

Testing of gastric contents for peanut proteins in a 13-year old anaphylaxis victim



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ABSTRACT

Background: We report the case of a 13-y female who went into anaphylactic shock following the ingestion of a meal suspected to be contaminated by peanuts. The teenager had a known sensitivity to peanuts, however, the restaurant claimed that no peanut products were used in the preparation of her meal. The gastric contents of the decedent were retained and tested for peanut proteins due to the possible legal liability of the proprietor.

Method: Using antibodies against peanut proteins (roasted and unroasted), we optimized a method to detect total soluble peanut proteins by Western-blot analysis in gastric contents. In addition, we validated two commercially available tests which were originally intended for detection of peanut proteins in food matrices to examine the same gastric sample. One was an enzyme-linked immunosorbent assay (ELISA) that utilized polyclonal antibodies against Ara h 1 (Tepnel Life Sciences). The other was a laminar-flow assay directed against Ara h 1, Ara h 2 and Ara h 3 (R-Biopharm). A positive food-based control was created by reducing bread and peanuts (1:1, w/w) with water (1:1, w/v) using a mortar and pestle. A food-based negative food control was created similar to the positive control, except the peanuts were omitted and the amount of bread was doubled.

Results: The Western-blot assay was sensitive down to 2.5 ng/ml of total peanut protein. The laminar flow was the most rapid and least complex. The ELISA was the most analytically sensitive with a cut-off of 1 ng/ml of Ara h 1 protein compared to the laminar flow which had a cut-off of 4 ng/ml Ara h 1 equivalent. Both ELISA and laminar flow assays were able to detect peanut proteins in the food matrices and positive controls, and not in negative controls. No peanut related proteins were detected in the decedent's gastric sample. The gastric sample spiked with peanuts was reliably detectable.

Conclusion: The anaphylaxis patient had no peanut allergens detected in her gastric contents by any of the three methods employed. Both commercially available assays are easily adaptable for testing peanut allergens in the gastric contents as judged by the results of the immunoassays as well as the Western blot analysis. Due to the rising need for detecting peanut proteins in various heterogeneous and complex matrices, the use of appropriate controls should be also considered in these unique investigations.

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1. Introduction

The decedent is a 13-y white female with a past medical history of asthma who ordered a deli sandwich which she had consumed on multiple previous occasions, with the only difference being a change in bread. Approximately 10 min after eating the sandwich, she began to have difficulty breathing, became nauseous, and began to vomit. She collapsed in a restroom and was found acutely cyanotic by bystanders. An Epi-Pen was unfortunately not available at the time of the reaction. Despite resuscitation by emergency personnel, she was declared dead

upon arrival at the local children's hospital. An autopsy was performed and revealed marked vocal cord edema and changes in the lungs consistent with chronic asthma. A retrospective review of her history revealed a reaction to peanut butter crackers at two years of age and a radioallergen sorbent test (RAST) which indicated an increased sensitivity to both peanuts and soy allergens. The decedent actively avoided peanuts, but not soy products.

A postmortem blood sample drawn from the decedent revealed an elevated tryptase level of 21 ug/l (RR 0.4–10.9), which was indicative of a hypersensitivity reaction. Postmortem allergen specific IgE levels were positive for both peanut at >100 kU/l and soybean at 29.10 kU/l. The cause of death was described by the medical examiner as an “anaphylactic reaction with vocal cord edema due to food allergy.” The gastric contents of the decedent were frozen and retained for additional testing. We received these samples for analysis to determine if any peanut protein was present in her final meal. Additionally, her gastric specimen was also tested for drugs, which revealed only caffeine correlating with the soda she drank with her meal.

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Table 1
Inter-assay precision profile for standard curve in the ELISA assay.

Standards	STD0	STD1	STD2	STD3	STD4	STD5
[Ara h 1], ng/ml	0	10	20	50	100	200
%CV	40	11.3	6.3	8.3	1.4	N/A

Table 2
Intra-assay precision profile for standard curve in the Elisa Assay.

Standards	STD0	STD1	STD2	STD3	STD4	STD5
[Ara h 1], ng/ml	0	10	20	50	100	200
%CV	51	2.6	2.9	4.6	0.3	3.1

2. Case investigation

The decedent's gastric contents were analyzed using an ELISA that utilized polyclonal antibodies against Ara h 1 (Tepnel Life Sciences) and a laminar-flow assay directed against Ara h 1, Ara h 2 and Ara h 3 (R-Biopharm, Darmstadt, Germany). Additionally, a Western blot analysis was targeted to detect total soluble peanut proteins. A positive food-based peanut extract control was created by reducing bread and peanuts (1:1, w/w) with water (1:1, w/v) using a mortar and pestle for 10 min to simulate mastication. Using concentrated hydrochloric acid, the pH was changed to 2.10 to simulate gastric acidity. A food-based negative food control was created exactly like the positive control, except the peanuts were omitted and the amount of bread was doubled.

3. Validation of assays for peanut proteins

3.1. Elisa assay

Linearity was determined using standards purchased from the manufacturer. The standards were measured in triplicate on two different days. The precision of this assay ranges from 1.4% at 100 ng/ml to 11.3% at 10 ng/mg, based on inter-assay precision data (Table 1). The positive control was the extraction buffer (hydroxymethyl methylamine) spiked with the peanut extract. Following serial dilution (1:2) of test samples and the positive control with their respective matrices, the samples were tested. In the extraction buffer 98% recovery was possible with up to 8-fold dilution. There was no measurable amount of Ara h 1 in the gastric sample, according to the ELISA assay (Table 2).

3.2. Laminar flow assay

In order to determine if we could detect peanut allergens using a laminar flow device, the spiked samples were used from the ELISA

experiment. In this assay there is a control line that appears to let the technician know that they did in fact place a sample on the device. The results show positive reactivity towards the positive control. The test failed to show reactivity towards the negative control and extraction buffer. The test had a cut-off of 4 ng/ml of Ara h 1 equivalent. The result of the gastric sample from the decedent was negative.

3.3. Western blot assay

Western blot analysis was targeted to total soluble peanut proteins. The results showed reactivity to the positive control. There was no reactivity to the patient sample, molecular weight marks, the negative controls or the Ara h 1 standards from Tepnel Biosciences. These results would seem to indicate that there were no peanut proteins present in the gastric contents (Fig. 1).

4. Resolution

We have proven that peanut protein was not present in the decedent's final meal by multiple tests with high analytical sensitivities. Other possible allergens that could have been contributory to her death are cross reacting substances such as soy and lupin. Cross reactivity between legumes is commonly described [1].

5. Discussion

Food is the most common cause of anaphylaxis seen in the hospital emergency department [2]. Approximately 30,000 food-induced anaphylactic events are seen in American emergency departments each year, 200 of which are fatal [3]. Peanuts or tree nuts cause >80% of these reactions [4]. Exposure to peanut containing products can lead to severe bronchospasm or even death in sensitized individuals. The number of individuals suffering from peanut allergies is constantly rising, especially in industrialized countries. In the U.S. about 4% of the adult population is affected and among children the rate is up to 6%. Therefore, sensitive and reliable tests are needed to detect peanut-related proteins in various matrices such as biological fluids (e.g. gastric content) or food products. Of the 10 allergenic proteins identified in peanuts, conarachin (Ara h) types 1 and 2 are believed to play a significant role in overall allergenicity, while Ara h 3 is believed to play a minor role. Due to its prevalence, persistence, and severity, peanut allergy is one of the most serious of the immediate hypersensitivity reactions to foods [5]. Currently the only method for assessing exposure to peanuts in biological fluids is to test for the presence of serum IgE antibodies. Peanut allergen-specific IgE antibodies can increase due to exposure to legumes, wheat, and chocolate. Currently, tests for peanut-

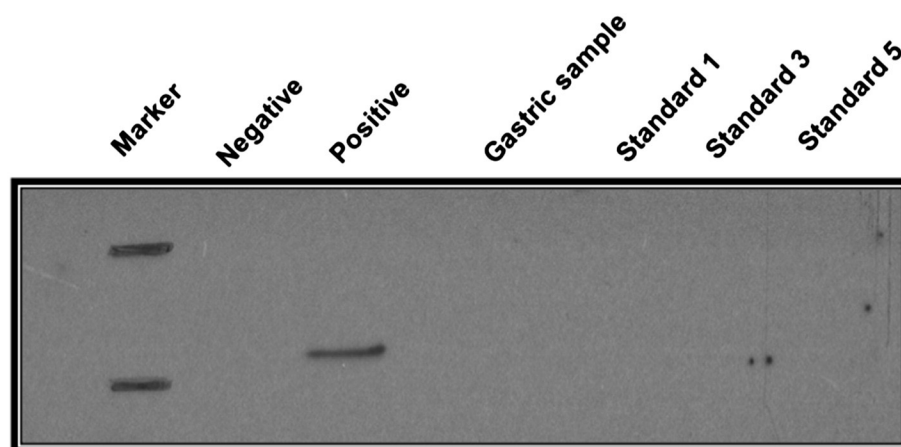


Fig. 1. Western blot against total soluble peanut protein demonstrating a valid control run and no peanut proteins present in the gastric contents of the decedent.

specific proteins are used in the food industry and are designed for food products. As emergency departments start to see more food-induced anaphylactic events, the clinical laboratory will be asked to provide information concerning food exposure.

The purpose of this work was to determine whether the individual had exposure to peanut allergens in her final meal, and if tests developed for the food industry could be adapted for clinical use. Despite high sensitivity techniques, many have assumed incorrectly that peanut proteins are degraded completely by the acidic environment of the stomach [6]. Strong allergens have the common characteristics of being both heat and acid stable [7]. Particularly Ara h 1, which degrades into acid stable fragments with as many as 23 IgE binding epitopes present following a pepsin digestion [6].

6. Conclusions

No peanut related proteins were detected in the decedent's gastric sample by 3 separate monoclonal antibody based assays, which relieved the restaurant of legal liability in this case. All three methods used (ELISA, laminar flow and Western blot analysis) were able to detect peanut proteins in the food matrices and positive controls, and not in negative controls [8]. The Western-blot assay was sensitive down to 2.5 ng/ml of total peanut protein. The laminar flow was the most rapid and least complex. The ELISA was the most analytically sensitive with a cut-off of 1 ng/ml of Ara h 1 protein compared to the laminar flow which had a cut-off of 4 ng/ml Ara h 1 equivalent. Both commercially available assays (ELISA and laminar flow) are easily adaptable for testing peanut allergens in the gastric contents as judged by the Western-blot analysis. This rising need for detecting peanut proteins

in various heterogeneous matrices necessitates the creation of a food-based matrix and control.

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