EFFECT OF CLEANING PROTOCOLS ON THE REMOVAL OF MILK, EGG, AND PEANUT ALLERGENS FROM ABRADED AND UNABRADED STAINLESS STEEL SURFACES

By

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To my loving family

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Food allergens represent a major threat for allergic consumers. Although a law is in place to protect individuals that suffer from allergies, the Food Allergen Labeling and Consumer Protection Act (FALCPA), the presence of undeclared allergens is still a huge concern for the food industry since it is known to have caused severe reactions in sensitive people. It has been proven that one of the critical control points for successful food allergen management is thorough cleaning of shared equipment and processing lines, as well as adherence to Good Manufacturing Practices (GMPs). However, present techniques are not 100% effective in preventing allergen cross-contact, and there is a lack of consensus on the validation of cleaning protocols within the food processing industry.

The objectives of this study were: 1) To determine the effectiveness of four cleaning protocols for the removal of egg, peanut and milk residues from abraded and unabraded stainless steel surfaces; and 2) To validate the technique that proves to be most efficient.

Potentially allergenic food products (peanut butter, pasteurized liquid egg, and milk) were applied in a controlled manner to abraded and unabraded stainless steel coupons (type 304, 2B finish). The food contact surfaces were subjected to four cleaning protocols that encompassed

a water rinse, and the application of a Juice Products Association (JPA) Type 4 wash, a chlorinated alkaline detergent (CAD) and food degreaser wash, an acid detergent (AD) and food degreaser wash, and a water only treatment, all applied at 63°C (145° F). Allergen residues were tested with commercial test kits (Veratox Allergen Test Kits, Neogen Corporation, Lansing, MI.) in conjunction with the development of a standard curve. SAS 9.2 software was used to compare treatments and surfaces ($\alpha = 0.05$). This experiment was conducted two times.

For all three allergens, JPA and CAD resulted in the highest % reductions (99.6% on average for all surfaces), while AD resulted in the least allergen % reduction (91.6% on average for all surfaces). The average reduction for water was 96.5% for all allergens and surfaces. According to the statistical analysis, there were no significant differences between CAD, JPA type 4 wash, and water protocols for egg and milk allergens, but these were significantly different from AD wash in all three allergens. For peanut allergens, there were no significant differences between CAD, JPA type 4 wash, and water protocols in the first study, but the second study showed that JPA and CAD were different from water and AD, and the latter two were different from each other.

For peanut allergens, water was the least successful cleaning protocol with a combined average reduction of 92.6% in both types of surfaces. CAD and JPA were equally effective. For milk allergens, the acid detergent (AD) reduced a combined average of 88% in both surfaces. CAD was the most successful method with over 99% reduction. For egg allergens, JAP, CAD, and water achieved 100% allergen reductions in all samples. AD wash achieved a combined average reduction of 93.4% for abraded and unabraded surfaces, respectively.

CHAPTER 1
INTRODUCTION

Food allergies have been a major concern for sensitive individuals for a very long time. It is estimated that about 3% of adult Americans and 7% of infants are affected by food allergies, and these numbers are continuously increasing (NIAID 2008). According to a recent study by the Centers for Disease Control and Prevention (CDC), the prevalence of food allergies amongst children has increased by 18% between 1997 and 2007, which translates to 1 in 26 from that age group (Branum, CDC 2008).

Food allergens, proteins in the food that cause adverse immune reactions, represent a serious problem in the food processing industry. Allergens known as the "big eight" are the cause of approximately 90% of all allergic reactions, and these are soy, eggs, milk, fish, wheat, shellfish, tree nuts, and peanuts. However, there are other numerous products capable of eliciting allergic reactions, as well as food additives and ingredients that can act as sensitizing agents (Jackson 2008).

There are no present treatments for food allergies, thus allergic consumers rely solely on labels to avoid potential allergens. Avoidance is not always easy, since allergens can be unintentionally introduced to a food product by cross-contact during processing. Food processors are responsible for developing and implementing procedures to address allergen control. Currently, each processor has its own allergen removal techniques, but cross-contamination can result in serious health risks, hence there is an immediate need to develop standard protocols that will ensure complete removal.

Four cleaning methods will be tested on abraded and unabraded stainless steel: a basic Juice Products Association (JPA) Type 4 wash, a chlorinated alkali detergent (CAD) wash, an acid detergent (AD) wash, and a water only treatment. Abraded and unabraded stainless steel

coupons (type 304, 2B finish) will be soiled with milk, egg, and peanut butter and the experimental cleaning protocols will be applied.

The benefits of an effective allergen removal protocol are countless, including prevention of cross-contamination, which leads to safer products on the shelves, and ultimately the well-being of the end user by avoiding dangerous allergic reactions. The food industry will benefit from these techniques by ensuring allergic consumers that their products do not accidentally contain allergens. At the same time, individuals suffering from allergies will have the peace of mind that they will not get sick by the presence of undeclared allergens in their food.

The main objective of the present study is to determine which protocol is most effective at removing one or more of the allergens. Statistical analyses of each protocol will decide which one(s) is/are more successful at achieving the highest allergen reduction. A secondary objective is to validate the technique that proves to be most efficient.

The strategy towards approaching this study was to choose one common cleaning method for the removal of allergens currently used in the food industry. This method was chosen as the base or control from where the other three methods originated. The hypothesis is that the spin off methods will actually be more effective than the control. If that was the case, verification would have to follow in order to validate the best method to ensure its effectiveness for the industry. The allergens chosen for this study are the three most prevalent for reactions in the U.S.

CHAPTER 2
LITERATURE REVIEW

2.1 Food Allergies Overview

Food allergies are a major health problem around the world, especially in developed countries where consumption of processed foods is highly common. In the U.S., an estimated 10 to 12 million people are affected by food allergies, constituting about 3% of adults and about 7% of infants and children. Every year, about 30,000 people are hospitalized due to food-related allergic reactions and about 150 individuals die from them. Individuals with a family history of food allergies are more predisposed to develop allergies themselves (NIAID 2008). Studies suggest that the incidence of food allergies has been on the rise in recent years, and one study has found that the prevalence of peanut allergy has doubled in the past five years (van Hengel 2007).

There are more than 160 foods known to produce allergic reactions. However, only eight are accounted for more than 90% of the reactions in the U.S. The eight most common foods, known as the "big eight", are: milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybeans (Jackson 2008). Adults are mostly affected by consumption of peanuts, tree nuts, fish, and shellfish, while children have most reactions after consuming milk, eggs, and peanuts (Sampson 2004). These foods contain what is known as food allergens which are proteins that cause immunological reactions in the body (Poms 2004). Once they enter the bloodstream, they are transported to specific organs such as the nose, skin, or lungs (NIAID 2008).

A food allergy is defined as an adverse reaction of the immune system to a protein (antigen) in a food or food ingredient. More specifically, when in contact with these antigens the body produces allergen specific antibodies called immunoglobin (Ig) E. IgEs are a type of protein that interact with specific allergens in food (NIAID 2008). Individuals can become sensitive to food allergens in a process called sensitization. When the immune system is exposed

to a food allergen, a stimulus is triggered to produce IgE specific antibodies. Sensitization occurs when these antibodies bind to the surfaces of mediator cells, mast cells in the tissues or basophils in the blood, and activate them. This process is known to be symptomless and it does not always result in hypersensitivity. However, when sensitization is effective, a subsequent exposure to the same allergen can produce an allergic reaction by releasing inflammatory molecules such as leukotrienes and histamine. In this case, the food allergen cross-links two IgE molecules on a mast cell or basophil, triggering allergic symptoms (Taylor 2006).

An allergic reaction can occur within minutes of ingesting the food or up to one hour after ingestion. Factors such as eating and digestion time can determine the onset and location of symptoms (NIAID 2008). The severity and duration of these symptoms depends on the amount of allergen consumed, the route of exposure, and the organs involved (FDA/CFSAN 2006). Symptoms range from mild to severe and can occur in the skin (eczema, urticaria), eyes (conjunctivitis), nose (sneezing, rhinitis), oral cavity (swelling and itching), and gastrointestinal tract (vomiting, diarrhea, nausea). A severe case of a food allergic reaction can cause anaphylaxis, a condition involving multiple organs in the body that requires immediate medical attention (FDA/CFSAN 2006). Anaphylaxis is a very serious allergic reaction that occurs rapidly and may lead to death. It may start with a tingling sensation, itching, or a metallic taste in the mouth. Other common symptoms include hives, a sensation of warmth, wheezing or other difficulty breathing, coughing, swelling of the mouth and throat area, vomiting, diarrhea, cramping, a drop in blood pressure, and loss of consciousness. The onset of these symptoms may begin right away or take up to two hours after exposure to the allergen, but life-threatening reactions may worsen after several hours upon ingestion (FAAN 2009).

Once an individual is diagnosed with a food allergy or allergies the only prevention-based treatment available to date is to eliminate the food or foods that trigger the reaction. Sometimes, allergy-producing ingredients are used in foods that people would not normally associate with them. For example, peanuts could be used as a protein source or a dressing could contain eggs or milk. There have been cases where food allergy reactions occurred due to undeclared ingredients in labels. For this reason, the Food and Drug Administration (FDA) passed a law in 2004 requiring to state food allergens in the labels called the Food Allergen Labeling and Consumer Protection Act (FALCPA), which became effective January 1, 2006 (FDA/CFSAN 2004). Under this law, food manufacturers are required to clearly label foods that are or contain one or more of the 'big eight" food allergens (FDA/CFSAN 2006). This law is extremely important because food allergic individuals do not have any preventive approaches to avoid food allergies other than avoiding the food altogether. However, it does not require FDA to establish a threshold level for any food allergen, nor does it require advisory labeling such as "may contain…" even if allergens may be present as a result of cross-contamination (FDA/CFSAN 2006).

Currently, it is not clear what the threshold doses of certain allergens are, so it is very difficult to determine if a person will suffer a reaction even if minimal consumption occurs. In some cases, traces amount of a food allergen will elicit a severe reaction; however, the food matrix and the sensitivity of the individual play a big role in determining the outcome (Taylor 2006). To date, the scientific community has not been able to reach a consensus on the minimum level of allergens required to cause a reaction, and the ranges observed for the different types of proteins change drastically from person to person (Jackson 2008). Current FDA regulation

requires manufacturers to remove all detectable allergenic residues from food processing surfaces in order to prevent cross-contamination (FDA/CFSAN 2006).

A clinical study was conducted to determine the thresholds of reactivity to milk, egg, and peanut. Results indicated a threshold equal or below to 65 mg of solid food or 0.8 mL of milk, resulted in 16% of egg allergies, 18% of peanut allergies, and 8% of milk allergies. A threshold equal or below to 15 mg of solid food or 0.3 mL of milk was seen in 5.6% of egg allergies, 3.9% of peanut allergies, and 1.7% of milk allergies. The lowest reactive doses were < 2 mg of crude egg white, 5 mg of peanut, and 0.1 mL of milk. These results suggest that the risk doubles for peanut compared to milk and egg. The findings in this study suggest that sensitivity limits of tests should be at least 10 ppm for egg, 24 ppm for peanut, and 30 ppm for milk, to guarantee a safety of 99%, 96%, and 98%, respectively, for individuals affected to these foods (Morisset 2003).

2.2 Egg Allergens

Eggs are one of the most common sources of food allergies, affecting an estimated 35% of children and 12% of adults (Poms 2004). Studies have shown that individuals have reacted to doses between 1 and 200 mg of egg, with a second study showing a reaction with as little as 0.03 mg of spray dried whole egg (Poms 2004). The main allergens in egg white are ovomucoid, ovalbumin, ovotransferring, and lysozyme, making up 80% of the total protein content in the white (Hildebrandt 2008). The principal allergen in egg yolk is α-livetin. Most reactions are caused by egg white compared to the yolk and the major agent identified is ovomucoid, the dominant allergen in hen's eggs (Poms 2004). According to Matsuda et al. (1993), individuals suffering from egg allergies are especially sensitive to ovomucoid in hard-boiled eggs which is a heat-stable glycoprotein.

Egg allergen stability has been extensively studied. One study suggested that in cooked eggs the yolk and the white coagulated, thus decreasing the egg's allergenicity. In the same study, researchers discovered that by heating the egg white for ten min at 90°C and feeding it to patients they decreased adverse reactions by 50% compared to the consumption of raw eggs (Demeulemester and Giovanacci 2006). Another study of similar nature was able to demonstrate that 21 out of 38 people who have had a positive allergic reaction to freeze-dried egg white did not have a reaction to heated egg white (Urisu 1997). However, that same study showed that ovomucoid was a heat stable allergen compared to other egg proteins that were previously labeled as heat-labile.

It has been shown that thermal processing and enzymatic hydrolysis can influence the hen's egg allergenicity. Enzymatic hydrolysis can efficiently reduce allergenic potential, especially if proteins are partially or fully denatured since this makes the proteins more available for enzymatic digestion. Pasteurization, or any other thermal process, will cause the enzymes to cleave the egg proteins more efficiently (Hildebrandt 2008).

Eggs are used as major ingredients in numerous foods and consumers generally can identify them in an ingredient list. However, issues arise when egg derivatives, such as lecithin, provitamin A, or other products are integrated in food systems, since they can cause allergic reactions to very sensitive individuals. When listed by their function, for example binder, coagulant, or emulsifier, people have a hard time recognizing they are present (Poms 2004). There are various detection methods for the presence of egg residues in foods, especially ELISA test kits and techniques based on gel-electrophoresis.

2.3 Milk Allergens

Milk allergens account for approximately 2% of food allergies in children ages 2 and under, but most of them (over 50%) become tolerant past 6 years old. However, in some cases

severe allergic reactions can occur in adults (Poms 2004). The major allergens present in cow's milk are caseins, whey proteins β-lactoglobulin and α-lactalbumin, bovine serum albumin (BSA), and bovine immunoglobulins (Igs) (Poms 2004). These proteins are known to be heat resistant and stable after industrial processes (Demeulemester and Giovanacci 2006). Generally, hydrolysis of milk significantly reduces its allergenicity (Wal 2001).

Studies have found that cow's milk contains between 30 and 35g of protein per liter (Wal 1998). When milk is acidified to pH 4.6 two fractions of milk can be obtained: whey and curd. The whey fraction contains approximately 20% of the proteins, which are essentially globular. The main ones are β-lactoglobulin and α-lactalbumin, which are synthesized in the mammary gland, while bovine serum albumin comes from the blood. Initially, it was believed that β-lactoglobulin was responsible for the majority of allergic reactions. However, recent studies on large populations suggest that the majority are sensitive to a wide variety of milk proteins besides β-lactoglobulin. In addition, proteins present in small quantities in the milk, such as bovine serum albumin and lactoferrin, seem to affect 35-50% of allergic patients. Besides, sensitivity to caseins and frequency of cases has been on the rise in recent years (Wal 2001).

Conversely, the curd contains the caseins that are comprised of four proteins: α_{S1}-, α_{S2}-, β-, and κ-caseins, and these are defined as phosphorylated proteins (Wal 1998). Caseins are known to be resistant to severe heat treatments but are affected by proteinases and exopeptidases.

Cow's milk allergens are present in a variety of sources, ranging from breast milk (due to the mother's consumption) and infant formulas, to other dairy products like cheese and butter. Other products classified as non-dairy may also contain these allergens, either accidentally or as additives not mentioned in the label. Extracted milk proteins are used as emulsifiers or protein sources (Poms 2004).

Similar to other allergens, there is very little information regarding the minimum quantities required to elicit severe reactions. One such example is the case of a 3-year-old boy who experienced a severe anaphylactic reaction after consumption of lemon sorbet containing trace amounts of milk (Laoprasert 1998). Some of the symptoms included angioadema, vomiting, and urticaria, within 20 min of ingesting 4 to 6 oz of the sorbet. The sample analyzed was found to contain minimal amounts of whey proteins (<200 µg), and it was traced to a plant that also produced and packaged ice cream. This is a prime example of individuals who are extremely sensitive to trace levels of allergen containing substances and it highlights the importance of proper and effective allergen removal practices.

As with egg residues, there are a number of detection methods for milk residues in the form of readily available test kits (ELISA). These have very low detection limits, as low as 1 mg kg^{-1} for the detection of β-lactoglobulin. They can also detect bovine serum albumin, casein, β-lactoglobulin, or whey proteins, with detection levels as low as 2.5 mg kg^{-1} in food products.

2.4 Peanut Allergens

Peanuts are probably the most analyzed food when it comes to allergenic reactions. Special attention has been paid to peanut allergens, since they are responsible for more than 50% of allergy-related deaths and have a prevalence rate of approximately 0.6% in the U.S general population (Poms 2004). They are also responsible for 10-47% of food-induced anaphylactic reactions. It has been reported that doses as low as 100 µg of peanut have caused mild to moderate reactions (Poms 2004).

There are two major storage proteins in peanuts that belong to two globulin families, arachin and conarachin. The main allergens are defined as Ara h 1 and Ara h 2, while Ara h 3 and Ara h 4, which are glycinin proteins, are classified as somewhat relevant allergens (Poms 2004). It has been reported that peanut seeds contain approximately 29% protein, with 20% of

the total protein belonging to the major allergen Ara h 1and 10% to Ara h 2 (Hefle 2006). Ara h 2 has been linked to the most severe cases of allergic reactions in people who are sensitive to it (Hefle 1996).

Studies have shown that the major peanut allergens are resistant to food processing methods and stable to digestion (Burks 1998). Other studies have come to the conclusion that roasting did not affect the allergenicity of Ara h 1, while one study claims that roasting actually enhanced it (Hefle 1996).

Most of the global peanut production is marketed as salted peanuts, peanut butter, in confectionary, or as oil. The majority of the time it is clearly stated in labels that peanuts are present; however, cross contamination during processing can result in trace amounts of peanuts in products supposed to be peanut-free. Mislabeling is also an issue and can result in severe reactions (Poms 2004).

2.5 Detection Methods

There are several detection methods for allergens in food products, and they either target the protein itself or have a marker that indicates the presence of the offending food. Some of the protein-based methods are radio-allergosorbent test (RAST), enzyme allergosorbent test (EAST), rocket immune-electrophoresis (RIE), immunoblotting, and enzyme-linked immunosorbent assay (ELISA), among others. Of all these techniques, ELISA is the method of choice in routine food analysis since it is quantitative and very accurate, simple to use, and has a high potential for standardization (Poms 2004).

ELISA test kits work based on the detection of allergens or specific marker proteins by colorimetric reaction after they bind with a specific enzyme-labeled antibody. By applying a standard curve the concentration of the antigen/antibody complex can be estimated. ELISA tests come in two formats, competitive and sandwich, with competitive ELISA mostly used for the

detection of small proteins. Sandwich ELISA tests are mostly used for the detection of food allergens (Poms 2004). Competitive ELISAs are based on an indirect ratio: the more allergenic residue in sample, fewer antibodies are bound and less color is developed. Sandwich ELISAs are based on direct ratios since the amount of color developed is directly proportional to the amount of allergens present in the sample (Immer 2006).

2.5.1 Factors Affecting Detection Methods of Allergenic Residues

Immunoassay effectiveness is highly dependent on the quality of the antigen being measured and the antibody used to do it, as well as the test itself being used. Generally, there are two main factors affecting assay efficacy: the extraction of the allergen and the assay (Immer 2006).

There are several factors that should be taken into account to avoid false or faulty results. 1) Contamination: it is imperative that contamination be avoided. Clean containers and solutions should be used to avoid either bacterial or allergenic contamination. A study showed that there were differences in the standard curves obtained between a fresh prepared buffer and a contaminated one (Immer 2006). 2) Extraction: this step should ideally extract the analyte 100% from the food. However, storage or even processing conditions of the food can significantly affect extraction effectiveness. Therefore, it is important to take into consideration the type of sample being analyzed to obtain better results. 3) Handling: pipetting, washing of plates, and precision, among others, all contribute to the end results. It is important that pipetting is performed correctly and that pipettes are calibrated. Proteins adsorb onto surfaces so pipette tips should be rinsed repeatedly before being used. Incubation time is also a factor in the number of samples run at once. If the incubation period is long then more samples can be run at the same time, but if it is short (~ 5 min) fewer samples should be analyzed because of the difference in time between the first and last sample pipetted (Immer 2006).

ELISA tests are mostly reliable, but since detection is achieved through binding of target proteins any changes to these proteins can affect results. For example, thermal processing, hydrolysis, and even oxidizing agents have an effect on the reactivity of the antibodies. Therefore, if a surface, piece of equipment, or a finished product were in contact with high temperatures or sanitizing agents, ELISAs may not be able to detect allergenic residues (Jackson 2008). It has also been shown that exposure to oxidizing chemicals, such as those present in cleaning solutions, may affect the solubility and immunoreactivity of proteins preventing ELISA to detect residues on surfaces exposed to cleaning. This is known as background noise and is a disadvantage of these types of analytical methods.

2.5.2 Factors Affecting Detection of Allergens on Equipment and Processed Foods

There are various limitations that affect the detection of allergens, such as extraction of the analyte from the food (as mentioned above), matrix interference, and changes in protein configuration due to processing (Crevel 2006).

Extraction of analyte from food is by far the most significant limitation. The efficacy of the extraction depends on the solubility of the protein, since most immunoassays like the ELISA method operate under aqueous conditions. Many products contain lipids as part of their formulation which can make it harder to extract. One study of edible oils found that total residual protein content recovery was only 50% by extraction in phosphate-buffered saline (Crevel 2006). Another study was only able to recover 2-3% of peanut protein added to chocolate (Crevel 2006). These findings show the importance of familiarizing with the matrix of the food as well as the protein being analyzed.

Matrix interference can be a problem when analyzing for allergens. High sugar content foods were proved to interfere with the recovery of β-lactoglobulin. Other materials commonly added to foods, such as colors, can also hinder the results obtained (Crevel 2006).

As previously mentioned, protein configuration changes can have a large effect on allergen detection. During processing, protein allergenicity may change or its ability to be detected in a particular food due to immunoreactivity alteration, reactivity between the protein and the food matrix, or a combination of both (Crevel 2006).

2.6 Cleaning Protocols and Prevention Programs

Although consumers rely on food labels to determine if a product is acceptable or not, allergens could still be unintentionally present in that product. A food allergen can be accidentally ingested in several ways, including incorrect labeling of the food product, improper handling, cross-contamination, and ineffective equipment cleaning and sanitation. Cross-contamination is a huge concern in the food industry and can occur at any time. One of the most common ways cross-contact can occur is through the transfer of allergens during processing or handling, especially if the same equipment or processing line is used for various foods, both allergenic and non-allergenic. It has been shown that cross-contamination has resulted in allergic reactions in several occasions (Jackson 2008).

Cleaning is an invaluable technique in the process of food allergen cross-contact and its effectiveness is crucial in combating adverse reactions. Adequate sanitation in shared equipment is pivotal, and when successful, it can cut costs in machinery and equipment. Rarely do food manufacturers have two sets of equipment for allergenic and non allergenic food materials. A study conducted by the FDA from 2002 to 2004 shows that around 80% of the facilities visited that shared equipment had one or more specific control measures to avoid cross-contamination. However, the same study determined that 96% of the inspected plants used at least one of the “big eight” allergenic foods as an ingredient. As a result, FDA inspectors estimated a 25% chance of producing cross-contaminated products during manufacture (Jackson 2008).

Food soils are defined as unwanted matter on food-contact surfaces (Schmidt 2003). Soils are usually classified depending on the method of removal from the surface cleaned. The two main categories are water soluble and non-water soluble. Water soluble soils are those that can dissolve in water that contains no cleaning agent, and these are inorganic salts, sugar, starches, and minerals (Marriot 2006). Among the water insoluble soils are those that can dissolve in alkaline detergents, solutions with a pH above 7.0, such as fatty acids and proteins, and those that dissolve in acid media (pH below 7.0) like mineral deposits (Marriot 2006). There are four types of food soils commonly found on equipment and these are carbohydrates, proteins, fats, and minerals. Of the four, proteins (including allergenic proteins) are the most difficult to remove, and it is even harder if they have been heat denatured and adhered to surfaces (Schmidt 2003).

Cleaning and sanitation are very important aspects when it comes to the development of a successful sanitation program (Schmidt 2003). Cleaning is defined as the complete removal of food soils using detergents under manufacturer's specifications (Schmidt 2003). The appropriate sequence should be 1) Rinse, 2) Clean, 3) Rinse, and 4) Sanitize. The last step is performed to reduce microorganisms to safe levels. Detergents are commonly used to aid in cleaning of food soils by lowering the surface tension of the water and loosening the mater (Marriot 2006). They can be classified according to the effect they produce upon contact with the soil. Two main classes of detergents are alkaline and acid cleaners. Alkaline detergents are those with a pH higher than 7.0 and they are sub-divided as the alkalinity (pH) increases. In general, fats, oils, and proteins require a pH of 11 or higher for effective removal. Conversely, acid detergents are those with a pH lower than 7.0. They are useful for removal of mineral deposits (Schmidt 2003). In addition to the soil, surface characteristics should be taken into account when choosing a

detergent. Other factors that affect detergent performance are time, temperature, and quality of the water (Marriot 2006).

Alkali-based detergents are commonly used to remove proteins and oxidizing agents can be added to solutions to further solubilize the soil (Jackson 2008). Removal of protein films requires alkaline detergents in conjunction with chlorine (usually hypochlorite) (Schmidt 2003). Fat based soils are present as emulsions and generally can be removed with hot water above the melting point or through alkaline detergents (Schmidt 2003). The condition of the soil also affects the ability to be removed. Fresh soils are easier to remove in cold solutions compared to old, dried, or baked-on deposits (Schmidt 2003). In addition, soils that fall into cracks or crevices or on uneven surfaces are harder to remove, thus soil removal also depends on surface qualities like smoothness, porosity, and wettability (Marriot 2006).

Allergen removal protocols can be divided into wet and dry cleaning. Wet cleaning is performed in facilities where high-water-activity foods are processed; therefore, they have accommodations to have water available everywhere as well as equipment that is resistant to constant moisture.

The existing four types of wet-cleaning methods are 1) clean in place, where equipment is not disassembled, 2) clean out of place, where equipment is partially disassembled, 3) foam or gel cleaning, and 4) manual cleaning, where fully dismantled equipment is cleaned by hand. Most of the facilities choose clean in place systems because they can be automated and constantly applied. The efficiency of a cleaning procedure depends on the cleaning time, the temperature and composition of the cleaning solution, and the force used to apply such solution (Jackson 2008).

Conversely, dry allergen cleaning is much more difficult to successfully achieve since it is applied in facilities where dry goods are produced and no water contact is desired. Usually, these are plants designed so that water is not readily available, if at all, to avoid contact with the product. Some of the most common used methods are vacuuming, sweeping, scraping, wiping, and using compressed air. According to an Institute of Food Technologists (IFT) report of allergen control practices in the food industry, more than 50% of the companies use these dry cleaning techniques for allergen removal (Jackson 2008).

As part of a safe allergen control program, many food plants are establishing several programs to tackle this ever increasing issue. There are various ways to control allergens in processing plants. Some factors that should be taken into consideration are manufacturing, separation of operations, sanitary design, hygienic practices, as well as verification and validation techniques (Newcomer 1999).

Separation of operations is extremely important, especially when allergenic and non-allergenic products are produced on the same line. Production of allergenic foods should be done before cleanup and within several hours of processing non-allergenic products. If possible, workers handling products containing allergens should not come in contact with non-allergen containing ones (Newcomer 1999).

Good sanitary designs should be in place, including selection of materials and utensils that are easy to clean and examined for food residues. Proper system design and cleaning should be available to minimize allergen cross-contamination. Visual verification should be easy to perform by providing clear access to all pieces of equipment. Besides, thorough cleaning between product runs is imperative to avoid contamination (Huggett and Hischenhuber 1998).

Having an effective Hazards Analysis Critical Control Points (HACCP) approach in place is a good way to establish an allergen-control prevention plan. Current Good Manufacturing Practices (cGMPs) should also be part of an aggressive approach for dealing with allergens (FDA/CFSAN 2004). The GMP approach includes implementing a preventive plan to avoid the unwanted presence of allergens in products they are not supposed to be a part of (Huggett and Hischenhuber 1998). The HAACP approach requires a team of personnel that needs to have a thorough understanding of the manufacturing process and the ingredients used, and that is close to the day-to-day operations (Clark 2005, Huggett and Hischenhuber 1998). The objective of HAACP is to identify the critical control points during production where steps can be taken towards minimizing the risk of having hidden allergens in finished products, and to establish a continuous monitoring plan for each critical control point (Huggett and Hischenhuber 1998).

Hygienic practices are fundamental and all plant workers should be taught about their importance (Newcomer 1999). Workers should be fully aware of the risks associated with cross-contamination and the consequences attached to it (Clark 2005). Tagging, color-coding, and other identification procedures can help employees quickly and correctly identify allergen sensitive ingredients or finished goods (Newcomer 1999).

Labeling is a key step as sometimes is the only source of information about the presence of allergens that alerts consumers. Having the correct label is important but verifying that the final formula matches what is written on the package is also imperative (Huggett and Hischenhuber 1998). In some cases, formulas are modified at the last minute but labels are not updated with the correct information that can cause distress for consumers.

2.7 Cleaning Studies

Stainless steel has been one of the preferred materials for food contact surfaces due to easy cleanability, mechanical strength and corrosion resistance, and cost benefit advantages (Milledge and Jowitt, 1980). Several finishes are used in the food industry depending on the application. One of the most used types for food applications is 304, polished to a #4 finish, or milled or rolled (2B) finish (Frank 1997, 2001).

A study by Jackson et al. (2005) was performed to determine the efficacy of several cleaning methods for the removal of hot milk soils and cold milk soils from a stainless steel surface. Plates (304A, 2B finish) were prepared by adding either 1) hot milk soils (0.75 mL whole milk, stored for 1.5hrs in 88°C oven) or 2) cold milk soils (0.75 mL whole milk, stored for 48hrs in 6°C refrigerator). Plates were washed with water or chlorinated alkali detergent (CAD) at several temperatures (ambient, 62.8°C, 73.8°C) for 30 min. Residues were determined using ELISA milk test kits. Results indicated that water alone removed all cold milk residues at 62.8°C and at 73.8°C, but was not able to remove hot milk residues from stainless steel. Chlorinated alkali cleaner removed hot milk soils at all temperatures. As demonstrated by these findings, it is imperative for food manufacturers to correctly determine the type of cleaning conditions that suits them best for each kind of soil.

In 2004, Jackson et al. performed a study to determine the removal of peanut allergens from several food-contact surfaces. Various food-contact materials (stainless steel, Teflon, polyethylene, urethane, and polycarbonate) were contaminated with peanut butter, and washed for 30 min with either water, chlorinated alkali detergent (CAD), or acid detergent (AD), each at room temperature and at 62.8°C. Results indicated that CAD and AD solutions removed residues from all food-contact surfaces at 62.8°C, but room temperature CAD was not effective

in some plates. Room temperature water did not remove residues from any of the materials, and hot water (62.8°C) was not effective in urethane and Teflon plates.

Frank and Chmielewski (2001) determined the effect of several finishes on the cleanability of stainless steel. A total of nine-type 304 samples of stainless steel were used, and several finishes were tested, including no finish, #4 finish, 2B mechanical polished, and electropolished. Coupon samples were soiled with either cultured milk inoculated with *Bacillus stearothermophilus* or a *Pseudomonas* spp. biofilm. Cleaning was performed by immersion in a turbulent bath containing 1.28% NaOH at 66°C for 3 min followed by a sterile water rinse, neutralization in 0.1% phosphoric acid for 30 sec, rinsing on phosphate buffer, sanitizing in 100 ppm hypochlorite, neutralization in sodium thiosulfate, and drying. Results indicated that removal of milk residues from soiled coupons depends on the surface defects rather than finish type. Also, biofilm residue was harder to remove than milk soil.

In a study conducted by Guzel-Seydim et al. (2000), the removal of dairy soil from heated stainless steel surfaces using ozonated water as a pre-rinse was investigated. Stainless steel coupons were cleaned, passivated, and soiled by autoclaving (121°C at 15 psi for 15 min) with reconstituted nonfat dry milk (20% solids). Plates were subjected to a 15 min treatment of either warm water (40°C) or ozonated cold water (10°C) to compare the pre-rinse cleanability. Results showed that the ozone treatment removed 84% of soil versus 51% by the warm water treatment. Using electron microscopy (at 200x and 2000x magnification), it was determined that the amount of soil present in the plates washed with ozonated water was significantly less than in those washed with warm water.

Frank and Chmielewski (1997) compared the effectiveness of sanitation of several materials used as food contact surfaces. Mechanically polished (type 304, #4 finish) and

electropolished stainless steel, polycarbonate, and mineral resin were tested. Samples were prepared by contamination with *Staphylococcus aureus* for 4 hrs until a population of 10^4 to 10^5 CFU/cm^2 was obtained. The cleaning procedure consisted on immersing the samples in either 1) sanitizer solution (200 mg of N-alkyl dimethyl benzyl alkonium chloride/L distilled water) for 10 sec at room temperature, followed by wiping with a Quaternary Ammonium Compound (QAC)-saturated cloth for 5 sec, or 2 sec) chlorine sanitizer (200 mg/L) for 20 sec, followed by wiping with a chlorine-saturated cloth for 5 sec. According to the results, the stainless steel and polycarbonate surfaces were better sanitized by QAC than the mineral resin surfaces. Chlorine was effective on the mechanically polished and electropolished stainless steel, as well as the polycarbonate samples. However, it was not as effective on the abraded electropolished stainless steel and mineral resin surfaces. Overall, the QAC and the chlorine were successful in reducing *Staphylococcus aureus* by 1000-fold on all surfaces except on unabraded mineral resin.

A study by Jensen (1970), determined the cleanability of milk-soiled stainless steel plates by using chlorinated-detergent solution. Stainless steel plates were either pretreated with 100 ppm chlorine or no chlorine, soiled with cold raw milk, and washed by 16 detergents at 0.35% concentration, ranging from 0.016 to 0.102% active alkalinity, and 0 to 100 ppm available chlorine. Non-pretreated plates soiled with milk were successfully cleaned by all nonchlorinated detergents, but soil build up occurred when washed in alkaline solutions with 25 ppm available chlorine. Less build up was noted from 50-54 ppm chlorine, and none from 75- 100 ppm chlorine. Pre-treated plates had a high build up when washed by alkaline solutions. The buildup was a result of adhesive nonsoluble chloro-protein, which occurs at low concentration of chlorine ions. At chlorine concentrations between 75 and 100 ppm, the chloro-protein was solubilized and nonadhesive.

Schlegel et al. (2007) tested a direct sampling method for verifying the cleanliness of equipment shared with peanut products. The peanut protein was prepared by mixing 20 g of ground peanuts with 100 mL of 20 mM bis-Tris propane (pH 7.2) for 2 hrs. The aqueous phase was separated and centrifuged several times to remove fats and insoluble particles. The peanut protein residues were placed directly onto stainless steel plates in 3 x 3 cm^2 areas. The coupons had a base or acid cleaning cycle, and quantitation of residues was achieved with a non-specific assay with SDS-PAGE verification. Results indicated between a 90-95% of peanut residue recovery.

According to Reinemann et al. (2000), cleaning of milk handling equipment should involve chemical, thermal, and physical action. They found that a water rinse, usually between 38-55°C, should be performed right away after the completion of milking. A chlorinated alkaline detergent should be used to remove organic soils, such as fat and protein, within a temperature range of 43-77°C. These detergents contain alkalies, phosphates, and other agents that help dissolve fat, protein, and carbohydrates (Jones 2001). Katsuyama (1993) had found that cleaning detergents should be applied at a temperature between 54-71°C. Reinemann et al. (2000) also discusses that an acid rinse should be applied to remove mineral deposits, such as those caused by salts like calcium, following the alkaline cleaner (Jones 2001). In addition, Jones (2001) has proposed that is necessary to know the water hardness for effective cleaning of milking equipment. He suggested that as the hardness increases, the detergent concentration should also increase.

Heat aids in the removal of soils because it can soften them, and they also allow for better chemical action in detergents. Heat also affects proteins because it denatures them, but some proteins retain their allergenic properties even after heat is applied. Therefore, the addition of

chlorine to alkaline detergents is recommended to aid in the protein removal process since it aids in peptizing or breakage of protein into smaller units for easier removal (Katsuyama 1999). Since temperature is a big factor in the removal of soils it has been extensively studied. Watrous (1975) showed that a 10°C increase in temperature between 32-42°C doubles the cleaning efficiency. However, he determined that at temperatures above 85°C the milk proteins bind more tightly to the surface due to heat-induced interactions, which ultimately decreases the cleaning efficiency and can leave residues. In 1982, Bradley found that 60°C (140°F) appeared to be an optimum temperature to remove all milk residues with respect to the detergent.

As discussed above, food allergies represent a big threat to sensitive individuals, so more reliable detection and cleaning methods are necessary to allow consumers to feel safe about the products they purchase. Validation of cleaning techniques plays a huge factor in trying to combat food allergens. Validation is defined as the "process of assuring that a defined cleaning procedure is able to effectively and reproducibly remove the allergenic food from the specific food processing line or equipment" (Jackson 2008). Some validation practices include visual inspection of equipment, inspection of finished goods, final rinse water, and diagnostic swab samples, or any combination thereof. Even though research has made great progress regarding the control of food allergens, undeclared allergens are still a concern in the food industry. More research is needed in the area of cleaning protocols for several allergen-contaminated soils from different food contact surfaces (Jackson 2008).

2.8 Objectives

The main objective of this study was to evaluate and compare four different cleaning protocols for the removal of peanut, egg, and milk allergens from stainless steel surfaces commonly used in the food industry. A polished and an abraded type of surface were chosen to compare and contrast the removal differences between the two. The abraded stainless steel

surface was chosen to mimic the abrasion that occurs in food plants over the years. The allergens were chosen due to their high impact in the U.S. population, since they are the cause of most adverse reactions. The first protocol chosen was a modified Juice Products Association (JPA) type 4 wash, which is currently used to remove allergens, especially in tankers. The chlorinated alkali detergent (CAD) and acid detergent (AD) methods were modified versions of the JPA type 4 wash. The water only treatment was applied to see the effects of the cleaning chemicals on the removal of the allergens. A secondary objective was to validate the method most efficient for each type of allergen for further use in manufacturing plants.

CHAPTER 3
MATERIALS AND METHODS

3.1 Stainless Steel Coupons Preparation

For this study, two types of stainless steel plates were used. Unabraded coupons were type 304-2B stainless steel panels (0.037 x 3 x 6”), and abraded coupons were obtained by grinding one of the sides of the 304-2B stainless steel panels (Q-Lab Corporation, Westlake, Ohio). The plates were first submerged in a solution of 10 mL Fisherbrand Sparkleen 1 detergent solution (Fisher Scientific, Pittsburgh, PA) for 24 hrs to remove impurities and grease, followed by a rinse with distilled water, and allowed to air-dry. Plates were wrapped in aluminum foil (Publix brand) and sterilized by autoclaving for 15 min at 121°C and 15 psi. After being autoclaved, the plates were allowed to cool down at room temperature and stored in closed containers until further use.

3.2 Samples Preparation and Inoculation

The food samples were purchased from a local supermarket. The peanut butter used was Publix brand (creamy type), the eggs were Publix brand pasteurized whole liquid eggs, and the milk was shelf-stable pasteurized whole milk (Borden brand).

The peanut butter samples had to be diluted in water before being applied to the stainless steel coupons. The solution was a 50% peanut butter and 50% de-ionized water mixture. One gram of this mixture was applied to a 15 in^2 area of the stainless steel plates in a uniform manner and spread out with a plastic hockey stick (Fisher Scientific, Pittsburgh, PA). After the application the plates were allowed to dry for 15 min.

The pasteurized liquid eggs were directly applied to the plates. One gram of product was applied to a 15 in^2 area of the stainless steel plates in a uniform manner with a spraying bottle,

and spread out using plastic hockey stick (Fisher Scientific, Pittsburgh, PA). After the application the plates were allowed to dry for 15 min.

The shelf-stable milk was directly applied to the plates. One gram of product was applied to a 15 in^2 area of the stainless steel plates in a uniform manner with a spraying bottle, and spread out using plastic hockey stick (Fisher Scientific, Pittsburgh, PA). After the application the plates were allowed to dry for 15 min.

For the three samples, the initial concentration of protein applied was calculated from the nutritional information provided on the label of the products. From this, it was determined that approximately 2259.8 ppm peanut allergen/g peanut butter/15 in^2, 1016.53 ppm egg allergen/g liquid egg/15 in^2, and 354.17 ppm milk allergen/ g milk/15 in^2 were the initial concentrations applied.

For this study, there were two controls to see the effects of the different types of washes and allergens. The negative control consisted on an empty stainless steel plate for each type of wash. The positive control consisted on a coupon soiled with the food allergen but no cleaning protocol was applied. The latter control was the baseline for each allergen.

3.3 Juice Products Association (JPA) Type 4 Wash

The Type 4 wash was designed to be applied to food tankers for the removal of food allergens as part of the Model Tanker Wash Guidelines for the Fruit Industry (JPA 2008). For this study, the Type 4 wash was modified to accommodate the needs of this research. The pH of the solution was 11.5. The protocol used for removing the soils from the coupons was as follows:

1. Rinse plates with room temperature water.
2. Wash stainless steel plates by immersing them in a hot (63°C) potable water and food grade degreaser solution (Dawn Liquid Detergent, P&G, Cincinnati, OH) (3fl oz/1 gallon water) for 15 min.
3. Rinse plates thoroughly with room temperature water.

4. Apply hot cleaning solution (63°C) utilizing a USDA-A1 rated cleaner (ZEP, Atlanta, GA) (3fl oz/1 gallon water) for 15 min.

5. Rinse plates thoroughly with room temperature water.

3.4 Chlorinated Alkali Detergent (CAD) Wash

The Chlorinated Alkali Detergent wash is a modified version of the JPA Type 4 wash and was adapted to fit the study requirements. The pH of the solution was 12.2. The protocol used for removing the soils from the coupons was as follows:

1. Rinse plates with room temperature water.

2. Wash plates by immersing them in hot (63°C) potable water with chlorinated alkali detergent (ZEP, Atlanta, GA) (3fl oz/1 gallon water) for 15 min.

3. Rinse plates with room temperature water.

4. Immerse plates in hot (63°C) potable water and food grade degreaser solution (Dawn Liquid Detergent, P&G, Cincinnati, OH) (3fl oz/1 gallon water) for 15 min.

5. Rinse plates thoroughly with room temperature water.

3.5 Acid Detergent (AD) Wash

The Acid Detergent wash is a modified version of the JPA Type 4 wash and was adapted to fit the study requirements. The pH of the solution was 1.35. The protocol used for removing the soils from the coupons was as follows:

1. Rinse plates with room temperature water.

2. Wash plates by immersing them in hot (63°C) potable water with acid detergent (ZEP, Atlanta, GA) (1:5 ratio) for 15 min.

3. Rinse plates with room temperature water.

4. Immerse plates in hot (63°C) potable water and food grade degreaser solution (Dawn Liquid Detergent, P&G, Cincinnati, OH) (3fl oz/1 gallon water) for 15 min.

5. Rinse plates thoroughly with room temperature water.

3.6 Water Only Wash

The protocol used for removing soils from the plates was as follows:

1. Rinse plates with room temperature water.
2. Wash plates by immersing them in hot (63°C) potable water for 15 min.
3. Rinse plates with room temperature water.
4. Immerse plates in hot (63°C) potable water for 15 min.
5. Rinse plates thoroughly with room temperature water.

3.7 Food Allergens Quantification and Analysis

After the wash was applied, a visual assessment was performed on the plates to determine if there were visible residues left. The residues from the plates were collected using a Spongesicle® (International BioProducts, St. Paul, MN) containing 10 mL of neutralizing buffer and returned to the sample bag. The collection was done using both sides of the sponge on a 5 x 3 in^2 surface area. To the bags, 90 mL of a 0.1% solution of sodium phosphate dibasic was added (pH ~ 7). The bags were thoroughly massaged and stored under refrigerated conditions for further testing.

3.7.1 Sample Preparation and Extraction

Prior to testing, the sample bags were taken out of the refrigerator and brought to room temperature. Once at ambient temperature, modified test kit procedures were followed for all three allergens using commercially available test kits (Veratox Quantitative Allergen Test Kits, Neogen Corporation, Lansing, MI, Peanut No. 8430, Total Milk No. 8470, and Egg No. 8450).

For all allergens, 5 mL were taken from the sample bag and transferred to a sterile 18 oz Whirl-Pak bag (Fisher Scientific, Pittsburgh, PA). One level scoop of extraction additive and 125 mL of the 60°C extraction solution were added to the sterile bag. The extraction was performed by stomaching each bag at high speed for 2 min (Seward Stomacher® 80 Biomaster Lab System, Brinkmann Instruments, Mississauga, ON). The extracts were allowed to cool down to room temperature before beginning the analysis. After the modified extraction, test procedures were

followed as indicated in the manual for each allergen. All wells were read using a micro-well reader (Stat Fax Model 321 Plus, Neogen Corporation, Lansing, MI) at 650 nm per manufacturer's specifications. All readings were done in triplicates for each well and then averaged. Standard curves were constructed for each kind of allergen in the form of part per million (ppm) of allergen present (0, 2.5, 5, 10, and 25 ppm) at an absorbance of 650 nm to calculate the residue left on the plates.

3.7.2 Statistical Analysis

Data was entered into Excel and sorted by surface type, treatment type, sample number, and peanut, milk, and egg concentrations. The data were then transferred onto SAS 9.2 software (Cary, NC) for analysis and comparison between surfaces, treatments, and allergens. Data were sorted into seven classifications for analysis of variance comparison (AOV): surface, treatment, sample, rep, egg concentration, milk concentration, and peanut concentration. Surface referred to either an abraded or unabraded coupon, treatment was the type of wash applied, sample was the negative, positive, or the sample number, and rep was the reading number on the samples. Differences were considered at an alpha level equal or less than 0.05 ($\alpha = 0.05$).

In addition, a separation of means was conducted on the surface and treatment AOV results to see if there were any differences between them. The two tests performed were Duncan's Multiple Range Test and Least Significant Difference (LSD) Test for more accurate results.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Results and Discussion

To test and compare the four different washes on the removal of allergens, abraded and unabraded stainless steel plates were soiled with approximately 1 g of each food allergen. The contaminated area was 15 in^2. After applying the food, the plates were allowed to air-dry for 15 min to mimic what would happen in a food plant.

After the 15 min were up, the plates were subjected to their corresponding washes. For each wash, kind of allergen, and type of coupon, there was a negative control, a positive control, and three coupons soiled with the same food allergen to obtain triplicate samples. Each sample was independent of each other. The plates were submerged in beakers containing the cleaning solution that corresponded to the wash being tested. The beakers were contained in a water bath set to a temperature of 63°C at all times. Following the wash, the coupons were swabbed with a Spongesicle® and test kit procedures were applied as described in Chapter 3. Standard curves were constructed foe each allergen and with each new test kit. Each standard curve contains three readings per data point (absorbance) and a linear regression equation was calculated using the mean of the three data points. A statistical analysis was performed to evaluate the performance of each cleaning protocol based on the surface type.

As can be seen in the results below, some of the positive controls showed some reduction. This was due to the fact that the positive control coupons were swabbed like the samples, thus not all of the food residues were removed by the swab. However, all the positive controls show little or no reduction, indicating the presence of allergens that was the goal. In addition, very few of the negative controls showed some allergen residue, which may have been caused by accidental cross-contamination. However, the most probable reason behind the apparent allergen

residue on the negative controls may have been due to background noise caused by the chemicals on the test kits. As previously mentioned, ELISA test kits may not be able to detect residues on surfaces that have been exposed to cleaning, since the oxidizing chemicals can affect the solubility and immunoreactivity of the proteins.

The tables of results show the initial allergen concentration, calculated from the amount of protein indicated on the labels, the absorbance for each sample (read at 650 nm), the final allergen concentration before dilution (calculated from the standard curve equation), the final allergen concentration after dilution, the average final concentration after dilution, and the % reduction.

4.2 Allergen Removal Study 1

4.2.1 Juice Products Association (JPA) Type 4 Wash

4.2.1.1 Peanut allergens

The source of peanut allergens was commercial peanut butter, which had to be diluted in water in a 1:1 ratio for easier application onto the plates. The initial content of peanut allergens before the dilution was calculated to be 2259.8 ppm allergen in 1 gram of peanut butter.

The results for the removal of peanut allergens from the unabraded stainless steel coupons are shown in Table 4-1, and they have taken the dilution into account. According to these, the JPA Type 4 protocol achieved over a 99% reduction of peanut allergens in two of the samples and 100% in the remaining one. The average combined reduction for the triplicates was 99.9%. These positive results were expected because the Type 4 wash is currently in use in the food industry so a high reduction was anticipated.

A standard curve was constructed for allergen quantification, as can be seen in Figure 1. The equation on the graph was used to determine the peanut concentration (ppm) left on the coupons by substituting the “y-value” with the absorbance obtained for each sample. The %

reduction was calculated by subtracting the final concentration from the initial concentration and dividing it by the initial concentration. The result was expressed as a percentage. The ideal was 100% reduction. The correlation obtained was 0.999 that is almost ideal.

The results from the peanut allergen reduction from abraded stainless steel plates are presented in Table 4-2. As with the unabraded stainless steel plates, the overall reduction was over 99% and the average combined reduction was 99.5%. A standard curve was used to quantitate the removal of allergens (Figure 4-1). No food residue was visually observed in any if the plates.

Even though the results from the abraded plates are extremely favorable, they were surprising due to the type of surface used. Our hypothesis was that there were going to be significant differences among the two types of surfaces when in reality there were none. Therefore, we can conclude that the JPA Type 4 wash was very successful at removing peanut allergens from both abraded and unabraded stainless steel surfaces.

4.2.1.2 Milk allergens

One gram of milk was directly applied to the coupons and spread evenly in a controlled manner. The estimated content of allergens present in a gram of milk was calculated to be 354.17 ppm (per 15^2 in).

The milk allergens removal results from the unabraded stainless steel coupons are presented in Table 4-3. These have taken into account the 1:10 dilution after the sodium phosphate was added. From the table it is possible to see that the reduction ranges from 87% to 100% in some of the plates, with a combined average of 96.1%, showing a high variability. No food residues were observed after the wash was applied. From visual observation it was possible to see that the milk formed a film on the coupons after 15 min.

A standard curve was constructed to determine the final allergen content using the Neogen test kits, as can be seen in Figure 4-2. The correlation for the standards was 0.945. According to the manufacturer's specifications, a high correlation is important for better quantification of allergens because it is more reliable.

Table 4-4 presents the results for the removal of milk allergens from the abraded stainless steel coupons. Allergen reduction ranged from 97% to 100%, with a combined average of 98.2% for all coupons. These results were obtained by using the standard curve used for the unabraded results (Figure 4-2). No food residues were present.

When comparing the results from the unabraded plates to the abraded plates it is possible to see that the abraded ones were "cleaner" overall. This was not expected because the abraded surfaces were supposed to capture more food than the smooth surfaces due to the ridges in them. In addition, one would assume that a smooth surface is much easier to clean than a rough one.

Overall, the JPA Type 4 wash was found to be a good option for removing milk allergens from stainless steel surfaces, more so from abraded ones. The average reduction for both types of surfaces was 97.2%, making this kind of wash a suitable option for cleaning milk allergen residues.

4.2.1.3 Egg allergens

One gram of pasteurized liquid egg was directly applied to the coupons and spread evenly in a controlled manner. The estimated content of allergens present in a gram of egg was calculated to be 1016.53 ppm (per 15^2 in).

The results for the unabraded stainless steel coupons are presented in Table 4-5. According to the results, the JPA Type 4 wash was extremely successful are removing egg allergens since 100% reduction was obtained for all the samples. A standard curve was constructed for the quantification of egg allergens, as shown in Figure 4-3. The correlation between the absorbance

at 650 nm and the allergen concentration was 0.89 that is still considered a good correlation for quantitation. From the visual assessment it was concluded that no residues were present after the wash.

The positive controls showed some reduction, but as mentioned before, it just means that when the coupons were swabbed not all the food was collected. However, allergens were collected from the swab that was the reason for the positive control. Some of the positive controls showed an absorbance higher than 3.000 which is the highest the instrument can read. This means that the % reduction calculated is the maximum amount of residue that could be present, but most likely that number is lower than shown in the table (63.61% reduction). It was observed that a film was formed on the positive control plates after 15 min of exposure.

The results for the removal of egg allergens from the abraded plates are very similar to those from the unabraded stainless steel plates since there was 100% reduction across all samples (Table 4-6). The standard curve presented in Figure 4-3 was used to quantitate allergen residues. The visual assessment did not render any food residues. The recorded absorbance for the positive controls was 3.000, which means that most likely the actual readings were higher than this value. Therefore, the maximum egg allergen reduction was 51.5% which only confirms the presence of egg allergens in the food.

From the results obtained, it is possible to conclude that the Juice Products Association Type 4 wash was a huge success for the removal of egg allergens. The egg allergens were removed 100% from both abraded and unabraded surfaces, proving that this method could be applied in the food industry on a regular basis.

4.2.2 Chlorinated Alkali Detergent (CAD) Wash

4.2.2.1 Peanut allergens

Approximately one gram of a solution of peanut butter and water (1:1) was applied in a controlled manner to 15 in^2 of abraded or unabraded stainless steel plates. The food was spread evenly throughout the surface.

Results for the removal of peanut allergen residues from unabraded coupons are shown in Table 4-7. These have already taken into account both dilutions, the water dilution and the sodium phosphate dilution. All the samples show a similar reduction value, between 98% and 99%, with a combined average reduction of 99.3%. These results were quantitated using the standard curve from Figure 4-1. The positive control values (54.6%) represent the maximum reduction at an absorbance of 3.000. As in previous cases, this absorbance is probably higher than 3.000 but it could not be read by the machine. However, it shows the presence of peanut allergens in the food. After the wash was applied, no food residues could be seen with the naked eye.

Table 4-8 shows the removal results of peanut allergens from the abraded coupons. These were obtained using the standard curve from Figure 4-1. From the table we can conclude that for most samples the reduction was over 99% or absolute (100%). The average reduction was 99.9%, slightly higher than that obtained from the unabraded plates (99.3%).

These results are surprising since expectations were to have higher reduction for the smooth surfaces. The same was seen for the removal of peanut allergens using the JPA Type 4 wash. This indicates that the surface type did not have a big effect on the effective removal as previously thought. Similar findings were reported in a study where it was shown that CAD at 62.8°C was able to successfully reduce peanut allergens from stainless steel surfaces (Jackson 2004).

4.2.2.2 Milk allergens

Results for the reduction of milk allergens from unabraded and abraded stainless steel are shown in Tables 4-9 and 4-10, respectively. As with the JPA Type 4 wash, the standard curve in Figure 4-2 was used to calculate the concentration of allergens present.

The reduction from the unabraded surfaces was between 97% and 100%, with a combined average of 98.6% for all samples. Reduction for positive controls was negative which means that there was no reduction at all. This is possible because the absorbance readings were 3.000.

Table 4-10 indicates that the allergen removal from the abraded plates was 100% for all samples except for one, resulting in a combined average of 99.8% for all samples.

Once again, the results for the abraded surfaces were better that for the unabraded ones, although not by a huge extent. The same was observed with the JPA Type 4 wash, where the milk allergens were better removed from the rough surfaces. Overall, the CAD wash reduced 99.2% of milk allergens among all samples from both types of surfaces, making it a valuable alternative to other washes for this type of allergens. These results are similar to those obtained by a study conducted by Jackson et al. (2005), where it was found that milk soils were completely removed from stainless steel surfaces using a CAD at 62.8°C. Visual assessments from all the coupons did not reveal any food residues left and, as previously mentioned, it was observed that the milk formed a film on the coupons after 15 min of exposure.

4.2.2.3 Egg allergens

The removal results for egg allergens for unabraded and abraded surfaces are presented in Tables 4-11 and 4-12. The standard curve shown in Figure 4-3 was used to determine the allergen reduction for all samples.

Results show that 100% reduction was achieved for all plates and surfaces. The presence of allergens was confirmed by obtaining some reduction from the positive control plates. It is

important to mention that there were some food residues left on two out of the three abraded plates, but none was observed on the unabraded coupons. It is possible that even though food was left on the surfaces, the allergenic components of the egg were deactivated (denatured) by the wash, thus leading to 100% reduction.

These results compare to those obtained with the JPA Type 4 wash where 100% reduction was observed for all the samples as well. In conclusion, the CAD wash was a success for removing egg allergens and with further studies it could be validated for regular use in the food industry.

4.2.3 Acid Detergent (AD) Wash

4.2.3.1 Peanut allergens

The results for the removal of peanut allergens from unabraded stainless steel are presented in Table 4-13. As in previous cases, the removal was calculated by using the peanut allergens controls standard curve presented in Figure 4-1. The reduction of the allergens varied from 85.7% to 96.9% for all plates, with a combined average of 90.1% reduction. The positive controls had some reduction, approximately 65.5%, confirming the presence of allergens.

Results from the abraded plates are displayed in Table 4-14. The removal of peanut allergens was lower than that for the abraded coupons, as expected. Reduction varied from 81.3% to 89.1%, with a combined average of 85.6%. The absorbance for the positive control plates was 3.000 for the triplicates, with a maximum reduction of 46.3%, once again confirming the presence of the allergens.

In both types of surfaces, there was reduction variability between plates. From visual observation it was possible to see large quantities of food residue left on the plates, thus the large variability in reduction can be explained from the amount of food that was picked up by the Spongesicle®. In some plates, more food may have been picked up compared to other, which

can account from the differences in reduction. Additionally, some protein may have been denatured by the heat, thus the food residues collected may have had deactivated proteins in them which were not recognized by the allergen test kit.

The acid detergent wash was less successful at removing the peanut allergens compared to the other methods. The objective is to obtain 100% reduction in most cases, and this protocol felt short at achieving optimum results. For both abraded and unabraded plates, the average reduction was 87.8%, which is not sufficient to ensure allergen-free surfaces. Therefore, it is possible to conclude that this cleaning method would not be suitable for peanut allergen removal, since a small part of the population could still be at risk for allergenic reactions resulting from cross-contamination. These results contradict findings in a study where it was observed complete removal of peanut allergens using an AD at 62.8°C for the removal of milk allergens from stainless steel plates (Jackson 2004).

4.2.3.2 Milk allergens

The results for milk allergen removal for unabraded and abraded surfaces are presented in Tables 4-15 and 4-16, respectively. As in previous cases, the standard curve from Figure 4-2 was used for allergen quantification. From Table 4-15, we can conclude that reduction ranged from 82.8% to 100%, which is a huge variability. The average reduction for all plates was 94.6%. As in the case of the peanut allergens, the variability can be attributed to the amount of food picked up for allergen quantitation. It is possible that for some plates more food was swabbed with the Spongesicle® compared to others or that proteins were denatured.

The reduction results for milk allergens from the abraded coupons showed variability ranging from 81% to almost 100% reduction, with a combined reduction average of 91.3% (Table 4-16). It was observed that some residue was left after the acid detergent was applied, but

some of it was removed after the detergent was applied, suggesting that the first part of the protocol was not as effective as the second portion.

As expected, less reduction was seen in the abraded surfaces compared to the not abraded ones. The overall reduction for all surfaces was 92.9% which is still not as effective as the other methods. From the visual assessment it is evident that the acid wash alone, without the detergent, does not remove food residues or reduce allergenicity as much.

4.2.3.3 Egg allergens

The results for the removal of egg allergens are presented in Tables 4-17 and 4-18. The standard curve in Figure 4-3 was used for the quantitation of egg allergens. In the case of the unabraded surfaces, the reduction varied from 97% to 100%, with an average of 98.7% reduction. These results were surprising because large quantities of residues were left on the plates after the wash was applied. However, the allergenicity of the eggs may have been reduced by the acid detergent, the soap detergent, or a combination of both. Due to some reduction of allergens in the positive controls it was possible to confirm that allergens were in the food.

Table 4-18 contains the results for the removal of egg allergens from the abraded plates. Reduction ranged from 85% to 88.7%, with an average of 86.6% removal. There were also large quantities of egg residue left and reduction was expected to be lower. However, as mentioned above, the allergenicity may have been reduced by a combination of the detergents and high heat.

From these results, we could conclude that the acid detergent may be an effective method for the removal of egg allergens from smooth surfaces, but not rough ones. The combined average reduction for both types of surfaces was 92.3% which would not qualify as being very effective. As expected, the reduction was higher for the polished plates but more testing is

needed to render it an efficient method, especially due to higher efficacy of the other methods discussed above.

4.2.4 Water Treatment

4.2.4.1 Peanut allergens

According to the results, the peanut allergen reduction varied from 88.6% to 96.5% for unabraded plates, with an average of 93.6% (Table 4-19). The results for the abraded coupons are shown in Table 4-20. The percent reduction ranged from 93.6 to 98.4, with a combined average of 96.0% removal. The results were quantitated using the standard curve in Figure 4-4.

After the first 15 min, the plates had most of the residue left on them, but it partially came out after the first water rinse. They also seemed to have grease in them, which can be attributed to the fat of the peanut butter that could not be removed since no degreaser detergent was used as in previous cases.

Unexpectedly, the results for the abraded plates were better than those for the unabraded ones, as was the case with the JPA and CAD washes. A study by Jackson et al. (2004) had found that water at 62.8°C was able to completely remove peanut butter allergens from stainless steel plates that had the treatment applied for 30 min.

4.2.4.2 Milk allergens

The results for the removal of milk allergens from unabraded and abraded surfaces are presented in Tables 4-21 and 4-22, respectively. These were quantified using the standard curve presented in Figure 4-5. For the unabraded plates, the reduction ranged from 91.1% to 98.0%, with an average of 94.8%. For the abraded coupons, removal ranged from 95.5% to 99.7% reduction, with a combined average of 97.6% for all samples. As in previous cases, the removal from abraded plates was a little more effective than the unabraded ones. There were no food residues visible by the naked eye.

These results were similar to those presented in a study by Jackson et al. (2005), where it was demonstrated that water alone was able to remove cold milk residues from stainless steel plates at 62.8°C and 73.8°C. In our study, most of the residues were removed by the water but the remaining present may have been a result of some proteins in the milk not being able to fully solubilize into solution.

4.2.4.3 Egg allergens

According to the results, there was 100% egg allergen reduction for all surfaces and samples as can be seen in Tables 4-23 and 4-24. The standard curve in Figure 4-6 was used to quantify allergen reduction. These results were surprising because it means that water alone can be applied to clean egg residues, proving it is as efficient as either the JPA Type 4 or CAD washes. They were even more unexpected due to the fact that much residue was left on the plates after the first half of the wash, but some of it came off with the first water rinse (as was the case of the peanut residues).

4.2.5 Statistical Analysis

From the statistical analysis performed with SAS 9.2 software, there were no differences between the JPA Type 4, the CAD, and the water wash in the reduction of milk and egg allergens (Tables A-2 and A-3). The acid wash was different from these three washes for both egg and milk allergens ($\alpha = 0.05$). In the case of the peanut allergens, there were no differences between the JPA Type 4 wash and the CAD wash. However, these two were different from the AD and the water. The water treatment was also different from the acid wash (Table A-1).

For egg allergens, there were significant differences between the removal from abraded and unabraded stainless steel surfaces (Table A-3). Conversely, there were no differences observed for the type of surfaces for either peanut or milk allergens (Tables A-1 and A-2).

4.1 Allergen Removal Study 2

4.3.1 Juice Products Association (JPA) Type 4 Wash

4.3.1.1 Peanut allergens

The results for the removal of peanut allergens from unabraded and abraded coupons are presented in Tables 4-25 and 4-26, respectively. The results were quantitated using the standard curve presented in Figure 4-7. According to the results, the JPA Type 4 wash achieved an average allergen reduction of 99.9% from the unabraded surfaces. These results exactly compare to those obtained in the first study, since then the average reduction was also 99.9%.

The average removal from the abraded surfaces was 100% for all samples. These results are slightly better than those obtained for the polished surfaces, which is somewhat surprising. However, they also compare to those seen in study 1, where the reduction was 99.5%, on average. The visual assessment did not render any food residues.

According to the observed results, it is safe to say that the JPA Type 4 wash was very effective for the removal of peanut allergens from both types of surfaces. The second study validated the previous results obtained for this wash, since in both studies the results were practically the same.

4.3.1.2 Milk allergens

Tables 4-27 and 4-28 show the reduction results for the removal of milk allergens from the smooth and rough surfaces, respectively. These were quantified using the standard curve from Figure 4-8. In both cases, the reduction was 100% for all samples and surfaces. These results were better than those obtained in the first study, where the averages were 96.1% and 98.2% for unabraded and abraded coupons, respectively, and more variability was observed.

As in the case of the peanut allergens, it is possible to conclude that this wash is very effective at removing milk allergens from both types of surfaces. The average reduction between

studies 1 and 2 was 98.1% for unabraded plates and 99.1% for abraded surfaces. Therefore, the JPA Type 4 wash is an excellent cleaning method to get rid of milk allergens.

4.3.1.3 Egg allergens

The results for the removal of egg allergens are shown in Table 4-29 and Table 4-30. Allergen quantification was calculated using the standard curve in Figure 4-9. For the unabraded and abraded surfaces the protocol achieved 100% reduction across all samples. These results measure up to those obtained in study 1, where 100% reduction was also obtained for all samples and surfaces. From these results we can conclude that the JPA Type 4 wash can be safely applied for the removal of egg from either abraded or unabraded stainless steel surfaces.

4.3.2 Chlorinated Alkali Detergent (CAD) Wash

4.3.2.1 Peanut allergens

The results from the removal of peanut allergens for unabraded and abraded plates are presented in Tables 4-31 and 4-32, respectively. These results were quantified using the standard curve from Figure 4-7. Results show that there was 100% reduction for all samples and both surfaces. In study 1, the average removal was 99.3% and 99.9% for unabraded and abraded plates. Overall, the average between both studies was 99.7% for unabraded surfaces and almost 100% for abraded ones, indicating that the CAD protocol was very effective for the removal of peanut allergens. As previously mentioned, these results match those found by Jackson et al. (2004). Hence, even more studies are necessary to completely validate these results.

4.3.2.2 Milk allergens

Tables 4-33 and 4-34 show the results obtained for the removal of milk allergens from the unabraded and abraded plates, respectively. These were calculated using the standard curve from Figure 4-8. The results show that there was 100% reduction across all samples and surfaces. In the first study, the average reductions were 98.6% and 99.8% for unabraded and abraded plates.

Thus, the average reduction between the two studies was 99.3% and 99.9%, respectively. These findings compare to those observed by Jackson et al. (2005), were CAD was able to completely remove milk allergens from stainless steel surfaces.

4.3.2.3 Egg allergens

The results for the removal of egg allergens are presented in Tables 4-35 and 4-36, for unabraded and abraded respectively. They were quantified by using the standard curve from Figure 4-9. According to the results, there was complete egg allergen removal (100%) from both types of surfaces. These exactly match the results from the first study where 100% was also achieved across the board. Since in both trials the findings were the same, it is possible to conclude that the CAD method can be safely applied to clean egg allergen residues from stainless steel surfaces.

4.3.3 Acid Detergent (AD) Wash

4.3.3.1 Peanut allergens

Results for the removal of peanut allergens from unabraded and abraded surfaces are shown in Tables 4-37 and 4-38. These were quantified using the standard curve from Figure 4-7. The average reductions were 99.0% and 99.6% for those surfaces, respectively. These results were surprising due to the high amount of food residue that was visible on the plates, thus less reduction was expected. We could speculate that the food residues left on the plates contained denatured proteins, and therefore were not recognized by the allergen test kits. In addition, less reduction was also expected in the abraded plates due to the type of surface, but this was not the case.

In study 1, the average reductions were 90.1% and 85.6% for unabraded and abraded plates. These were the kind of results expected in the second study. As previously mentioned, a study by Jackson et al. (2004) had reported complete peanut protein reduction from the plates.

This was observed in the second study of this research but not in the first one. Between the two trials, the average reductions were 94.5% and 92.6% (unabraded and abraded).

4.3.3.2 Milk allergens

The results for the milk allergens removal utilizing the AD wash are presented in Tables 4-39 and 4-40. Allergen reduction was quantified using the standard curve from Figure 4-8. It is possible to see a large variation in reduction of milk allergens from the unabraded plates, ranging from 41.7% to 98.1%. The combined average was 66.7%. These results are very different from the ones obtained in the first study, where the average reduction was 94.6% and the removal ranged from 82.8% to 100%. Visually, the coupons had extensive food residue left on them.

One possible explanation for the second study results is to take into account the amount of food residue that was swabbed from the plates. One theory is that more residues were collected from some plates compared to others. However, in study 1 there was a significant amount of food left on the coupons and there was also the issue of the amount swabbed with the Spongesicle®. Therefore, since in both studies the amount of residue was the same and the food picked up by the swab was variable, the results from the second study could mean that the protein present in the food was not completely, or partially, denatured as was the case in study 1.

Caseins are known to be heat resistant due to the random folding of their structure (Gregory 2009). In addition, whey proteins are stable to acid but are sensitive to heat. In general, milk solubility increases with increasing pH, and it is at its lowest around the isoelectric point of milk at pH = 4.6 (Zayas 1997). Besides, it has been shown that at high incubation temperatures, between 60 and 70°C, and at low pH the solubility of milk proteins is not very high (Zayas 1997). Therefore, since the pH of the AD was very low (pH = 1.35) and the temperature of the wash was 63°C, the proteins that stayed on the coupon that were collected were not denatured.

Conversely, the removal of milk allergens from the abraded plates was much higher than the unabraded, with an average of 95.1% reduction. As in previous AD washes, there was a significant amount of food residues left on the plates. The large difference between the two surfaces can be attributed to the amount of food that was rinsed off with ambient water in between solutions of the AD wash. Additionally, the quantity collected with the swab could have impacted the amount of protein residue present.

4.3.3.3 Egg allergens

The results for the removal of egg allergens from unabraded and abraded surfaces are shown in Tables 4-41 and 4-42. Allergen reduction was calculated using the standard curve depicted in Figure 4-9. Results show a combined reduction of 92.5% for both types of surfaces.

Again, this is surprising since in theory it would be harder to remove allergens from a rough surface rather than a smooth one. In addition, the variability range for reduction was very similar for the two kinds of coupons, ranging from approximately 91% to 94%. In study 1, the average reduction for unabraded plates was 98.7% while that for abraded was 86.7%, which was expected for the second study. Once more, the variability can be partly attributed to the quantity of food that was swabbed. It can also be attributed to the fact that egg proteins are heat sensitive and the solubility increases with increasing pH (Zayas 1997). In fact, it has been shown that at 70°C there was a significant increase in solubility when the pH varied from 2, 3, 4, and 5 (Zayas 1997). Thus, some of the egg proteins may have been denatured by the heat, but the portions that were not soluble in the solution may have still had undenatured proteins in them, which were captured by the allergen test kit.

4.3.4 Water Treatment

4.3.4.1 Peanut allergens

Reduction for peanut allergens from unabraded surfaces varied from 89.2% to 99.6% for all samples, with an average of 93.5% (Table 4-43). Results for the removal from abraded surfaces show a range between 84.7 to 91.5%, with an average of 87.1% reduction (Table 4-44). Allergen reduction was quantified using the standard curve from Figure 4-4.

As expected, reduction was lower for the rough surfaces, but these results differed from those in study 1. In the first study, the combined average for the unabraded surfaces was lower than for the abraded. However, the average removal for the unabraded plates in study 1 compared almost exactly to that in study 2 (93.6% vs. 93.5%), indicating good reproducibility. As for the abraded plates, the second study had a lower average in relation to the first study (87.1% vs. 96.0%). The average between both studies was 91.5% reduction. Like in the first study, most of the initial food was left on the plates but was partially removed by the water rinse.

4.3.4.2 Milk allergens

The reduction of the unabraded samples ranged from 94.4% to 97.7%, and 95.5% reduction on average (Table 4-45). These results compare to those obtained in the first study where the average was 94.8%. The overall removal between the 2 studies was 95.1%. Once again, no food residues were observed.

Milk allergen reduction for the abraded plates ranged from 98.3% to 100% removal, and 99.6% reduction on average (Table 4-46). These numbers were slightly better than those in the first study (97.6%), and between the two the reduction was 98.6%, which is still very effective. The results for both types of surfaces were quantified using the equation from Figure 4-5.

4.3.4.3 Egg allergens

Results for the removal of egg allergens are presented in Tables 4-47 and 4-48, for unabraded and abraded surfaces. These were quantitated using the equation presented in Figure 4-6. Reduction was 100% for all samples and surfaces, as was the case in study 1, which shows that hot water (62.8°C) is a very effective tool for removing egg residues. This can be explained by the high solubility of the proteins present in the egg at the neutral pH of water. At pH ~ 7, the proteins present in the egg are still far from their isoelectric point (pH ~ 4.8) which is the point of lowest solubility (Gregory 2009). There were residues left after the first half of the wash, but they were mostly removed after the first water rinse (as was the case in study 1).

4.3.5 Statistical Analysis

For all three allergens, the acid wash was different from the other 3 washes, but these were not different from each other (Tables A-4 to A-6). In terms of surfaces, there were no differences among abraded and unabraded plates for peanut allergens (Table A-4). However, surfaces showed differences for milk and egg allergens (Tables A-5 and A-6).

4.4 Overall Results

Side by side comparison of all treatments and surfaces for studies 1 and 2 are presented in Figures 4-10 through 4-15. Each set of data is colored differently from the others for better understanding. The bars in each set represent the samples for a total of 9 observations per set. The standard deviation is also presented in the graph. For each pair of bars, the bar on the left represents the unabraded coupons and the one on the right corresponds to the abraded surfaces. The order of the treatments is JPA Type 4, CAD, AD, and Water.

In general, results are similar for both studies, especially for the JPA and CAD washes. For the peanut allergens, there was more variability for the water and AD washes between studies 1 and 2, and the results from the second study appear to be more consistent (Figures 4-10 and 4-

11). For the milk allergens, there was more consistency in the second study once again, but there was a large variability between AD washed samples in unabraded plates (Figures 4-12 and 4-13). Additionally, the standard deviation among samples of the first study was larger compared to those of the second one. In the case of egg allergens, there was no variability for JPA, CAD, and water washes in either study because they all achieved 100% reduction for all surfaces (Figures 4-14 and 4-15). In study 1, reduction for unabraded plates was higher than study 2. Conversely, reduction for abraded plates was lower in the first study.

Table 4-1. JPA type 4 wash removal results for peanut allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after Dilution (ppm)	%Reduction
	1	1.8	4067.6	2.068	43.24	864.8	825.01	78.7
+	2	1.8	4067.6	1.950	40.28	805.8		80.2
	3	1.8	4067.6	1.948	40.23	804.5		80.2
	1	0.0	0.0	0.341	<2.5	0.00	0.18	-
-	2	0.0	0.0	0.346	<2.5	0.00		-
	3	0.0	0.0	0.348	<2.5	0.55		-
	1	1.3	2937.7	0.336	<2.5	0.00	0.54	100
1	2	1.3	2937.7	0.347	<2.5	0.05		100
	3	1.3	2937.7	0.350	<2.5	1.56		99.9
	1	1.1	2485.7	0.349	<2.5	1.06	4.57	99.9
2	2	1.1	2485.7	0.360	<2.5	6.58		99.7
	3	1.1	2485.7	0.359	<2.5	6.08		99.7
	1	1.6	3615.6	0.350	<2.5	1.56	3.40	99.9
3	2	1.6	3615.6	0.357	<2.5	5.08		99.8
	3	1.6	3615.6	0.354	<2.5	3.57		99.9

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-2. JPA type 4 wash removal results for peanut allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.8	1807.8	2.318	49.52	990.5	1001.89	45.2
+	2	0.8	1807.8	2.357	50.50	1010.1		44.1
	3	0.8	1807.8	2.347	50.25	1005.0		44.4
	1	0.0	0.0	0.365	<2.5	9.10	13.79	-
-	2	0.0	0.0	0.375	<2.5	14.12		-
	3	0.0	0.0	0.383	<2.5	18.14		-
	1	0.9	2033.8	0.361	<2.5	7.09	4.41	99.7
1	2	0.9	2033.8	0.356	<2.5	4.57		99.8
	3	0.9	2033.8	0.350	<2.5	1.56		99.9
	1	1.2	2711.8	0.364	<2.5	8.59	6.25	99.7
2	2	1.2	2711.8	0.355	<2.5	4.07		99.9
	3	1.2	2711.8	0.359	<2.5	6.08		99.8
	1	1.0	2259.8	0.387	<2.5	20.15	20.99	99.1
3	2	1.0	2259.8	0.390	<2.5	21.66		99.0
	3	1.0	2259.8	0.389	<2.5	21.16		99.1

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

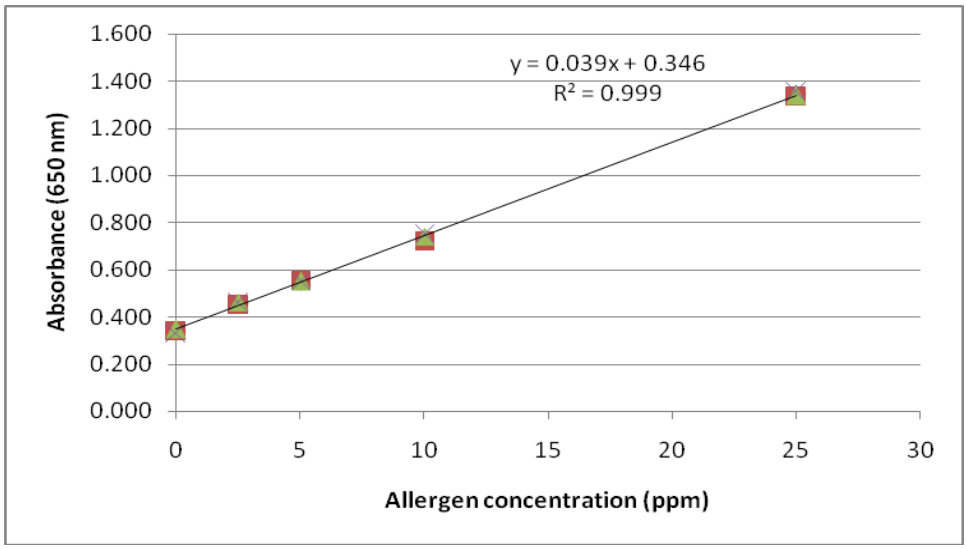

Figure 4-1. Standard curve for peanut allergen quantification test 1. Peanut controls contained 0, 2.5, 5, 10, and 25 ppm of allergen. Each concentration was read 3 times at an absorbance of 650 nm and plotted in the graph. The linear regression equation was calculated using the average of the 3 readings.

Table 4-3. JPA type 4 wash removal results for milk allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.9	318.8	2.987	57.21	572.1	573.53	-79.5
+	2	0.9	318.8	2.998	57.46	574.6		-80.3
	3	0.9	318.8	2.995	57.39	573.9		-80.1
	1	0.0	0.0	0.496	<2.5	6.0	1.98	-
-	2	0.0	0.0	0.442	<2.5	0.0		-
	3	0.0	0.0	0.460	<2.5	0.0		-
	1	1.2	425.0	0.711	5.482	54.82	40.50	87.1
1	2	1.2	425.0	0.616	3.323	33.23		92.2
	3	1.2	425.0	0.617	3.345	33.45		92.1
	1	1.0	354.2	0.525	<2.5	12.55	4.18	96.5
2	2	1.0	354.2	0.445	<2.5	0.0		100
	3	1.0	354.2	0.444	<2.5	0.0		100
	1	1.0	354.2	0.518	<2.5	10.95	3.65	96.9
3	2	1.0	354.2	0.447	<2.5	0.0		100
	3	1.0	354.2	0.453	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-4. JPA type 4 wash removal results for milk allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.9	318.8	2.961	56.62	566.2	565.8	-77.6
+	2	0.9	318.8	2.944	56.23	562.3		-76.4
	3	0.9	318.8	2.973	56.89	568.9		-78.5
	1	0.0	0.0	0.560	<2.5	20.5	6.83	-
-	2	0.0	0.0	0.466	<2.5	0.0		-
	3	0.0	0.0	0.467	<2.5	0.0		-
	1	0.8	283.3	0.502	<2.5	7.318	5.50	97.4
1	2	0.8	283.3	0.484	<2.5	3.227		98.9
	3	0.8	283.3	0.496	<2.5	5.955		97.9
	1	1.0	354.2	0.578	<2.5	24.59	11.86	93.1
2	2	1.0	354.2	0.498	<2.5	6.409		98.2
	3	1.0	354.2	0.490	<2.5	4.591		98.7
	1	1.0	354.2	0.432	<2.5	0.0	0.00	100
3	2	1.0	354.2	0.437	<2.5	0.0		100
	3	1.0	354.2	0.453	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

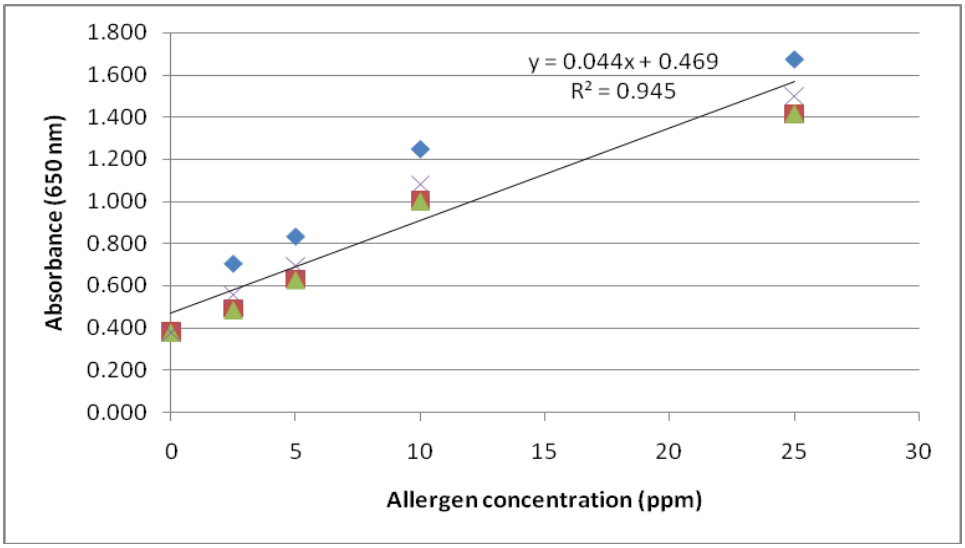

Figure 4-2. Standard curve for milk allergen quantification test 1. Milk controls contained 0, 2.5, 5, 10, and 25 ppm of allergen. Each concentration was read 3 times at an absorbance of 650 nm and plotted in the graph. The linear regression equation was calculated using the average of the 3 readings.

Table 4-5. JPA type 4 wash removal results for egg allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.8	813.2	2.932	28.66	286.6	292.8	64.8
+	2	0.8	813.2	3.000	29.59	295.9		63.6
	3	0.8	813.2	3.000	29.59	295.9		63.6
	1	0.0	0.0	0.479	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.469	<2.5	0.0		-
	3	0.0	0.0	0.466	<2.5	0.0		-
	1	1.1	1118.2	0.520	<2.5	0.0	0.00	100
1	2	1.1	1118.2	0.459	<2.5	0.0		100
	3	1.1	1118.2	0.448	<2.5	0.0		100
	1	1.0	1016.5	0.423	<2.5	0.0	0.00	100
2	2	1.0	1016.5	0.421	<2.5	0.0		100
	3	1.0	1016.5	0.415	<2.5	0.0		100
	1	1.0	1016.5	0.472	<2.5	0.0	0.00	100
3	2	1.0	1016.5	0.458	<2.5	0.0		100
	3	1.0	1016.5	0.451	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-6. JPA type 4 wash removal results for egg allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.6	609.9	3.000	29.59	295.9	295.9	51.5
+	2	0.6	609.9	3.000	29.59	295.9		51.5
	3	0.6	609.9	3.000	29.59	295.9		51.5
	1	0.0	0.0	0.569	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.557	<2.5	0.0		-
	3	0.0	0.0	0.568	<2.5	0.0		-
	1	1.2	1219.8	0.622	<2.5	0.0	0.00	100
1	2	1.2	1219.8	0.616	<2.5	0.0		100
	3	1.2	1219.8	0.631	<2.5	0.0		100
	1	1.1	1118.2	0.472	<2.5	0.0	0.00	100
2	2	1.1	1118.2	0.483	<2.5	0.0		100
	3	1.1	1118.2	0.508	<2.5	0.0		100
	1	0.7	711.6	0.478	<2.5	0.0	0.00	100
3	2	0.7	711.6	0.496	<2.5	0.0		100
	3	0.7	711.6	0.496	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

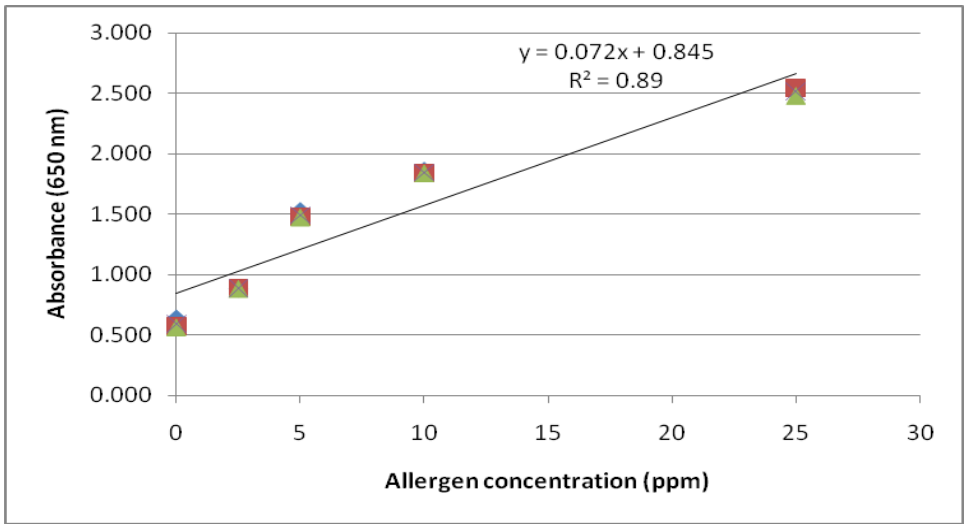

Figure 4-3. Standard curve for egg allergen quantification test 1. Egg controls contained 0, 2.5, 5, 10, and 25 ppm of allergen. Each concentration was read 3 times at an absorbance of 650 nm and plotted in the graph. The linear regression equation was calculated using the average of the 3 readings.

Table 4-7. CAD wash removal results for peanut allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.3	2937.7	3.000	66.66	1333.2	1333.2	54.6
+	2	1.3	2937.7	3.000	66.66	1333.2		54.6
	3	1.3	2937.7	3.000	66.66	1333.2		54.6
	1	0.0	0.0	0.378	<2.5	15.63	19.48	-
-	2	0.0	0.0	0.388	<2.5	20.65		-
	3	0.0	0.0	0.391	<2.5	22.16		-
	1	0.6	1355.9	0.380	<2.5	16.63	15.80	98.8
1	2	0.6	1355.9	0.377	<2.5	15.13		98.9
	3	0.6	1355.9	0.378	<2.5	15.63		98.8
	1	1.1	2485.8	0.355	<2.5	4.07	4.07	99.8
2	2	1.1	2485.8	0.355	<2.5	4.07		99.8
	3	1.1	2485.8	0.355	<2.5	4.07		99.8
	1	1.3	2937.7	0.375	<2.5	14.12	11.27	99.5
3	2	1.3	2937.7	0.368	<2.5	10.60		99.6
	3	1.3	2937.7	0.365	<2.5	9.10		99.7

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2]An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-8. CAD wash removal results for peanut allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.1	2485.8	2.579	56.08	1121.7	1131.5	54.9
+	2	1.1	2485.8	2.615	56.99	1139.6		54.2
	3	1.1	2485.8	2.602	56.66	1133.2		54.4
	1	0.0	0.0	0.356	<2.5	4.57	3.57	-
-	2	0.0	0.0	0.354	<2.5	3.57		-
	3	0.0	0.0	0.352	<2.5	2.56		-
	1	1.6	3615.7	0.343	<2.5	0.00	0.00	100
1	2	1.6	3615.7	0.342	<2.5	0.00		100
	3	1.6	3615.7	0.337	<2.5	0.00		100
	1	1.1	2485.8	0.36	<2.5	6.58	3.40	99.7
2	2	1.1	2485.8	0.350	<2.5	1.56		99.9
	3	1.1	2485.8	0.351	<2.5	2.06		99.9
	1	1.0	2259.8	0.361	<2.5	7.09	6.42	99.7
3	2	1.0	2259.8	0.359	<2.5	6.08		99.7
	3	1.0	2259.8	0.359	<2.5	6.08		99.7

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-9. CAD wash removal results for milk allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.0	354.2	3.000	57.51	575.1	575.1	-62.4
+	2	1.0	354.2	3.000	57.51	575.1		-62.4
	3	1.0	354.2	3.000	57.51	575.1		-62.4
	1	0.0	0.0	0.402	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.417	<2.5	0.0		-
	3	0.0	0.0	0.418	<2.5	0.0		-
	1	1.0	354.2	0.485	<2.5	3.455	3.83	99.0
1	2	1.0	354.2	0.488	<2.5	4.136		98.8
	3	1.0	354.2	0.487	<2.5	3.909		98.9
	1	1.1	389.6	0.522	<2.5	11.86	3.95	96.9
2	2	1.1	389.6	0.435	<2.5	0.0		100
	3	1.1	389.6	0.439	<2.5	0.0		100
	1	1.1	389.6	0.507	<2.5	8.455	8.45	97.8
3	2	1.1	389.6	0.512	<2.5	9.591		97.5
	3	1.1	389.6	0.502	<2.5	7.318		98.1

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-10. CAD wash removal results for milk allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.8	283.3	3.000	57.51	575.1	575.1	-103
+	2	0.8	283.3	3.000	57.51	575.1		-103
	3	0.8	283.3	3.000	57.51	575.1		-103
	1	0.0	0.0	0.515	<2.5	10.27	9.59	-
-	2	0.0	0.0	0.51	<2.5	9.136		-
	3	0.0	0.0	0.511	<2.5	9.364		-
	1	1.0	354.2	0.494	<2.5	5.500	1.83	98.4
1	2	1.0	354.2	0.436	<2.5	0.0		100
	3	1.0	354.2	0.442	<2.5	0.0		100
	1	1.1	389.6	0.457	<2.5	0.0	0.00	100
2	2	1.1	389.6	0.453	<2.5	0.0		100
	3	1.1	389.6	0.459	<2.5	0.0		100
	1	1.0	354.2	0.455	<2.5	0.0	0.00	100
3	2	1.0	354.2	0.449	<2.5	0.0		100
	3	1.0	354.2	0.453	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-11. CAD wash removal results for egg allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.1	1118.2	3.000	29.59	295.9	295.9	73.5
+	2	1.1	1118.2	3.000	29.59	295.9		73.5
	3	1.1	1118.2	3.000	29.59	295.9		73.5
	1	0.0	0.0	0.491	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.520	<2.5	0.0		-
	3	0.0	0.0	0.513	<2.5	0.0		-
	1	0.8	813.2	0.480	<2.5	0.0	0.00	100
1	2	0.8	813.2	0.525	<2.5	0.0		100
	3	0.8	813.2	0.502	<2.5	0.0		100
	1	0.8	813.2	0.533	<2.5	0.0	0.00	100
2	2	0.8	813.2	0.533	<2.5	0.0		100
	3	0.8	813.2	0.537	<2.5	0.0		100
	1	1.1	1118.2	0.533	<2.5	0.0	0.00	100
3	2	1.1	1118.2	0.489	<2.5	0.0		100
	3	1.1	1118.2	0.488	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-12. CAD wash removal results for egg allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.8	813.2	3.000	29.59	295.9	295.9	63.6
+	2	0.8	813.2	3.000	29.59	295.9		63.6
	3	0.8	813.2	3.000	29.59	295.9		63.6
	1	0.0	0.0	0.610	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.621	<2.5	0.0		-
	3	0.0	0.0	0.621	<2.5	0.0		-
	1	0.7	711.6	0.582	<2.5	0.0	0.00	100
1	2	0.7	711.6	0.600	<2.5	0.0		100
	3	0.7	711.6	0.583	<2.5	0.0		100
	1	0.7	711.6	0.495	<2.5	0.0	0.00	100
2	2	0.7	711.6	0.510	<2.5	0.0		100
	3	0.7	711.6	0.540	<2.5	0.0		100
	1	0.9	914.9	0.638	<2.5	0.0	0.00	100
3	2	0.9	914.9	0.674	<2.5	0.0		100
	3	0.9	914.9	0.667	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-13. AD wash removal results for peanut allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.4	3163.7	2.492	53.90	1077.9	1084.8	65.9
+	2	1.4	3163.7	2.519	54.58	1091.5		65.5
	3	1.4	3163.7	2.506	54.25	1085.0		65.7
	1	0.0	0.0	0.351	<2.5	2.06	0.69	-
-	2	0.0	0.0	0.342	<2.5	0.00		-
	3	0.0	0.0	0.344	<2.5	0.00		-
	1	1.3	2937.7	0.554	5.204	104.1	98.71	96.5
1	2	1.3	2937.7	0.551	5.128	102.6		96.5
	3	1.3	2937.7	0.525	4.475	89.50		96.9
	1	1.2	2711.8	1.014	16.76	335.2	328.53	87.6
2	2	1.2	2711.8	0.995	16.28	325.7		88.0
	3	1.2	2711.8	0.993	16.23	324.7		88.0
	1	1.0	2259.8	0.986	16.05	321.2	321.49	85.8
3	2	1.0	2259.8	0.985	16.03	320.6		85.8
	3	1.0	2259.8	0.989	16.13	322.6		85.7

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-14. AD wash removal results for peanut allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.1	2485.8	3.000	66.66	1333.2	1333.2	46.4
+	2	1.1	2485.8	3.000	66.66	1333.2		46.4
	3	1.1	2485.8	3.000	66.66	1333.2		46.4
	1	0.0	0.0	0.350	<2.5	1.56	1.04	-
-	2	0.0	0.0	0.345	<2.5	0.00		-
	3	0.0	0.0	0.35	<2.5	1.56		-
	1	1.3	2937.7	1.156	20.33	406.6	405.9	86.2
1	2	1.3	2937.7	1.153	20.25	405.1		86.2
	3	1.3	2937.7	1.155	20.30	406.1		86.2
	1	1.3	2937.7	1.437	27.39	547.8	543.4	81.3
2	2	1.3	2937.7	1.418	26.91	538.2		81.7
	3	1.3	2937.7	1.430	27.21	544.3		81.5
	1	1.1	2485.8	0.886	13.54	270.9	270.9	89.1
3	2	1.1	2485.8	0.889	13.62	272.4		89.0
	3	1.1	2485.8	0.883	13.47	269.4		89.2

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2]An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-15. AD wash removal results for milk allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.8	283.3	2.970	56.82	568.2	555.9	-100
+	2	0.8	283.3	2.885	54.89	548.9		-93.7
	3	0.8	283.4	2.892	55.05	550.5		-94.3
	1	0.0	0.0	0.532	<2.5	14.1	4.71	-
-	2	0.0	0.0	0.433	<2.5	0.0		-
	3	0.0	0.0	0.428	<2.5	0.0		-
	1	0.9	318.6	0.711	5.482	54.82	44.59	82.8
1	2	0.9	318.6	0.644	3.959	39.59		87.6
	3	0.9	318.6	0.643	3.936	39.36		87.6
	1	1.0	354.2	0.537	<2.5	15.27	5.09	95.7
2	2	1.0	354.2	0.465	<2.5	0.0		100
	3	1.0	354.2	0.469	<2.5	0.0		100
	1	1.2	425.0	0.507	<2.5	8.455	2.82	98.0
3	2	1.2	425.0	0.422	<2.5	0.0		100
	3	1.2	425.0	0.423	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-16. AD wash removal results for milk allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.1	389.6	2.985	57.16	571.6	571.3	-46.7
+	2	1.1	389.6	2.980	57.05	570.5		-46.4
	3	1.1	389.6	2.985	57.16	571.6		-46.7
	1	0.0	0.0	0.485	<2.5	3.455	1.15	-
-	2	0.0	0.0	0.458	<2.5	0.0		-
	3	0.0	0.0	0.443	<2.5	0.0		-
	1	1.2	425.0	0.482	<2.5	2.773	1.86	99.3
1	2	1.2	425.0	0.481	<2.5	2.545		99.4
	3	1.2	425.0	0.471	<2.5	0.273		99.9
	1	1.1	389.6	0.815	7.845	78.45	74.21	79.9
2	2	1.1	389.6	0.794	7.368	73.68		81.1
	3	1.1	389.6	0.780	7.050	70.50		81.9
	1	1.1	389.6	0.582	2.550	25.50	25.05	93.5
3	2	1.1	389.6	0.577	<2.5	24.36		93.8
	3	1.1	389.6	0.581	2.527	25.27		93.5

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-17. AD wash removal results for egg allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.3	1321.5	2.947	28.86	288.6	286.9	78.2
+	2	1.3	1321.5	2.937	28.73	287.3		78.3
	3	1.3	1321.5	2.921	28.51	285.1		78.4
	1	0.0	0.0	0.468	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.473	<2.5	0.0		-
	3	0.0	0.0	0.476	<2.5	0.0		-
	1	0.9	914.9	1.042	2.695	26.95	22.92	97.1
1	2	0.9	914.9	0.995	<2.5	20.49		97.8
	3	0.9	914.9	1.001	<2.5	21.32		97.7
	1	1.1	1118.2	0.958	<2.5	15.41	14.45	98.6
2	2	1.1	1118.2	0.954	<2.5	14.86		98.7
	3	1.1	1118.2	0.941	<2.5	13.08		98.8
	1	1.1	1118.2	0.674	<2.5	0.0	0.00	100
3	2	1.1	1118.2	0.672	<2.5	0.0		100
	3	1.1	1118.2	0.683	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-18. AD wash removal results for egg allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.9	914.9	3.000	29.59	295.9	295.9	67.7
+	2	0.9	914.9	3.000	29.59	295.9		67.7
	3	0.9	914.9	3.000	29.59	295.9		67.7
	1	0.0	0.0	0.539	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.550	<2.5	0.0		-
	3	0.0	0.0	0.552	<2.5	0.0		-
	1	0.9	914.9	1.637	10.86	108.7	107.4	88.1
1	2	0.9	914.9	1.651	11.06	110.6		87.9
	3	0.9	914.9	1.595	10.29	102.9		88.8
	1	0.9	914.9	1.735	12.21	122.1	123.8	86.7
2	2	0.9	914.9	1.760	12.56	125.6		86.3
	3	0.9	914.9	1.746	12.36	123.7		86.5
	1	0.9	914.9	1.840	13.66	136.6	134.5	85.1
3	2	0.9	914.9	1.829	13.51	135.1		85.2
	3	0.9	914.9	1.807	13.20	132.0		85.6

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-19. Water wash removal results for peanut allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.5	3389.7	2.086	16.68	333.6	335.0	90.2
+	2	1.5	3389.7	2.095	16.78	335.6		90.1
	3	1.5	3389.7	2.096	16.79	335.9		90.1
	1	0.0	0.0	0.540	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.531	<2.5	0.0		-
	3	0.0	0.0	0.521	<2.5	0.0		-
	1	1.0	2259.8	1.752	12.88	257.6	257.6	88.6
1	2	1.0	2259.8	1.786	13.27	265.3		88.3
	3	1.0	2259.8	1.718	12.49	249.9		88.9
	1	1.0	2259.8	1.034	4.711	94.22	94.22	95.8
2	2	1.0	2259.8	1.041	4.791	95.81		95.8
	3	1.0	2259.8	1.027	4.631	92.63		95.9
	1	1.2	2711.8	1.040	4.779	95.59	94.60	96.5
3	2	1.2	2711.8	1.039	4.768	95.36		96.5
	3	1.2	2711.8	1.028	4.643	92.86		96.6

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-20. Water wash removal results for peanut allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.5	3389.7	2.429	20.58	411.6	412.3	87.9
+	2	1.5	3389.7	2.420	20.48	409.6		87.9
	3	1.5	3389.7	2.447	20.79	415.7		87.7
	1	0.0	0.0	0.432	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.435	<2.5	0.0		-
	3	0.0	0.0	0.429	<2.5	0.0		-
	1	1.3	2937.7	1.130	5.803	116.1	113.0	96.0
1	2	1.3	2937.7	1.121	5.701	114.0		96.1
	3	1.3	2937.7	1.099	5.451	109.0		96.3
	1	1.1	2485.8	0.820	2.276	116.1	40.83	98.2
2	2	1.1	2485.8	0.811	2.174	114.0		98.3
	3	1.1	2485.8	0.767	1.673	109.0		98.7
	1	1.1	2485.8	1.323	7.999	45.53	159.4	93.6
3	2	1.1	2485.8	1.323	7.999	43.48		93.6
	3	1.1	2485.8	1.316	7.919	33.47		93.6

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2]An absorbance reading equal to 3.000 indicates the highest value read by the machine.

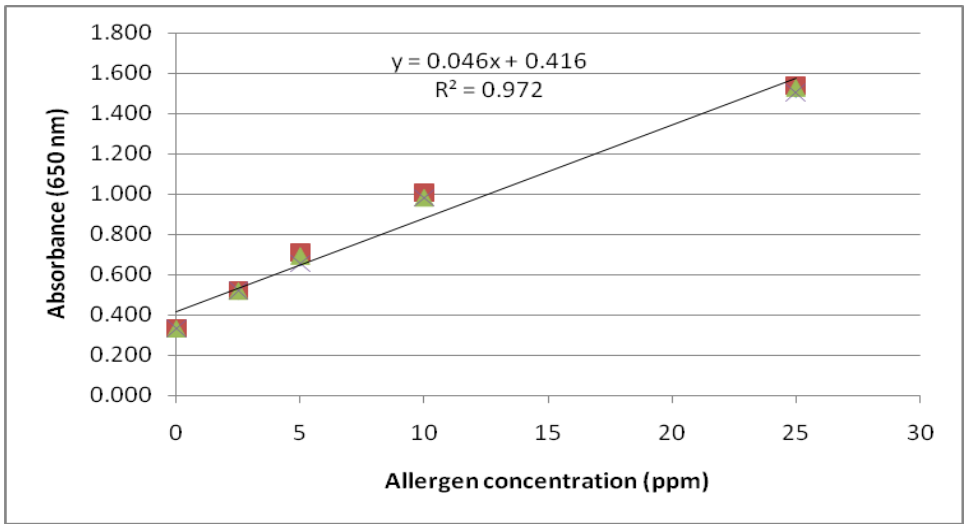

Figure 4-4. Standard curve for peanut allergen quantification test 2. Peanut controls contained 0, 2.5, 5, 10, and 25 ppm of allergen. Each concentration was read 3 times at an absorbance of 650 nm and plotted in the graph. The linear regression equation was calculated using the average of the 3 readings.

Table 4-21. Water wash removal results for milk allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.9	318.8	2.945	33.30	333.0	555.9	-101
+	2	0.9	318.8	2.959	33.48	334.8		-93.7
	3	0.9	318.8	2.923	33.01	330.1		-94.3
	1	0.0	0.0	0.431	<2.5	6.93	4.71	-
-	2	0.0	0.0	0.437	<2.5	7.70		-
	3	0.0	0.0	0.429	<2.5	6.67		-
	1	0.9	354.2	0.443	<2.5	8.48	44.59	82.8
1	2	0.9	354.2	0.437	<2.5	7.70		87.6
	3	0.9	354.2	0.419	<2.5	5.37		87.7
	1	1.0	354.2	0.620	3.144	31.44	5.09	95.7
2	2	1.0	354.2	0.624	3.196	31.96		100
	3	1.0	354.2	0.621	3.157	31.57		100
	1	1.0	354.2	0.508	<2.5	16.91	2.82	98.0
3	2	1.0	354.2	0.510	<2.5	17.17		100
	3	1.0	354.2	0.495	<2.5	15.23		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-22. Water wash removal results for milk allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.9	318.8	3.000	34.01	340.1	336.5	-6.71
+	2	0.9	318.8	2.993	33.92	339.2		-6.42
	3	0.9	318.8	2.923	33.01	330.1		-3.57
	1	0.0	0.0	0.393	<2.5	1.997	2.39	-
-	2	0.0	0.0	0.415	<2.5	4.851		-
	3	0.0	0.0	0.380	<2.5	0.311		-
	1	1.0	354.2	0.439	<2.5	7.964	8.53	97.8
1	2	1.0	354.2	0.456	<2.5	10.17		97.1
	3	1.0	354.2	0.435	<2.5	7.445		97.9
	1	1.0	354.2	0.494	<2.5	15.10	15.96	95.7
2	2	1.0	354.2	0.513	<2.5	17.56		95.0
	3	1.0	354.2	0.495	<2.5	15.23		95.7
	1	1.0	354.2	0.387	<2.5	1.219	0.83	99.7
3	2	1.0	354.2	0.385	<2.5	0.960		99.7
	3	1.0	354.2	0.380	<2.5	0.311		99.9

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2]An absorbance reading equal to 3.000 indicates the highest value read by the machine.

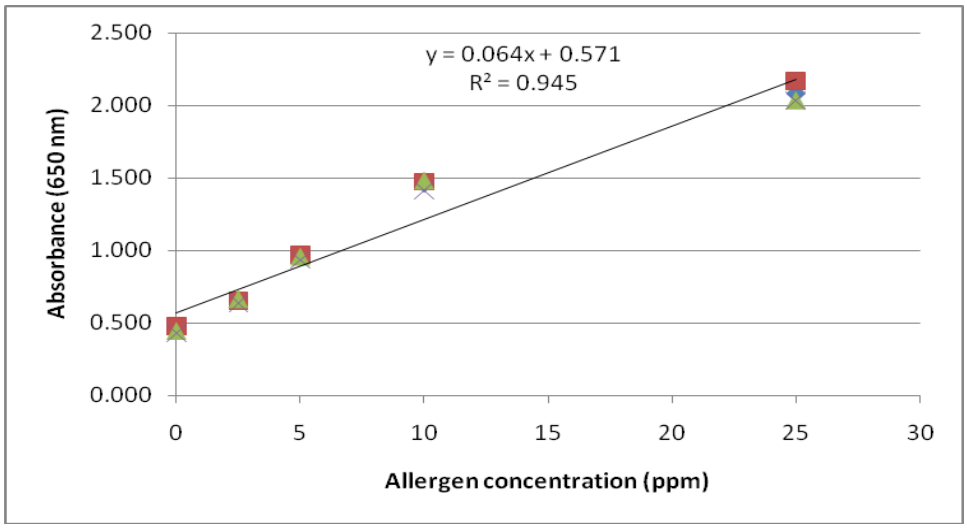

Figure 4-5. Standard curve for milk allergen quantification test 2. Milk controls contained 0, 2.5, 5, 10, and 25 ppm of allergen. Each concentration was read 3 times at an absorbance of 650 nm and plotted in the graph. The linear regression equation was calculated using the average of the 3 readings.

Table 4-23. Water wash removal results for egg allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.1	1118.2	2.775	27.70	277.0	273.9	75.2
+	2	1.1	1118.2	2.747	27.26	272.6		75.6
	3	1.1	1118.2	2.744	27.22	272.2		75.7
	1	0.0	0.0	0.621	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.625	<2.5	0.0		-
	3	0.0	0.0	0.625	<2.5	0.0		-
	1	0.9	914.9	0.640	<2.5	0.0	0.00	100
1	2	0.9	914.9	0.653	<2.5	0.0		100
	3	0.9	914.9	0.641	<2.5	0.0		100
	1	0.9	914.9	0.706	<2.5	0.0	0.00	100
2	2	0.9	914.9	0.698	<2.5	0.0		100
	3	0.9	914.9	0.703	<2.5	0.0		100
	1	0.8	813.2	0.828	<2.5	0.0	0.00	100
3	2	0.8	813.2	0.815	<2.5	0.0		100
	3	0.8	813.2	0.83	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-24. Water wash removal results for egg allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.1	1118.2	2.696	26.46	264.6	254.7	76.3
+	2	1.1	1118.2	2.585	24.72	247.2		77.9
	3	1.1	1118.2	2.618	25.24	252.4		77.4
	1	0.0	0.0	0.730	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.711	<2.5	0.0		-
	3	0.0	0.0	0.700	<2.5	0.0		-
	1	1.1	1118.2	0.703	<2.5	0.0	0.00	100
1	2	1.1	1118.2	0.709	<2.5	0.0		100
	3	1.1	1118.2	0.703	<2.5	0.0		100
	1	1.2	1219.8	0.661	<2.5	0.0	0.00	100
2	2	1.2	1219.8	0.639	<2.5	0.0		100
	3	1.2	1219.8	0.636	<2.5	0.0		100
	1	1.0	1016.5	0.513	<2.5	0.0	0.00	100
3	2	1.0	1016.5	0.512	<2.5	0.0		100
	3	1.0	1016.5	0.512	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

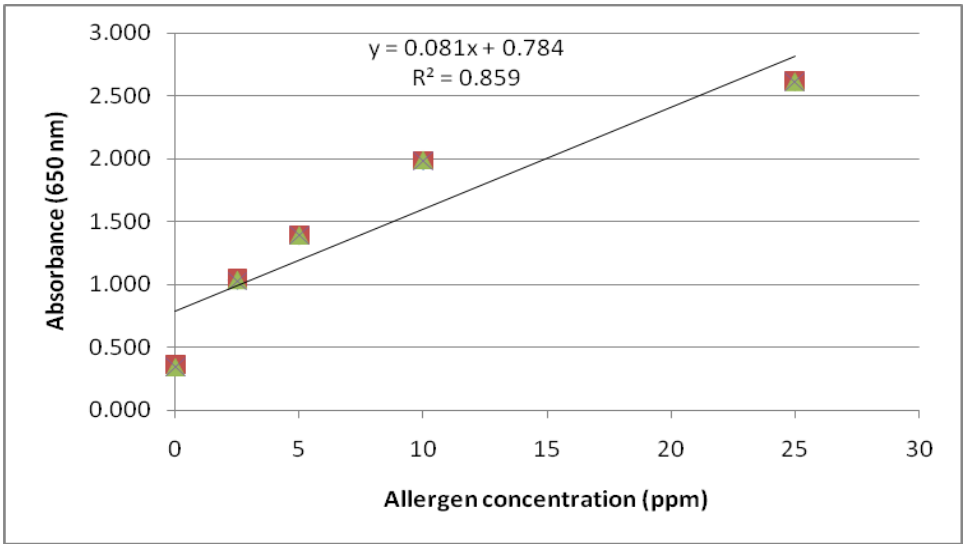

Figure 4-6. Standard curve for egg allergen quantification test 2. Egg controls contained 0, 2.5, 5, 10, and 25 ppm of allergen. Each concentration was read 3 times at an absorbance of 650 nm and plotted in the graph. The linear regression equation was calculated using the average of the 3 readings.

Table 4-25. JPA type 4 wash removal results for peanut allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.8	4067.6	2.068	43.24	864.9	825.0	78.7
+	2	1.8	4067.6	1.950	40.28	805.6		80.2
	3	1.8	4067.6	1.948	40.23	804.6		80.2
	1	0.0	0.0	0.340	<2.5	0.0	0.02	-
-	2	0.0	0.0	0.347	<2.5	0.1		-
	3	0.0	0.0	0.341	<2.5	0.0		-
	1	1.0	2259.8	0.338	<2.5	0.0	1.21	100
1	2	1.0	2259.8	0.351	<2.5	2.1		99.9
	3	1.0	2259.8	0.350	<2.5	1.6		99.9
	1	1.2	2711.8	0.358	<2.5	5.6	7.09	99.8
2	2	1.2	2711.8	0.367	<2.5	10.1		99.6
	3	1.2	2711.8	0.358	<2.5	5.6		99.8
	1	0.9	2033.8	0.332	<2.5	0.0	0.00	100
3	2	0.9	2033.8	0.340	<2.5	0.0		100
	3	0.9	2033.8	0.337	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-26. JPA type 4 wash removal results for peanut allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.8	1807.8	2.318	49.52	990.5	1001.9	45.2
+	2	0.8	1807.8	2.357	50.50	1010.1		44.1
	3	0.8	1807.8	2.347	50.25	1005.1		44.4
	1	0.0	0.0	0.339	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.339	<2.5	0.0		-
	3	0.0	0.0	0.337	<2.5	0.0		-
	1	0.9	2033.8	0.339	<2.5	0.0	0.00	100
1	2	0.9	2033.8	0.344	<2.5	0.0		100
	3	0.9	2033.8	0.346	<2.5	0.0		100
	1	1.0	2259.8	0.333	<2.5	0.0	0.00	100
2	2	1.0	2259.8	0.334	<2.5	0.0		100
	3	1.0	2259.8	0.332	<2.5	0.0		100
	1	1.2	2711.8	0.355	<2.5	0.0	0.00	100
3	2	1.2	2711.8	0.360	<2.5	0.0		100
	3	1.2	2711.8	0.356	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

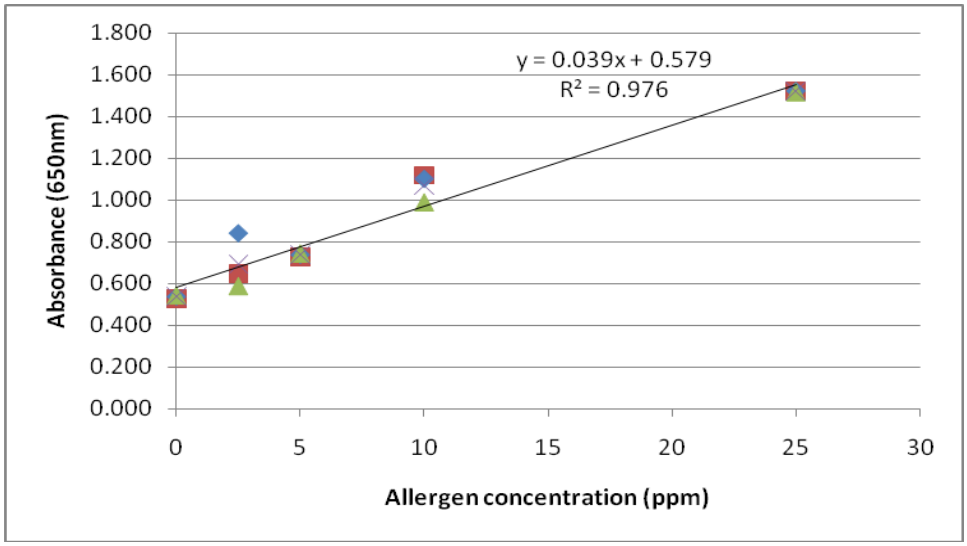

Figure 4-7. Standard curve for peanut allergen quantification for water treatment. Peanut controls contained 0, 2.5, 5, 10, and 25 ppm of allergen. Each concentration was read 3 times at an absorbance of 650 nm and plotted in the graph. The linear regression equation was calculated using the average of the 3 readings.

Table 4-27. JPA type 4 wash removal results for milk allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.9	318.6	2.987	57.21	572.1	573.5	-79.5
+	2	0.9	318.6	2.998	57.46	574.6		-80.3
	3	0.9	318.6	2.995	57.39	573.9		-80.1
	1	0.0	0.0	0.444	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.443	<2.5	0.0		-
	3	0.0	0.0	0.439	<2.5	0.0		-
	1	1.1	389.6	0.497	<2.5	0.0	0.00	100
1	2	1.1	389.6	0.493	<2.5	0.0		100
	3	1.1	389.6	0.485	<2.5	0.0		100
	1	1.2	425.0	0.456	<2.5	0.0	0.00	100
2	2	1.2	425.0	0.453	<2.5	0.0		100
	3	1.2	425.0	0.452	<2.5	0.0		100
	1	1.2	425.0	0.444	<2.5	0.0	0.00	100
3	2	1.2	425.0	0.447	<2.5	0.0		100
	3	1.2	425.0	0.439	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-28. JPA type 4 wash removal results for milk allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.9	318.6	2.961	56.62	566.2	565.8	-77.6
+	2	0.9	318.6	2.944	56.23	562.3		-76.4
	3	0.9	318.6	2.973	56.89	568.9		-78.5
	1	0.0	0.0	0.432	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.435	<2.5	0.0		-
	3	0.0	0.0	0.428	<2.5	0.0		-
	1	1.1	389.6	0.448	<2.5	0.0	0.00	100
1	2	1.1	389.6	0.456	<2.5	0.0		100
	3	1.1	389.6	0.438	<2.5	0.0		100
	1	1.0	354.2	0.393	<2.5	0.0	0.00	100
2	2	1.0	354.2	0.397	<2.5	0.0		100
	3	1.0	354.2	0.392	<2.5	0.0		100
	1	1.0	354.2	0.412	<2.5	0.0	0.00	100
3	2	1.0	354.2	0.416	<2.5	0.0		100
	3	1.0	354.2	0.409	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

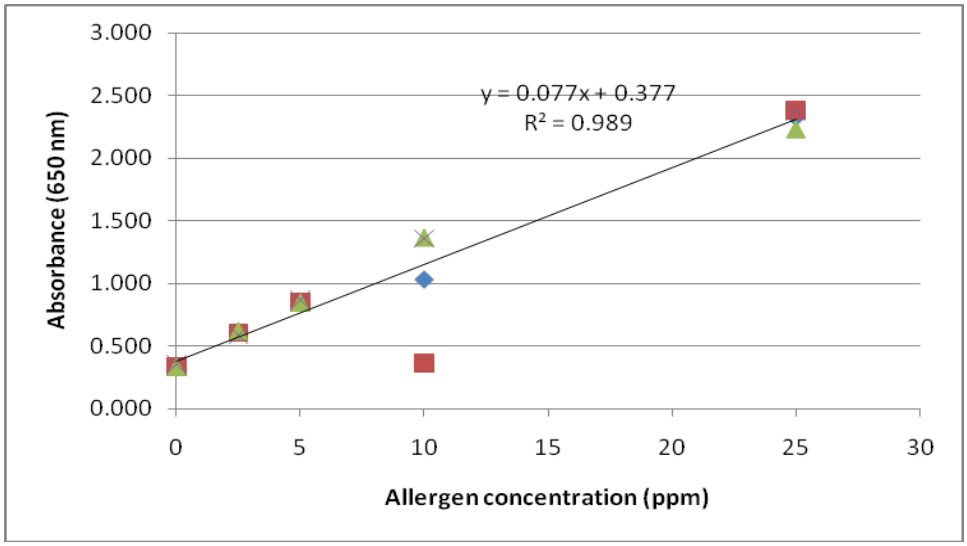

Figure 4-8. Standard curve for milk allergen quantification for water treatment. Milk controls contained 0, 2.5, 5, 10, and 25 ppm of allergen. Each concentration was read 3 times at an absorbance of 650 nm and plotted in the graph. The linear regression equation was calculated using the average of the 3 readings.

Table 4-29. JPA type 4 wash removal results for egg allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.8	813.2	2.932	28.66	286.6	292.8	64.8
+	2	0.8	813.2	3.000	29.59	295.9		63.6
	3	0.8	813.2	3.000	29.59	295.9		63.6
	1	0.0	0.0	0.335	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.329	<2.5	0.0		-
	3	0.0	0.0	0.320	<2.5	0.0		-
	1	1.0	1016.5	0.325	<2.5	0.0	0.00	100
1	2	1.0	1016.5	0.323	<2.5	0.0		100
	3	1.0	1016.5	0.318	<2.5	0.0		100
	1	1.1	1118.2	0.317	<2.5	0.0	0.00	100
2	2	1.1	1118.2	0.315	<2.5	0.0		100
	3	1.1	1118.2	0.309	<2.5	0.0		100
	1	1.0	1016.5	0.363	<2.5	0.0	0.00	100
3	2	1.0	1016.5	0.356	<2.5	0.0		100
	3	1.0	1016.5	0.320	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-30. JPA type 4 wash removal results for egg allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.6	609.9	3.000	29.59	295.9	295.9	51.5
+	2	0.6	609.9	3.000	29.59	295.9		51.5
	3	0.6	609.9	3.000	29.59	295.9		51.5
	1	0.0	0.0	0.285	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.285	<2.5	0.0		-
	3	0.0	0.0	0.286	<2.5	0.0		-
	1	1.0	1016.5	0.332	<2.5	0.0	0.00	100
1	2	1.0	1016.5	0.320	<2.5	0.0		100
	3	1.0	1016.5	0.317	<2.5	0.0		100
	1	1.1	1118.2	0.306	<2.5	0.0	0.00	100
2	2	1.1	1118.2	0.307	<2.5	0.0		100
	3	1.1	1118.2	0.307	<2.5	0.0		100
	1	1.0	1016.5	0.312	<2.5	0.0	0.00	100
3	2	1.0	1016.5	0.314	<2.5	0.0		100
	3	1.0	1016.5	0.308	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

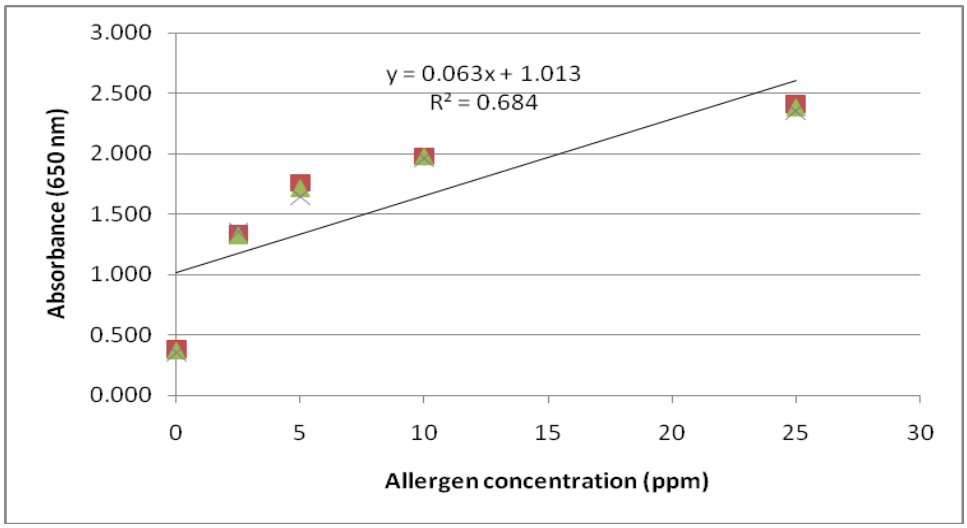

Figure 4-9. Standard curve for egg allergen quantification for water treatment. Egg controls contained 0, 2.5, 5, 10, and 25 ppm of allergen. Each concentration was read 3 times at an absorbance of 650 nm and plotted in the graph. The linear regression equation was calculated using the average of the 3 readings.

Table 4-31. CAD wash removal results for peanut allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.3	2937.7	3.000	66.66	1333.2	1333.2	54.6
+	2	1.3	2937.7	3.000	66.66	1333.2		54.6
	3	1.3	2937.7	3.000	66.66	1333.2		54.6
	1	0.0	0.0	0.411	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.361	<2.5	0.0		-
	3	0.0	0.0	0.354	<2.5	0.0		-
	1	1.0	2259.8	0.337	<2.5	0.0	0.00	100
1	2	1.0	2259.8	0.342	<2.5	0.0		100
	3	1.0	2259.8	0.336	<2.5	0.0		100
	1	1.2	2711.8	0.340	<2.5	0.0	0.00	100
2	2	1.2	2711.8	0.346	<2.5	0.0		100
	3	1.2	2711.8	0.339	<2.5	0.0		100
	1	1.1	2485.78	0.337	<2.5	0.0	0.00	100
3	2	1.1	2485.78	0.342	<2.5	0.0		100
	3	1.1	2485.78	0.336	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-32. CAD wash removal results for peanut allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.1	2485.8	2.579	56.08	1121.7	1131.5	54.9
+	2	1.1	2485.8	2.615	56.98	1139.8		54.2
	3	1.1	2485.8	2.602	56.66	1133.2		54.4
	1	0.0	0.0	0.359	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.352	<2.5	0.0		-
	3	0.0	0.0	0.355	<2.5	0.0		-
	1	1.0	2259.8	0.352	<2.5	0.0	0.00	100
1	2	1.0	2259.8	0.335	<2.5	0.0		100
	3	1.0	2259.8	0.343	<2.5	0.0		100
	1	0.9	2033.8	0.338	<2.5	0.0	0.00	100
2	2	0.9	2033.8	0.335	<2.5	0.0		100
	3	0.9	2033.8	0.335	<2.5	0.0		100
	1	1.2	2711.8	0.339	<2.5	0.0	0.00	100
3	2	1.2	2711.8	0.331	<2.5	0.0		100
	3	1.2	2711.8	0.334	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-33. CAD wash removal results for milk allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.0	354.2	3.000	57.51	575.1	575.1	-62.4
+	2	1.0	354.2	3.000	57.51	575.1		-62.4
	3	1.0	354.2	3.000	57.51	575.1		-62.4
	1	0.0	0.0	0.448	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.449	<2.5	0.0		-
	3	0.0	0.0	0.447	<2.5	0.0		-
	1	0.9	318.7	0.413	<2.5	0.0	0.00	100
1	2	0.9	318.7	0.419	<2.5	0.0		100
	3	0.9	318.7	0.417	<2.5	0.0		100
	1	1.0	354.2	0.425	<2.5	0.0	0.00	100
2	2	1.0	354.2	0.402	<2.5	0.0		100
	3	1.0	354.2	0.401	<2.5	0.0		100
	1	1.0	354.2	0.495	<2.5	0.0	0.00	100
3	2	1.0	354.2	0.431	<2.5	0.0		100
	3	1.0	354.2	0.426	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-34. CAD wash removal results for milk allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.8	283.3	3.000	57.51	575.1	575.1	-103
+	2	0.8	283.3	3.000	57.51	575.1		-103
	3	0.8	283.3	3.000	57.51	575.1		-103
	1	0.0	0.0	0.495	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.497	<2.5	0.0		-
	3	0.0	0.0	0.490	<2.5	0.0		-
	1	1.0	354.2	0.407	<2.5	0.0	0.00	100
1	2	1.0	354.2	0.408	<2.5	0.0		100
	3	1.0	354.2	0.406	<2.5	0.0		100
	1	0.9	318.8	0.407	<2.5	0.0	0.00	100
2	2	0.9	318.8	0.404	<2.5	0.0		100
	3	0.9	318.	0.408	<2.5	0.0		100
	1	1.2	425.0	0.537	<2.5	0.0	0.00	100
3	2	1.2	425.0	0.536	<2.5	0.0		100
	3	1.2	425.0	0.535	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-35. CAD wash removal results for egg allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.1	1118.2	3.000	29.59	295.9	295.9	73.5
+	2	1.1	1118.2	3.000	29.59	295.9		73.5
	3	1.1	1118.2	3.000	29.59	295.9		73.5
	1	0.0	0.0	0.337	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.335	<2.5	0.0		-
	3	0.0	0.0	0.329	<2.5	0.0		-
	1	1.0	1016.5	0.424	<2.5	0.0	0.00	100
1	2	1.0	1016.5	0.412	<2.5	0.0		100
	3	1.0	1016.5	0.451	<2.5	0.0		100
	1	1.1	1118.2	0.314	<2.5	0.0	0.00	100
2	2	1.1	1118.2	0.313	<2.5	0.0		100
	3	1.1	1118.2	0.306	<2.5	0.0		100
	1	1.0	1016.5	0.327	<2.5	0.0	0.00	100
3	2	1.0	1016.5	0.322	<2.5	0.0		100
	3	1.0	1016.5	0.314	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-36. CAD wash removal results for egg allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.8	813.2	3.000	29.59	295.9	295.9	63.6
+	2	0.8	813.2	3.000	29.59	295.9		63.6
	3	0.8	813.2	3.000	29.59	295.9		63.6
	1	0.0	0.0	0.322	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.314	<2.5	0.0		-
	3	0.0	0.0	0.319	<2.5	0.0		-
	1	0.9	914.9	0.326	<2.5	0.0	0.00	100
1	2	0.9	914.9	0.320	<2.5	0.0		100
	3	0.9	914.9	0.321	<2.5	0.0		100
	1	1.1	1118.2	0.311	<2.5	0.0	0.00	100
2	2	1.1	1118.2	0.314	<2.5	0.0		100
	3	1.1	1118.2	0.313	<2.5	0.0		100
	1	1.2	1219.8	0.349	<2.5	0.0	0.00	100
3	2	1.2	1219.8	0.322	<2.5	0.0		100
	3	1.2	1219.8	0.330	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-37. AD wash removal results for peanut allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.4	3163.7	2.492	53.90	1077.9	1084.8	65.9
+	2	1.4	3163.7	2.519	54.58	1091.5		65.5
	3	1.4	3163.7	2.506	54.25	1085.0		65.7
	1	0.0	0.0	0.362	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.370	<2.5	0.00		-
	3	0.0	0.0	0.370	<2.5	0.00		-
	1	1.0	2259.8	0.578	3.522	70.43	69.86	96.9
1	2	1.0	2259.8	0.574	3.435	68.70		97.0
	3	1.0	2259.8	0.578	3.522	70.43		96.9
	1	1.1	2485.8	0.397	<2.5	0.0	0.00	100
2	2	1.1	2485.8	0.398	<2.5	0.0		100
	3	1.1	2485.8	0.397	<2.5	0.0		100
	1	1.0	2259.8	0.364	<2.5	0.0	0.00	100
3	2	1.0	2259.8	0.369	<2.5	0.0		100
	3	1.0	2259.8	0.370	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-38. AD wash removal results for peanut allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.1	2485.8	3.000	66.66	1333.2	1333.2	46.4
+	2	1.1	2485.8	3.000	66.66	1333.2		46.4
	3	1.1	2485.8	3.000	66.66	1333.2		46.4
	1	0.0	0.0	0.357	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.344	<2.5	0.0		-
	3	0.0	0.0	0.339	<2.5	0.0		-
	1	1.0	2259.8	0.458	0.913	18.26	15.22	99.2
1	2	1.0	2259.8	0.447	0.674	13.48		99.4
	3	1.0	2259.8	0.448	0.696	13.91		99.4
	1	1.0	2259.8	0.441	0.543	10.87	9.42	99.5
2	2	1.0	2259.8	0.437	0.457	9.13		99.6
	3	1.0	2259.8	0.435	0.413	8.26		99.6
	1	1.1	2485.8	0.372	<2.5	0.0	0.00	100
3	2	1.1	2485.8	0.369	<2.5	0.0		100
	3	1.1	2485.8	0.366	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-39. AD wash removal results for milk allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.8	283.3	2.970	56.82	568.2	555.9	-100
+	2	0.8	283.3	2.885	54.89	548.9		-93.7
	3	0.8	283.3	2.892	55.05	550.5		-94.3
	1	0.0	0.0	0.431	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.433	<2.5	0.0		-
	3	0.0	0.0	0.424	<2.5	0.0		-
	1	0.9	318.8	1.380	12.64	126.4	124.8	60.3
1	2	0.9	318.8	1.381	12.66	126.6		60.3
	3	0.9	318.8	1.349	12.16	121.6		61.9
	1	1	354.2	0.623	<2.5	8.13	8.02	97.7
2	2	1	354.2	0.629	<2.5	9.06		97.4
	3	1	354.2	0.615	<2.5	6.88		98.1
	1	1.2	425.0	2.156	24.77	247.7	246.2	41.7
3	2	1.2	425.0	2.147	24.63	246.3		42.1
	3	1.2	425.0	2.137	24.47	244.7		42.4

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-40. AD wash removal results for milk allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.1	389.6	2.985	57.16	571.6	571.3	-46.7
+	2	1.1	389.6	2.980	57.05	570.5		-46.4
	3	1.1	389.6	2.985	57.16	571.6		-46.7
	1	0.0	0.0	0.443	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.439	<2.5	0.0		-
	3	0.0	0.0	0.438	<2.5	0.0		-
	1	1.0	354.2	0.765	3.031	30.31	25.94	91.4
1	2	1.0	354.2	0.737	2.594	25.94		92.7
	3	1.0	354.2	0.709	<2.5	21.56		93.9
	1	0.9	318.6	0.652	<2.5	12.66	12.66	96.0
2	2	0.9	318.6	0.651	<2.5	12.50		96.1
	3	0.9	318.6	0.653	<2.5	12.81		96.0
	1	1.0	354.2	0.642	<2.5	11.09	12.19	96.9
3	2	1.0	354.2	0.647	<2.5	11.88		96.7
	3	1.0	354.2	0.658	<2.5	13.59		96.2

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-41. AD wash removal results for egg allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.3	1321.5	2.947	28.86	288.6	287.0	78.2
+	2	1.3	1321.5	2.937	28.72	287.3		78.2
	3	1.3	1321.5	2.921	28.51	285.1		78.4
	1	0.0	0.0	0.318	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.318	<2.5	0.0		-
	3	0.0	0.0	0.318	<2.5	0.0		-
	1	0.9	914.9	1.374	7.284	72.84	72.76	92.0
1	2	0.9	914.9	1.376	7.309	73.09		92.0
	3	0.9	914.9	1.370	7.235	72.35		92.1
	1	1.0	1016.5	1.305	6.432	64.32	64.77	93.7
2	2	1.0	1016.5	1.312	6.519	65.19		93.6
	3	1.0	1016.5	1.309	6.481	64.81		93.6
	1	0.9	914.9	1.382	7.383	73.83	74.28	91.9
3	2	0.9	914.9	1.391	7.494	74.94		91.8
	3	0.9	914.9	1.384	7.407	74.07		91.9

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-42. AD wash removal results for egg allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.9	914.9	3.000	29.59	295.9	295.9	67.7
+	2	0.9	914.9	3.000	29.59	295.9		67.7
	3	0.9	914.9	3.000	29.59	295.9		67.7
	1	0.0	0.0	0.321	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.314	<2.5	0.0		-
	3	0.0	0.0	0.316	<2.5	0.0		-
	1	1.0	1016.5	1.524	9.136	91.4	83.00	91.0
1	2	1.0	1016.5	1.418	7.827	78.3		92.3
	3	1.0	1016.5	1.427	7.938	79.4		92.2
	1	1.0	1016.5	1.432	8.000	80.0	77.82	92.1
2	2	1.0	1016.5	1.407	7.691	76.9		92.4
	3	1.0	1016.5	1.404	7.654	76.5		92.5
	1	1.2	1219.8	1.657	10.78	108	83.05	91.2
3	2	1.2	1219.8	1.356	7.061	70.6		94.2
	3	1.2	1219.8	1.357	7.074	70.7		94.2

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-43. Water wash removal results for peanut allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.5	3389.7	2.086	16.68	333.6	335.0	90.2
+	2	1.5	3389.7	2.095	16.78	335.6		90.1
	3	1.5	3389.7	2.096	16.79	335.9		90.1
	1	0.0	0.0	0.540	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.531	<2.5	0.0		-
	3	0.0	0.0	0.521	<2.5	0.0		-
	1	1.1	2485.8	1.791	13.32	266.5	267.4	89.3
1	2	1.1	2485.8	1.803	13.46	269.2		89.2
	3	1.1	2485.8	1.791	13.32	266.5		89.3
	1	1.0	2259.8	1.449	9.432	188.6	187.9	91.7
2	2	1.0	2259.8	1.448	9.421	188.4		91.7
	3	1.0	2259.8	1.440	9.330	186.6		91.7
	1	1.1	2485.8	0.672	<2.5	11.85	11.17	99.5
3	2	1.1	2485.8	0.675	<2.5	12.54		99.5
	3	1.1	2485.8	0.660	<2.5	9.124		99.6

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-44. Water wash removal results for peanut allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.5	3389.7	2.429	52.31	1046.3	1047.8	69.1
+	2	1.5	3389.7	2.420	52.09	1041.8		69.3
	3	1.5	3389.7	2.447	52.77	1055.3		68.9
	1	0.0	0.0	0.432	<2.5	6.957	6.96	-
-	2	0.0	0.0	0.435	<2.5	8.261		-
	3	0.0	0.0	0.429	<2.5	5.652		-
	1	0.9	2033.8	1.132	15.57	311.3	307.8	84.7
1	2	0.9	2033.8	1.121	15.33	306.5		84.9
	3	0.9	2033.8	1.119	15.28	305.7		85.0
	1	0.9	2033.8	1.150	15.96	319.1	307.1	84.3
2	2	0.9	2033.8	1.120	15.30	306.1		85.0
	3	0.9	2033.8	1.097	14.80	296.1		85.4
	1	0.9	2033.8	0.818	8.739	174.8	173.3	91.4
3	2	0.9	2033.8	0.815	8.674	173.5		91.5
	3	0.9	2033.8	0.811	8.587	171.7		91.6

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-45. Water wash removal results for milk allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.9	2033.8	2.945	33.30	666.0	665.3	67.3
+	2	0.9	2033.8	2.959	33.48	669.6		67.1
	3	0.9	2033.8	2.923	33.01	660.3		67.5
	1	0.0	0.0	0.431	<2.5	6.926	7.099	-
-	2	0.0	0.0	0.437	<2.5	7.704		-
	3	0.0	0.0	0.429	<2.5	6.667		-
	1	1.1	389.6	0.540	<2.5	21.06	19.72	94.6
1	2	1.1	389.6	0.522	<2.5	18.73		95.2
	3	1.1	389.6	0.527	<2.5	19.38		95.0
	1	1.1	389.6	0.546	<2.5	21.84	18.12	94.4
2	2	1.1	389.6	0.535	<2.5	20.42		94.8
	3	1.1	389.6	0.471	<2.5	12.11		96.9
	1	1.1	389.6	0.549	<2.5	22.23	14.75	94.3
3	2	1.1	389.6	0.477	<2.5	12.89		96.7
	3	1.1	389.6	0.448	<2.5	9.131		97.7

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-46. Water wash removal results for milk allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	0.9	2033.8	3.000	34.01	340.1	336.5	83.3
+	2	0.9	2033.8	2.993	33.92	339.2		83.3
	3	0.9	2033.8	2.923	33.01	330.1		83.8
	1	0.0	0.0	0.393	<2.5	1.997	2.387	-
-	2	0.0	0.0	0.415	<2.5	4.851		-
	3	0.0	0.0	0.380	<2.5	0.311		-
	1	1.1	389.6	0.428	<2.5	6.537	4.375	98.3
1	2	1.1	389.6	0.421	<2.5	5.629		98.6
	3	1.1	389.6	0.385	<2.5	0.960		99.8
	1	1.2	425.0	0.348	<2.5	0.0	0.00	100
2	2	1.2	425.0	0.345	<2.5	0.0		100
	3	1.2	425.0	0.343	<2.5	0.0		100
	1	1.0	354.2	0.354	<2.5	0.0	0.00	100
3	2	1.0	354.2	0.353	<2.5	0.0		100
	3	1.0	354.2	0.346	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm
[2] An absorbance reading equal to 3.000 indicates the highest value read by the machine.

Table 4-47. Water wash removal results for egg allergens on unabraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.1	2485.8	2.775	27.70	277.0	273.95	88.9
+	2	1.1	2485.8	2.747	27.26	272.6		89.0
	3	1.1	2485.8	2.744	27.22	272.2		89.1
	1	0.0	0.0	0.621	<2.5	0.0	0.00	-
-	2	0.0	0.0	0.625	<2.5	0.0		-
	3	0.0	0.0	0.625	<2.5	0.0		-
	1	0.9	914.9	0.913	<2.5	0.0	0.00	100
1	2	0.9	914.9	0.920	<2.5	0.0		100
	3	0.9	914.9	0.948	<2.5	0.0		100
	1	1.0	1016.5	0.674	<2.5	0.0	0.00	100
2	2	1.0	1016.5	0.671	<2.5	0.0		100
	3	1.0	1016.5	0.675	<2.5	0.0		100
	1	1.1	1118.2	0.677	<2.5	0.0	0.00	100
3	2	1.1	1118.2	0.678	<2.5	0.0		100
	3	1.1	1118.2	0.676	<2.5	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

Table 4-48. Water wash removal results for egg allergens on abraded surfaces. Initial allergen concentration was calculated from the amount of protein indicated on the label, absorbance for each sample was read at 650 nm, final allergen concentration before dilution was calculated from the standard curve regression equation and multiplied by dilution factor, final concentrations for triplicates were averaged, and % reduction [(initial – final conc.)/ initial conc.]

Sample	Trial #	Initial weight (g)	Initial conc (ppm)	Absorbance (650 nm)	Final concentration[1] (ppm)	Conc after dilution (ppm)	Avg conc after dilution (ppm)	%Reduction
	1	1.1	2485.8	2.696	26.46	264.6	254.72	89.4
+	2	1.1	2485.8	2.585	24.72	247.2		90.1
	3	1.1	2485.8	2.618	25.24	252.4		89.8
	1	0.0	0.0	0.730	-4.450	0.0	0.00	-
-	2	0.0	0.0	0.711	-4.748	0.0		-
	3	0.0	0.0	0.700	-4.921	0.0		-
	1	0.9	914.9	0.844	-2.657	0.0	0.00	100
1	2	0.9	914.9	0.798	-3.381	0.0		100
	3	0.9	914.9	0.791	-3.491	0.0		100
	1	0.9	914.9	0.606	-6.399	0.0	0.00	100
2	2	0.9	914.9	0.599	-6.509	0.0		100
	3	0.9	914.9	0.598	-6.525	0.0		100
	1	0.9	914.9	0.610	-6.336	0.0	0.00	100
3	2	0.9	914.9	0.598	-6.525	0.0		100
	3	0.9	914.9	0.593	-6.604	0.0		100

[1]Allergen concentrations lower than the limit of quantitation of the test kit (2.5 ppm) are expressed as <2.5 ppm

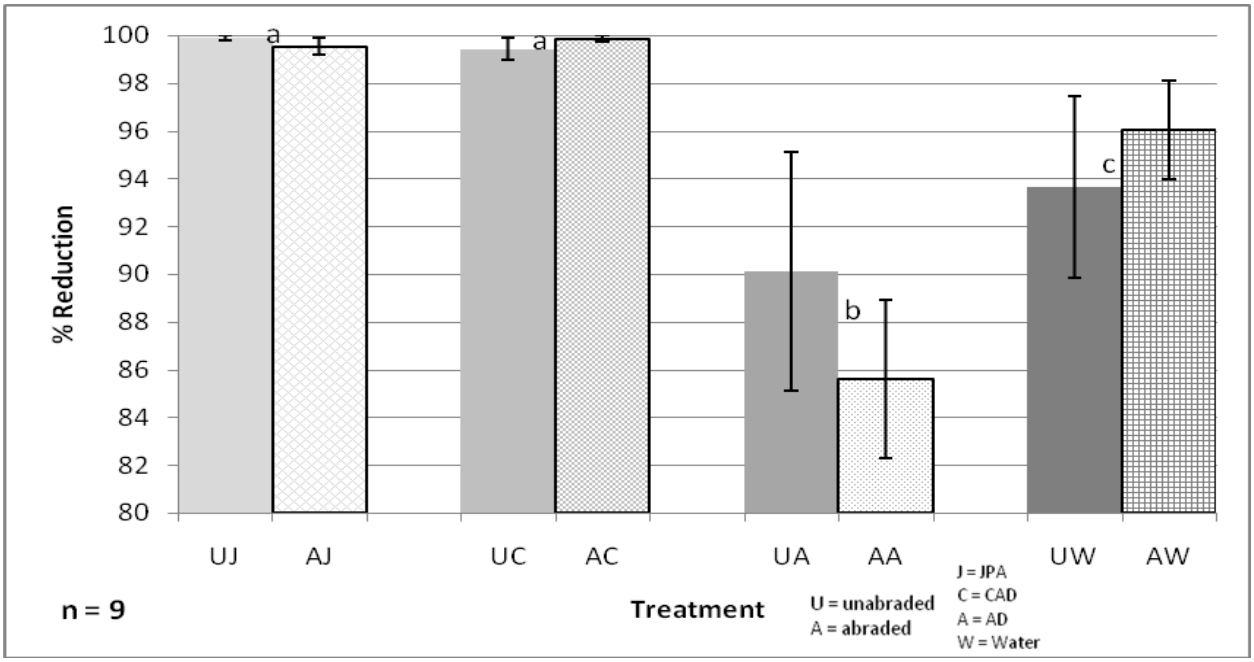

Figure 4-10. Peanut allergen reduction across methods in study 1. Each set of bars corresponds to unabraded surface (U) and abraded surface (A). From left to right, the order of treatments was JPA Type 4, CAD, AD, and Water. Each bar contains 9 observations. The variability is shown with standard error bars.

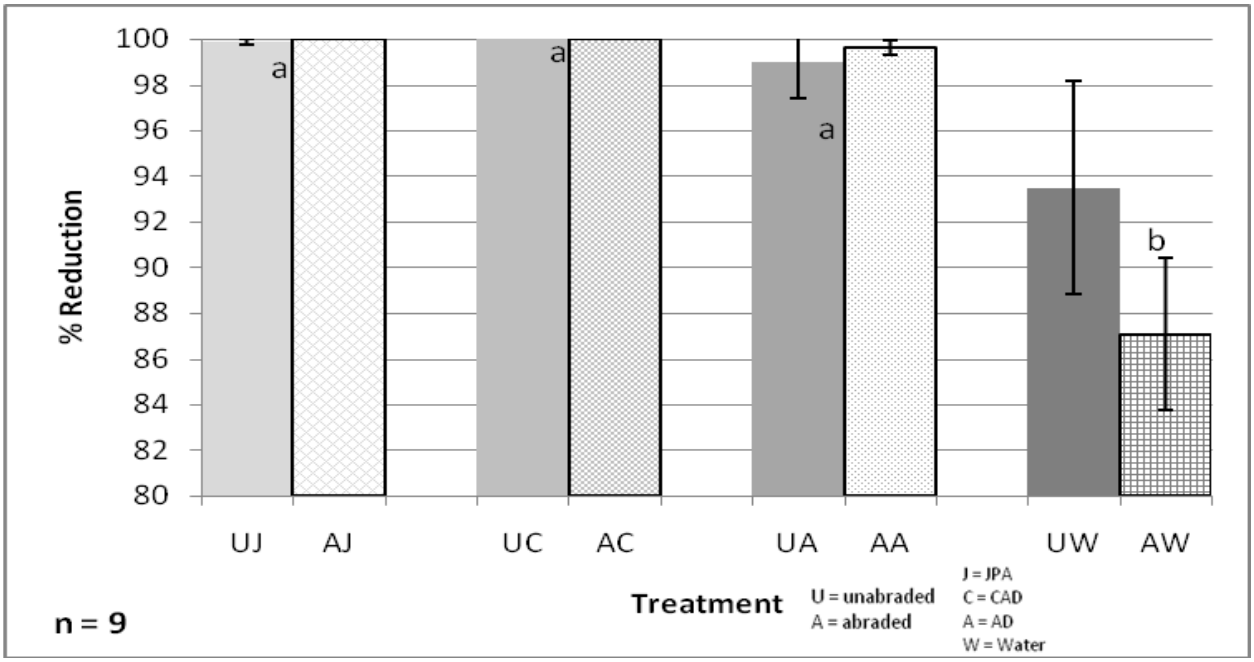

Figure 4-11. Peanut allergen reduction across methods in study 2. Each set of bars corresponds to unabraded surface (U) and abraded surface (A). From left to right, the order of treatments was JPA Type 4, CAD, AD, and Water. Each bar contains 9 observations. The variability is shown with standard error bars.

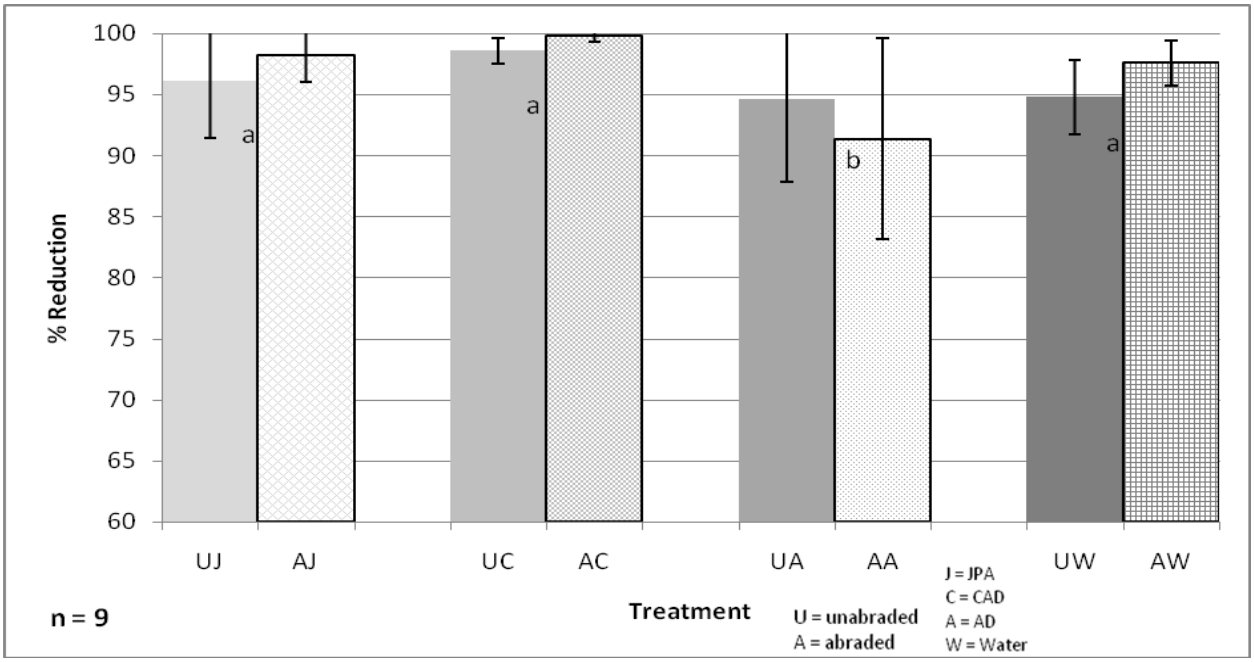

Figure 4-12. Milk allergen reduction across methods in study 1. Each set of bars corresponds to unabraded surface (U) and abraded surface (A). From left to right, the order of treatments was JPA Type 4, CAD, AD, and Water. Each bar contains 9 observations. The variability is shown with standard error bars.

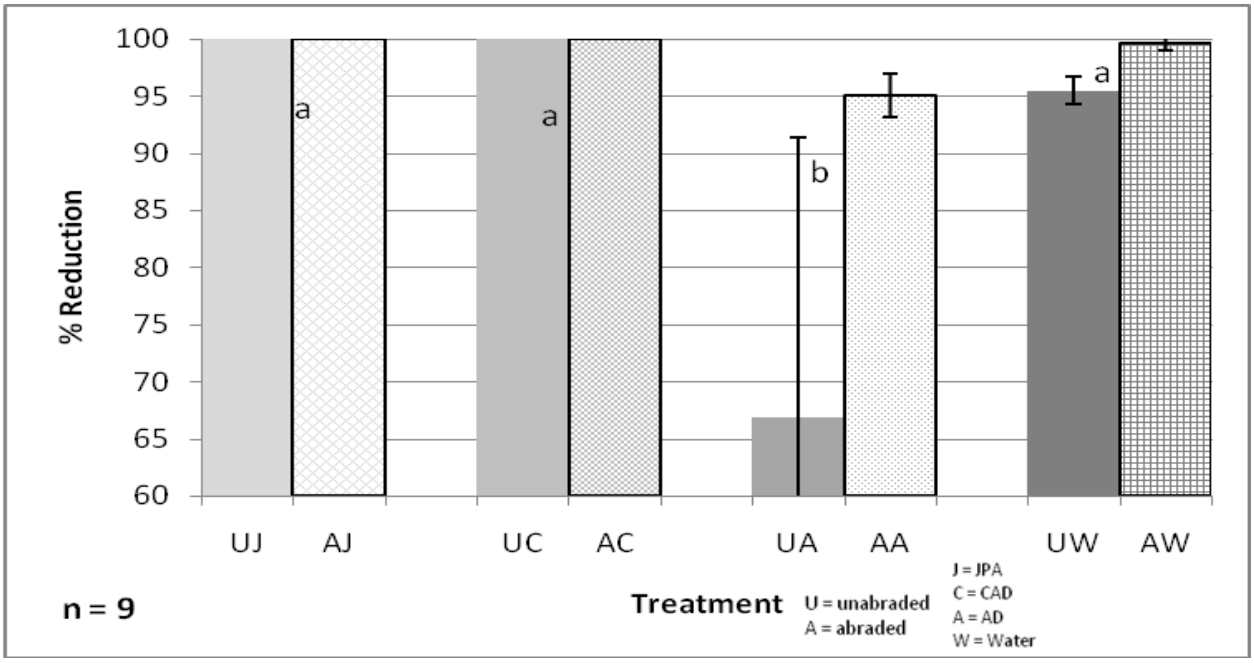

Figure 4-13. Milk allergen reduction across methods in study 2. Each set of bars corresponds to unabraded surface (U) and abraded surface (A). From left to right, the order of treatments was JPA Type 4, CAD, AD, and Water. Each bar contains 9 observations. The variability is shown with standard error bars.

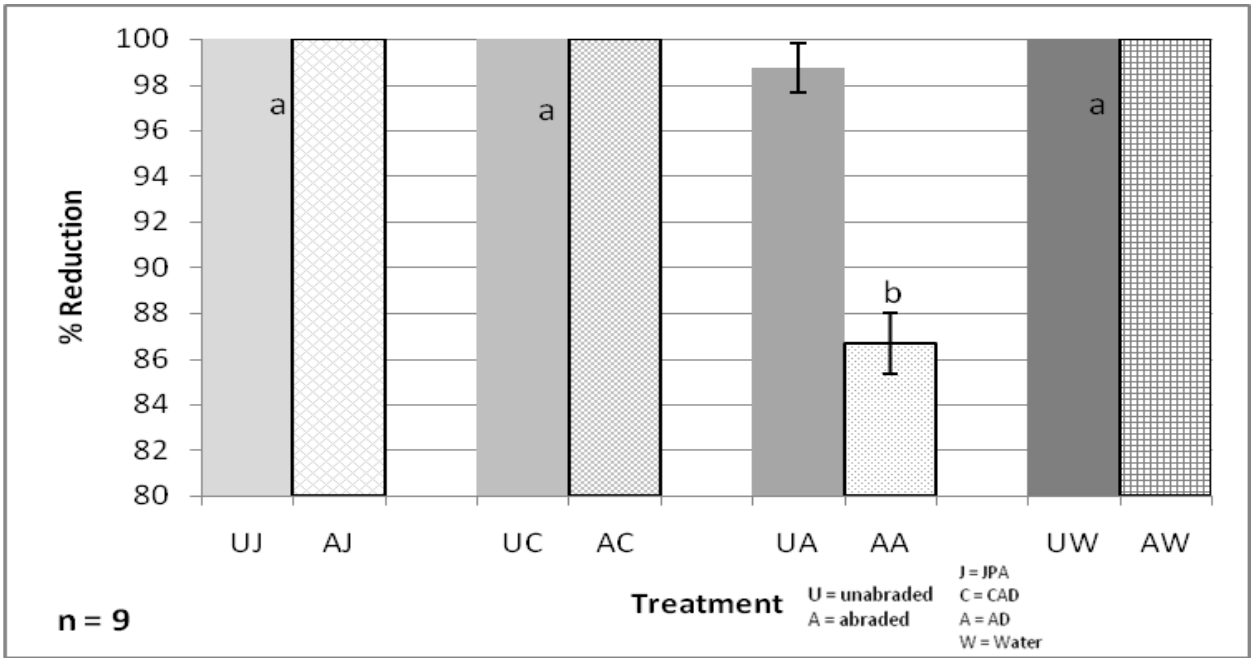

Figure 4-14. Egg allergen reduction across methods in study 1. Each set of bars corresponds to unabraded surface (U) and abraded surface (A). From left to right, the order of treatments was JPA Type 4, CAD, AD, and Water. Each bar contains 9 observations. The variability is shown with standard error bars.

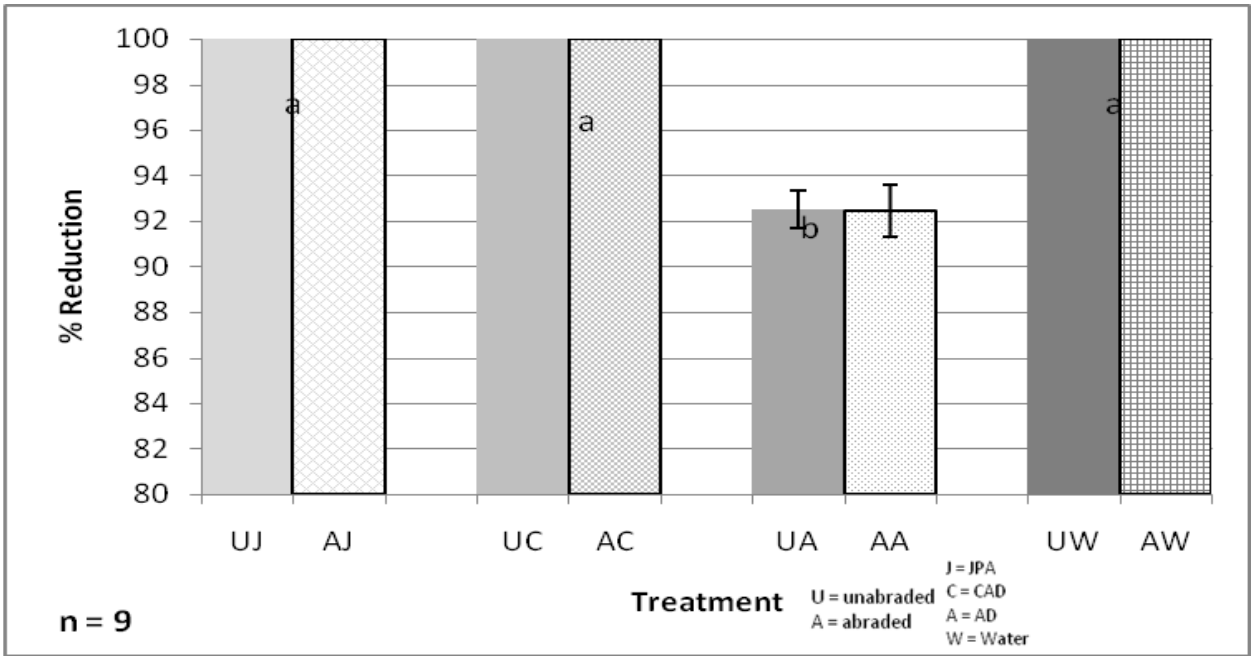

Figure 4-15. Egg allergen reduction across methods in study 2. Each set of bars corresponds to unabraded surface (U) and abraded surface (A). From left to right, the order of treatments was JPA Type 4, CAD, AD, and Water. Each bar contains 9 observations. The variability is shown with standard error bars.

CHAPTER 5
CONCLUSION

Food allergies are a major concern for a segment of consumers and have been on the rise in recent years. The Food Allergen Labeling and Consumer Protection Act, FALCPA, is a law that is currently in place to protect people that suffer from allergies, but their inadvertent or accidental presence in foods still remains a big concern for those severely affected. The only proven method towards preventing reactions lies in the avoidance of food allergens. Therefore, complete allergen removal from food processing plants that share equipment for allergen and non-allergen containing products is key. However, present techniques are not 100% effective in preventing allergen cross-contamination, and a consensus on the validation of cleaning protocols has not been reached within the food processing industry.

The present study compared four types of washes and two types of surfaces in the removal of peanut, milk, and egg allergens. In general, the JPA Type 4 wash and the CAD wash were the most effective methods for all allergens and surfaces. The acid detergent wash was not effective at all for removing milk allergens (88.0%), and had a fair performance for peanut and egg allergens. In addition, the water only wash performed better than the AD protocol, resulting in 100% reduction for egg allergens and almost 97% removal of milk allergens. The latter results were unexpected, especially since water removed more milk residues than the acid, which is a type of detergent commonly used in the dairy industry for cleaning.

Overall percent reductions were calculated for each allergen, where both types of surfaces and the two studies were taken into account. For peanut allergens, both JPA Type 4 and CAD washes had 99.8% reduction, followed by AD and water with 93.6 and 92.6% reduction, respectively. For milk allergens, CAD achieved 99.6% reduction, followed by JPA (98.6%),

water (96.9%), and finally AD (88.0%). Lastly, there was 100% reduction for egg allergens with the JPA, CAD, and water washes. The AD obtained a combined reduction of 93.4%.

Based on these results, it is possible to say that both the JPA and the CAD washes were the most effective methods across the board. Water was also very successful at removing egg and milk allergens to a certain extent. Conversely, the AD was unexpectedly ineffective, especially for milk residues. It is important to keep in mind that efficacy is based on 100% reduction which is needed for complete avoidance. The majority of the results have an average of 88% reduction and above, but the higher the achieved reduction, the lower the chances of someone getting sick from the presence of food allergens.

A second objective of this research was to validate the cleaning protocol that proved more successful at removing allergens. From the results obtained, we could say that for egg allergens all except for the acid detergent worked and could be validated. Even though high levels of reduction were seen for milk and peanut allergens with the CAD and modified JPA protocols, more studies need to be done to ensure reproducibility especially since there was variability in some of the samples for the JPA unabraded in study 1. For the peanut allergens, both the modified JPA and CAD methods seemed to work without so much variability and they could be validated if further studies are conducted proving these results.

The benefits of an effective allergen removal protocol are countless, including prevention of cross-contamination, which leads to safer products on the shelves, and ultimately the well-being of the end user by avoiding dangerous allergic reactions. The food industry will benefit from these techniques by ensuring allergic consumers that their products do not accidentally contain allergens. Interestingly, this study shows that three common cleaning protocols regularly used in the food industry did not yield the same results.

APPENDIX
STATISTICAL ANALYSIS

First Study

Table A-1. Statistical analysis of peanut allergen reduction across methods.
A) ANOVA. B) Means by surface. C) Means by treatment.

A)

The ANOVA Procedure

Dependent Variable: peanutconc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1314953.241	187850.463	40.73	<.0001
Error	64	295150.616	4611.728		
Corrected Total	71	1610103.857			

R-Square	Coeff Var	Root MSE	peanutconc Mean
0.816688	56.46638	67.90971	120.2657

Source	DF	Anova SS	Mean Square	F Value	Pr > F
surface	1	8688.630	8688.630	1.88	0.1747
treatment	3	1193271.742	397757.247	86.25	<.0001
surface*treatment	3	112992.869	37664.290	8.17	0.0001

B)

Duncan's Multiple Range Test for peanutconc

Alpha	0.05
Error Degrees of Freedom	64
Error Mean Square	4611.728

Number of Means	2
Critical Range	31.98

Duncan Grouping	Mean	N	surface
A	131.25	36	AB
A			
A	109.28	36	UN

C)

Duncan's Multiple Range Test for peanutconc

Alpha	0.05
Error Degrees of Freedom	64
Error Mean Square	4611.728

Number of Means	2	3	4
Critical Range	45.22	47.57	49.13

Duncan Grouping	Mean	N	treatment
A	328.16	18	AD
B	126.62	18	W
C	19.46	18	JPA
C			
C	6.83	18	CAD

Table A-2. Statistical analysis of milk allergen reduction across methods.
A) ANOVA. B) Means by surface. C) Means by treatment.

A)

The ANOVA Procedure

Dependent Variable: milkconc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	6942.94871	991.84982	3.74	0.0019
Error	64	16970.98449	265.17163		
Corrected Total	71	23913.93320			

R-Square	Coeff Var	Root MSE	milkconc Mean
0.290331	122.9051	16.28409	13.24932

Source	DF	Anova SS	Mean Square	F Value	Pr > F
surface	1	89.229214	89.229214	0.34	0.5639
treatment	3	4728.944782	1576.314927	5.94	0.0012
surface*treatment	3	2124.774710	708.258237	2.67	0.0549

B)

Duncan's Multiple Range Test for milkconc

Alpha	0.05
Error Degrees of Freedom	64
Error Mean Square	265.1716

Number of Means	2
Critical Range	7.668

Duncan Grouping	Mean	N	surface
A	14.363	36	UN
A			
A	12.136	36	AB

C)

Duncan's Multiple Range Test for milkconc

Alpha	0.05
Error Degrees of Freedom	64
Error Mean Square	265.1716

Number of Means	2	3	4
Critical Range	10.84	11.41	11.78

Duncan Grouping	Mean	N	treatment
A	25.603	18	AD
B	13.433	18	W
B			
B	10.949	18	JPA
B			
B	3.012	18	CAD

Table A-3. Statistical analysis of egg allergen reduction across methods.
A) ANOVA. B) Means by surface. C) Means by treatment.

A)

The ANOVA Procedure

Dependent Variable: eggconc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	114866.8748	16409.5535	523.59	<.0001
Error	64	2005.7778	31.3403		
Corrected Total	71	116872.6526			

R-Square	Coeff Var	Root MSE	eggconc Mean
0.982838	33.32808	5.598239	16.79736

Source	DF	Anova SS	Mean Square	F Value	Pr > F
surface	1	13480.54633	13480.54633	430.13	<.0001
treatment	3	60944.68950	20314.89650	648.20	<.0001
surface*treatment	3	40441.63900	13480.54633	430.13	<.0001

B)

Duncan's Multiple Range Test for eggconc

Alpha	0.05
Error Degrees of Freedom	64
Error Mean Square	31.34028

Number of Means	2
Critical Range	2.636

Duncan Grouping	Mean	N	surface
A	30.481	36	AB
B	3.114	36	UN

C)

Duncan's Multiple Range Test for eggconc

Alpha	0.05
Error Degrees of Freedom	64
Error Mean Square	31.34028

Number of Means	2	3	4
Critical Range	3.728	3.922	4.050

Duncan Grouping	Mean	N	treatment
A	67.189	18	AD
B	0.000	18	CAD
B			
B	0.000	18	JPA
B			
B	0.000	18	W

Second Study

Table A-4. Statistical analysis of peanut allergen reduction across methods. ANOVA. B) Means by surface. C) Means by treatment.

A)

The ANOVA Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	614106.5228	87729.5033	37.51	<.0001
Error	64	149704.6851	2339.1357		
Corrected Total	71	763811.2079			

R-Square	Coeff Var	Root MSE	peanutconc Mean
0.804003	85.51009	48.36461	56.56013

Source	DF	Anova SS	Mean Square	F Value	Pr > F
surface	1	9001.2641	9001.2641	3.85	0.0542
treatment	3	561254.6781	187084.8927	79.98	<.0001
surface*treatment	3	43850.5807	14616.8602	6.25	0.0009

B)

Duncan's Multiple Range Test for peanutconc

Alpha	0.05
Error Degrees of Freedom	64
Error Mean Square	2339.136

Number of Means	2
Critical Range	22.77

Duncan Grouping	Mean	N	surface
A	67.74	36	AB
A			
A	45.38	36	UN

C)

Duncan's Multiple Range Test for peanutconc

Alpha	0.05
Error Degrees of Freedom	64
Error Mean Square	2339.136

Number of Means	2	3	4
Critical Range	32.21	33.88	34.99

Duncan Grouping	Mean	N	treatment
A	209.11	18	W
B	15.75	18	AD
B			
B	1.38	18	JPA
B			
B	0.00	18	CAD

Table A-5. Statistical analysis of milk allergen reduction across methods.
A) ANOVA. B) Means by surface. C) Means by treatment.

A)

The ANOVA Procedure

Dependent Variable: milkconc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	5060593.339	722941.906	238.69	<.0001
Error	64	193838.520	3028.727		
Corrected Total	71	5254431.859			

R-Square	Coeff Var	Root MSE	milkconc Mean
0.963110	45.94537	55.03387	119.7811

Source	DF	Anova SS	Mean Square	F Value	Pr > F
surface	1	505676.018	505676.018	166.96	<.0001
treatment	3	2938571.223	979523.741	323.41	<.0001
surface*treatment	3	1616346.098	538782.033	177.89	<.0001

B)

Duncan's Multiple Range Test for milkconc

Alpha	0.05
Error Degrees of Freedom	64
Error Mean Square	3028.727

Number of Means	2
Critical Range	25.91

Duncan Grouping	Mean	N	surface
A	203.59	36	AB
B	35.98	36	UN

C)

Duncan's Multiple Range Test for milkconc

Alpha	0.05
Error Degrees of Freedom	64
Error Mean Square	3028.727

Number of Means	2	3	4
Critical Range	36.65	38.55	39.81

Duncan Grouping	Mean	N	treatment
A	469.63	18	AD
B	9.49	18	W
B			
B	0.00	18	JPA
B			
B	0.00	18	CAD

Table A-6. Statistical analysis of egg allergen reduction across methods.
A) ANOVA. B) Means by surface. C) Means by treatment.

A)

The ANOVA Procedure

Dependent Variable: eggconc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	78382.79862	11197.54266	576.39	<.0001
Error	64	1243.33591	19.42712		
Corrected Total	71	79626.13453			

R-Square	Coeff Var	Root MSE	eggconc Mean
0.984385	23.21396	4.407621	18.98694

Source	DF	Anova SS	Mean Square	F Value	Pr > F
surface	1	128.48045	128.48045	6.61	0.0125
treatment	3	77868.87682	25956.29227	1336.09	<.0001
surface*treatment	3	385.44135	128.48045	6.61	0.0006

B)

Duncan's Multiple Range Test for eggconc

Alpha	0.05
Error Degrees of Freedom	64
Error Mean Square	19.42712

Number of Means	2
Critical Range	2.075

Duncan Grouping	Mean	N	surface
A	20.323	36	AB
B	17.651	36	UN

C)

Duncan's Multiple Range Test for eggconc

Alpha	0.05
Error Degrees of Freedom	64
Error Mean Square	19.42712

Number of Means	2	3	4
Critical Range	2.935	3.088	3.189

Duncan Grouping	Mean	N	treatment
A	75.948	18	AD
B	0.000	18	CAD
B			
B	0.000	18	JPA
B			
B	0.000	18	W

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BIOGRAPHICAL SKETCH

Yael Spektor was born in 1985 in Montevideo, Uruguay. After moving to the U.S. in 2003, she attended the University of Florida from 2004-2007 and graduated Summa cum Laude with her B.S. in Food Science. Following her graduation, Yael was offered an assistantship at the University of Florida to pursue her master's degree in Food Science, under the supervision of Dr. Renée Goodrich. In the summer of 2009, Yael obtained an internship with Coca-Cola North America at their Juice R&D Department in Apopka, FL, where she gained invaluable experience about the business. In December 2009, she graduated with her M.S. in Food Science and she also received a minor in Packaging Sciences.

During the course of her graduate degree, Yael was the President of the Florida Association for Food Protection (FAFP), Gator Chapter. She was also an avid member of the Food Science and Human Nutrition club, where she served as Treasurer and FL Section IFT Ambassador. Yael has been an active leader and member of the Product Development Competition, and has participated in the Annual Collegiate Dairy Products Evaluation Contest and College Bowl teams. She has also been an active member in organizations in her field, such as the Institute of Food Technologists (IFT), since 2006, and the International Association for Food Protection (IAFP), since 2008.