clin Immunol 112(1):190–5.
Malmheden Yman I, Eriksson A, Everitt G, Yman L, Karlsson T. 1994. Analysis of food proteins for verification of contamination or mislabeling. Food Agric Immunol 6:167–72.
May CD. 1976. Objective clinical and laboratory studies of immediate hypersensitivity reactions to foods in asthmatic children. J Allergy Clin Immunol 58(4):500–15.
McKenna C, Klontz KC. 1997. Systemic allergic reaction following ingestion of undeclared peanut flour in a peanut-sensitive woman. Ann Allergy Asthma Immunol 79(3):234–6.
McNeish AS. 1984. Enzymatic maturation of the gastrointestinal tract and its relevance to food allergy and intolerance in infancy. Ann Allergy 53(6):643–8.
Merz B. 2003. Studying peanut anaphylaxis. N Engl J Med 348(11):975–6.
Mills ENC, Shewry PR. 2004. Plant food allergens. Oxford, U.K.: Blackwell Science Ltd.
Mills ENC, Potts A, Plumb GW, Lambert N, Morgan MRA. 1997. Development of a rapid dipstick immunoassay for the detection of peanut contamination of food. Food Agric Immunol 9:37–50.
Mills ENC, Jenkins J, Marigheto N, Belton PS, Gunning AP, Morris VJ. 2002. Allergens of the cupin superfamily. Biochem Soc Trans 30(6):925–9.
Mittag D, Akkerdaas J, Ballmer-Weber BK, Vogel L, Wensing M, Becker WM, Koppelman SJ, Knulst AC, Helbling A, Hefle SL, Van Ree R, Vieths S. 2004. Ara h8, a Bet v 1-homologous allergen from peanut, is a major allergen in patients with combined birch pollen and peanut allergy. J Allergy Clin Immunol 114(6):1410–7.
Mondoulet L, Paty E, Drumare MF, Ah-Leung S, Scheinmann P, Willemot RM, Wal JM, Bernard H. 2005. Influence of thermal processing on the allergenicity of peanut proteins. J Agric Food Chem 53(11):4547–53.
Moneret-Vautrin DA, Hatahet R, Kanny G.1994. Risks of milk formulas containing peanut oil contaminated with peanut allergens in infants with atopic dermatitis. Pediatr Allergy Immunol 5(3):184–8.
Moneret-Vautrin DA, Rance F, Kanny G, Olsewski A, Gueant JL, Dutau G, Guerin L. 1998. Food allergy to peanuts in France—evaluation of 142 observations. Clin Exp Allergy 28(9):1113–9.
Newsome WH, Abbot M. 1999. An immunoaffinity column for the determination of peanut protein in chocolate. J AOAC Int 82(3):666–8.
Nowak-Wegrzyn A, Sampson HA. 2004. Food allergy therapy. Immunol Allergy Clin North Am 24(4):705–25.
Park DL, Coates S, Brewer VA, Garber EA, Abouzied M, Johnson K, Ritter B, McKenzie D. 2005. Performance tested method multiple laboratory validation study of ELISA-based assays for the detection of peanuts in food. J AOAC Int 88(1):156–60.
Pastorello EA, Incorvaia C, Pravettoni V, Ortolani C. 1997. Crossreactions in food allergy. Clin Rev Allergy Immunol 15(4):415–27.
Peanut Inst. 2005. Available from: www.peanut-institute.org/PeanutFAQs.html. Accessed July 5, 2006.
Pomes A, Helm RM, Bannon GA, Burks AW, Tsay A, Chapman MD. 2003. Monitoring peanut allergen in food products by measuring Ara h1. J Allergy Clin Immunol 111(3):640–5.
Pomes A, Vinton R, Chapman MD. 2004. Peanut allergen (Ara h1) detection in foods containing chocolate. J Food Prot 67(4):793–8.
Poms RE, Capelletti C, Anklam E. 2002. Effect of roasting history and buffer composition on peanut protein extraction efficiency. Mol Nutr Food Res 48:459–64.
Poms RE, Lisi C, Summa C, Stroka J, Anklam E. 2003. In-house validation of commercially avaiable ELISA kits for peanut allergents in food. Report from the Inst. for Reference Material and Measurements. Joint Research Center, European Commission, EUR 20767 EN.
Poms RE, Klein CL, Anklam E. 2004. Methods for allergen analysis in food: a review. Food Addit Contam 21(1):1–31.
Poms RE, Agazzi ME, Bau A, Brohee M, Capelletti C, Norgaard JV, Anklam E. 2005. Interlaboratory validation study of five commercial ELISA test kits for the determination of peanut proteins in biscuits and dark chocolate. Food Addit Contam 22(2):104–12.
Rabjohn P, Helm EM, Stanley JS, West CM, Sampson HA, Burks AW, Bannon GA. 1999. Molecular cloning and epitope analysis of the peanut allergen Ara h3. J Clin Invest 103(4):535–42.
Rabjohn P, West CM, Connaughton C, Sampson HA, Helm RM, Burks AW, Bannon GA. 2002. Modification of peanut allergen Ara h3: effects on IgE binding and T cell stimulation. Int Arch Allergy Immunol 128(1):15–23.
Romagnani S. 1991. Human TH1 and TH2 subsets: doubt no more. Immunol Today 12(8):256–7.
Roy K, Mao HQ, Huang SK, Leong KW. 1999. Oral gene delivery with chitosan-DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. Nat Med 5(4):387–91.
Saavedra-Delgado AM. 1989. The many faces of the peanut. Allergy Proc 10(4):291–4.
Sampson HA. 1988a. Immunologically mediated food allergy: the importance of food challenge procedures. Ann Allergy 60(3):262–9.
Sampson HA. 1988b. Comparative study of commercial food antigen extracts for the diagnosis of food hypersensitivity. J Allergy Clin Immunol 82(5):718–26.
Sampson HA. 1996. Managing peanut allergy. BMJ 312(7038):1050–1.
Sampson HA. 1999a. Food allergy. Part 1: immunopathogenesis and clinical disorders. J Allergy Clin Immunol 103(5):717–28.
Sampson HA. 1999b. Food allergy. Part 2: diagnosis and management. J Allergy Clin Immunol 103(6):981–9.
Sampson HA. 2000. What should we be doing for children with peanut allergy? J Pediatr 137(6):741–3.
Sampson HA. 2001a. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. J Allergy Clin Immunol 107(5):891–6.
Sampson HA. 2001b. Immunological approaches to the treatment of food allergy. Pediatr Allergy Immunol 12(14):91–6.
Sampson HA. 2002. Clinical practice. Peanut allergy. N Engl J Med 346(17):1294–9.
Sampson HA. 2003. Anaphylaxis and emergency treatment. Pediatrics 111(6):1601–8.
Sampson HA. 2004. Update on food allergy. J Allergy Clin Immunol 113(5):805–19.

Sampson HA, Albergo R. 1984. Comparison of results of skin tests, RAST, and double-blind, placebo-controlled food challenges in children with atopic dermatitis. J Allergy Clin Immunol 74(1):26–33.
Sampson HA, Ho DG. 1997. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. J Allergy Clin Immunol 100(4):444–51.
Sampson HA, Mendelson L, Rosen JP. 1992. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. N Engl J Med 327(6):380–4.
Sampson HA, Srivastava K, Li XM, Burks AW. 2003. New perspectives for the treatment of food allergy (peanut). Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankfurt/Main (94):236–44.
Schappi GF, Konrad V, Imhof D, Etter R, Wuthrich B. 2001. Hidden peanut allergens detected in various foods: findings and legal measures. Allergy 56(12):1216–20.
Scurlock AM, Burks AW. 2004. Peanut allergenicity. Ann Allergy Asthma Immunol 93(5): S12–8.
Sen M, Kopper R, Pons L, Abraham EC, Burks AW, Bannon GA. 2002. Protein structure plays a critical role in peanut allergen stability and may determine immunodominant IgE-binding epitopes. J Immunol 169(2):882–7.
Shefcheck KJ, Musser SM. 2004. Confirmation of the allergenic peanut protein, Ara h1, in a model food matrix using liquid chromatography/tandem mass spectrometry (LC/MS/MS). J Agric Food Chem 52(10):2785–90.
Shin DS, Compadre CM, Maleki SJ, Kopper RA, Sampson H, Huang SK, Burks AW, Bannon GA. 1998. Biochemical and structural analysis of the IgE binding sites on ara h1, an abundant and highly allergenic peanut protein. J Biol Chem 273(22):13753–9.
Sicherer SH. 1999. Food allergy: when and how to perform oral food challenges. Pediatr Allergy Immunol 10(4):226–34.
Sicherer SH. 2002. Clinical update on peanut allergy. Ann Allergy Asthma Immunol 88(4):350–61.
Sicherer SH, Sampson HA. 2000. Peanut and tree nut allergy. Curr Opin Pediatr 12(6):567–73.
Sicherer SH, Sampson HA. 2006. 9. Food allergy. J Allergy Clin Immunol 117(2 Suppl): S470–5.
Sicherer SH, Burks AW, Sampson HA. 1998. Clinical features of acute allergic reactions to peanut and tree nuts in children. Pediatrics 102(1):e6.
Sicherer SH, Munoz-Furlong A, Burks AW, Sampson HA. 1999. Prevalence of peanut and tree nut allergy in the US determined by a random digit dial telephone survey. J Allergy Clin Immunol 103(4):559–62.
Sicherer SH, Morrow EH, Sampson HA. 2000. Dose-response in double-blind, placebo-controlled oral food challenges in children with atopic dermatitis. J Allergy Clin Immunol 105(3):582–6.
Sicherer SH, Furlong TJ, Desimone J, Sampson HA. 2001a. The US Peanut and Tree Nut Allergy Registry: characteristics of reactions in schools and day care. J Pediatr 138(4): 560–5.
Sicherer SH, Furlong TJ, Munoz-Furlong A, Burks AW, Sampson HA. 2001b. A voluntary registry for peanut and tree nut allergy: characteristics of the first 5149 registrants. J Allergy Clin Immunol 108(1):128–32.
Sicherer SH, Munoz-Furlong A, Sampson HA. 2003. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey: a 5-year follow-up study. J Allergy Clin Immunol 112(6):1203–7.
Sicherer SH, Munoz-Furlong A, Sampson HA. 2004. Prevalence of seafood allergy in the United States determined by a random telephone survey. J Allergy Clin Immunol 114(1):159–65.
Skolnick HS, Conover-Walker MK, Koerner CB, Sampson HA, Burks W, Wood RA. 2001. The natural history of peanut allergy. J Allergy Clin Immunol 107(2):367–74.
Spergel JM, Fiedler JM. 2001. Natural history of peanut allergy. Curr Opin Pediatr 13(6):517–22.
Spiegelberg HL, Orozco EM, Roman M, Raz E. 1997. DNA immunization: a novel approach to allergen-specific immunotherapy. Allergy 52(10):964–70.
Srivastava KD, Kattan JD, Zou ZM, Li JH, Zhang L, Wallenstein S, Goldfarb J, Sampson HA, Li XM. 2005. The Chinese herbal medicine formula FAHF-2 completely blocks anaphylactic reactions in a murine model of peanut allergy. J Allergy Clin Immunol 115(1):171–8.
Stanley JS, King N, Burks AW, Huang SK, Sampson H, Cockrell G, Helm RM, West CM, Bannon GA. 1997. Identification and mutational analysis of the immunodominant IgE binding epitopes of the major peanut allergen Ara h2. Arch Biochem Biophys 342(2):244–53.
Stark BJ, Sullivan TJ. 1986. Biphasic and protracted anaphylaxis. J Allergy Clin Immunol 78(1):76–83.
Staswick PE, Hermodson MA, Nielsen NC. 1981. Identification of the acidic and basic subunit complexes of glycinin. J Biol Chem 256(16):8752–5.
Stephan O, Vieths S. 2004. Development of a real-time PCR and a sandwich ELISA for detection of potentially allergenic trace amounts of peanut (*Arachis hypogaea*) in processed foods. J Agric Food Chem 52(12):3754–60.
Stephan O, Möller N, Lehmann S, Holzhauser T, Vieths S. 2002. Development and validation of two dipstick type immunoassays for determination of trace amounts of peanut and hazelnut in processed foods. Eur Food Res Technol 215(5):431–6.
Suhr M, Wicklein D, Lepp U, Becker WM. 2004. Isolation and characterization of natural Ara h6: Evidence for a further peanut allergen with putative clinical relevance based on resistance to pepsin digestion and heat. Mol Nutr Food Res 48(5):390–9.
Swagerty DL Jr, Walling AD, Klein RM. 2002. Lactose intolerance. Am Fam Physician 65(9):1845–50.
Tariq SM, Stevens M, Matthews S, Ridout S, Twiselton R, Hide DW. 1996. Cohort study of peanut and tree nut sensitisation by age of 4 years. BMJ 313(7056):514–7.
Taylor SL, Lehrer SB. 1996. Principles and characteristics of food allergens. Crit Rev Food Sci Nutr (36):S91–118.
Taylor SL, Busse WW, Sachs MI, Parker JL, Yunginger JW. 1981. Peanut oil is not allergenic to peanut-sensitive individuals. J Allergy Clin Immunol 68(5):372–5.
Taylor SL, Hefle SL, Bindslev-Jensen C, Bock SA, Burks AW Jr., Christie L, Hill DJ, Host A, Hourihane JO, Lack G, Metcalfe DD, Moneret-Vautrin DA, Vadas PA, Rance F, Skrypec DJ, Trautman TA, Yman IM, Zeiger RS. 2002. Factors affecting the determination of threshold doses for allergenic foods: how much is too much? J Allergy Clin Immunol 109(1): 24–30.
[USFDA] U.S. Food and Drug Administration: The Threshold Working Group. 2005. Approaches to establish thresholds for major food allergens and for gluten in food. Available from: http://www.cfsan.fda.gov/~dms/alrgn.html. Accessed July 5, 2006.
Vadas P, Wai Y, Burks W, Perelman B. 2001. Detection of peanut allergens in breast milk of lactating women. JAMA 285(13):1746–8.
Valenta R, Duchene M, Ebner C, Valent P, Sillaber C, Deviller P, Ferreira F, Tejkl M, Edelmann H, Kraft D. 1992. Profilins constitute a novel family of functional plant pan-allergens. J Exp Med 175(2):377–85.
Wen HW, Borejsza-Wysocki W, Decory RT, Baeumner A, Durst RA. 2005a. A novel extraction method for peanut allergenic proteins in chocolate and their detection by a liposome-based lateral flow assay. Eur Food Res Technol 221(3–4):564–9
Wen HW, Borejsza-Wysocki W, Decory RT, Durst RA. 2005b. Development of a competitive liposome-based lateral flow assay for the rapid detection of the allergenic peanut protein Ara h1. Anal Bioanal Chem 382(5):1217–26.
Wensing M, Penninks AH, Hefle SL, Koppelman SJ, Bruijnzeel-Koomen CA, Knulst AC. 2002. The distribution of individual threshold doses eliciting allergic reactions in a population with peanut allergy. J Allergy Clin Immunol 110(6):915–20.
Whitaker TB, Williams KM, Trucksess MW, Slate AB. 2005. Immunochemical analytical methods for the determination of peanut proteins in foods. J AOAC Int 88(1):161–74.
Wood RA. 2003. The natural history of food allergy. Pediatrics 111(6 Pt 3):1631–7.
Wraith DC, Smilek DE, Mitchell DJ, Steinman L, Mcdevitt HO. 1989. Antigen recognition in autoimmune encephalomyelitis and the potential for peptide-mediated immunotherapy. Cell 59(2):247–55.
Wuthrich B, Dascher M, Borelli S. 2001. Kiss-induced allergy to peanut. Allergy 56(9):913.
Yeung JM, Collins PG. 1996. Enzyme immunoassay for determination of peanut proteins in food products. J AOAC Int 79(6):1411–6.
Yu J, Mohawed SM, Bhatnagar D, Cleveland TE. 2003. Substrate-induced lipase gene expression and aflatoxin production in *Aspergillus parasiticus* and *Aspergillus flavus*. J Appl Microbiol 95(6):1334–42.
Yunginger JW, Gauerke MB, Jones RT, Dahlberg MJE, Ackerman SJ. 1983. Use of radioimmunoassay to determine the nature, quantity and source of allergic contamination of sunflower butter. J Food Prot 46:625–8.
Zeiger RS. 2003. Food allergen avoidance in the prevention of food allergy in infants and children. Pediatrics 111(6):1662–71.
Zimmerman B, Forsyth S, Gold M.1989. Highly atopic children: formation of IgE antibody to food protein, especially peanut. J Allergy Clin Immunol 83(4):764–70.