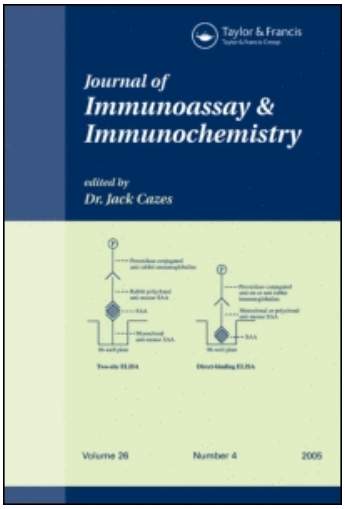

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A COMPARISON OF COMMERCIALLY AVAILABLE PEANUT ELISA TEST KITS ON THE ANALYSIS OF SAMPLES OF DARK AND MILK CHOCOLATE

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ABSTRACT

Reactions to peanut proteins by certain sensitive members of the population can result in dramatic and potentially catastrophic consequences. While peanut is not the only food known to cause allergies, it is the one that is most closely monitored. To address concerns related to peanut in food products, four commercial test kits have been developed to quantitatively analyze for peanut protein in finished products. This manuscript describes a study undertaken to compare these kits on reference samples of dark and milk chocolate containing known amounts of peanut. The results are mixed

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with the data suggesting that all kits were suitable for qualitative screening but were not suitable for general quantitative assays.

INTRODUCTION

Adverse reactions to food can be divided into two broad categories; food intolerance and food allergies, with approximately 2–3% of the adult population and 8% of children having some type of food allergy.[1] There are over 160 foods known to cause some allergic reactions with 8 of them said to be the major food allergens with peanut being a member of this smaller class.[2] The reasons for concern about peanut allergy are numerous, with the major one being that, while in many instances, children grow out of some food allergies, individuals who are allergic to peanut are said to be allergic for life.[3] The reactions to peanut allergen can range in severity from itching and urticaria to death.[4] Additionally, there are increasing uses of peanut in foods sometimes as a protein source and food previously thought to be peanut free is not, causing additional concerns for parents and adults with the trend likely to continue.

In 1991 and 1992, Burks reported on the isolation and characterization of two major glycoproteins, Ara h1 and Ara h2, which were isolated from peanuts and found to account for a substantial part of the allergic reaction in peanuts.[5] These proteins were reported to have molecular weights of 63.5 and 17 Kd, respectively, with pI's of 4.55 and 5.2.[6] In 1998, DeJong reported that peanuts could contain up to 6 major allergens including Ara h1 and Ara h2.[7] What makes peanut proteins especially troublesome is that they are heat resistant and even maintain their functionality when the carbohydrate portion of the glycoprotein is removed.[8,9] While additional work continues in this area, these papers provided the initial impetus. In 1994, Hefle first reported the production of monoclonal antibodies against selected peanut allergens and later in that year discussed the development of a sandwich ELISA for the determination of selected peanut proteins and it's use in the analysis of vanilla ice cream.[10,11] In 1996, Yeung reported the development of another ELISA for the determination of peanut proteins in processed food[12] with Newsome reporting in 1999 the development and use of an immunoassay column to improve the extraction and determination of peanut in chocolate products.[13] Prior to these assays, those interested in this type of determination would have used RAST, immunoblotting or some other mode of analysis.

In the late 1990's, as a result of this research and other studies, commercial kits came onto the market offering those modestly equipped

laboratories the opportunity to analyze for peanut protein in finished products or at various places in their process stream. Prior to this time, many of the assays would require special reagents or isotopes, which was beyond the scope of many food analysis laboratories. What resulted were four quantitative analysis kits for this analysis, with the individual laboratory left with the choice as to which one to use. This choice was not straightforward, since each of the kits had undergone development at different sites using different reagents and different antibody sources, with the only common denominator being that each was a sandwich ELISA. This can place the food industry and regulatory agencies in a difficult position, since it is possible to use different kits and develop substantially different data. Additionally, anecdotal information indicates that some regulators may chose a kit based on geographical rather than technical considerations.

Many of these kits could be used in general food analysis labs unaccustomed to ELISA methods. While ELISA is commonplace in some industry segments, such is not the case in many food laboratories; hence, the laboratory had not only to learn a new method, but sometimes a new analytical technique. This manuscript describes the results obtained on a series of reference chocolate samples that have been analyzed by each of the commercially available test kits.

EXPERIMENTAL

Four homogeneous samples were obtained each being prepared by an external source. The samples consisted of two dark chocolate and two milk chocolate samples with values ranging from 0 to 200 ppm. Table 1 provides the description and peanut concentration for each sample.

Test kits were obtained through commercial channels; Table 2 lists the supplier and their respective kits.

In addition to the four kits mentioned, Neogen also has an Alert kit which is suitable for qualitative analysis, which was not included in these

Table 1. Sample Table Values Provided by External Organization

Sample ID Description	Concentration*
1	Dark Chocolate 0 mg/kg
2	Milk Chocolate 10 mg/kg
3	Dark Chocolate 40 mg/kg
4	Milk Chocolate 200 mg/kg

Table 2. Commercially Available Kits Used in This Study

Supplier	Kit
Neogen	Veratox® Peanut
Canadian food testing laboratories	Prolisa™
Elisa-technologies	ELISA-TEK™
R-Biopharm	Ridascreen®

studies. All kits were used as received and samples were analyzed blind, according to instructions contained in that particular kit, ensuring that special sample preparation instructions for chocolate products were followed.[14–17] The only deviation from some of the instructions was that samples were analyzed in duplicate rather than single as some of the instructions indicated. For completeness, the parameters for each kit are listed in Table 3.

RESULTS AND DISCUSSION

The results for these assays are reported in Table 4, with each of the kits being designated by a number, with the number having no significance other than as a unique identifier.

Additionally, each sample extract was analyzed 7 times to establish precision data for the method. These data can be seen in Table 5.

The data seen in Table 4 indicates that all kits are able to detect peanut in samples that contain peanut and not detect peanut in those samples negative for peanut; hence, it would seem that the incidence of false positives and negatives in this studies were minimal. Based on the data that were developed, the results of this study are mixed. All of the methods were able to detect the presence of peanut in samples containing peanut and did not detect peanut in those samples that were negative, but the accuracy of the methods varied widely. The cause for concern from the data is the quantitative aspects of the study, since in the 10 PPM region, sample values reported ranged from <1 to >25 PPM. The data, furthermore, indicate that, should a sample have a concentration in our case in excess of 200 ppm, all kits indicated levels that were, in many cases, outside the limits of the assay. In this study, the region between 0 and 200 was problematic, since the data indicated neither a positive nor negative bias. This evaluation has, furthermore, indicated that dark chocolate can be problematic, since samples of dark chocolate can give low recoveries. One must also be aware that, with the data, there was a tacit

Table 3. Commercial Kit Operating Parameters

Parameter	Neogen's Veratox Kit	Ridascreen Peanut Kit	CFTL Prolisa Peanut PAK Kit	ELISA-TEK Peanut Protein Kit
Sample prep.	Homogenous	Homogenous	Homogenous	Homogenous
Sample amount	5 g	1 g	4 g	10 g
Extraction buffer	25 mL/g 60°C	20 mL/g 60°C	10 mL/g	5 mL/g
Extraction	Shake in 60°C water bath 15 min let stand 10 min	60°C water bath for 20 min	60°C water bath 10 min, vortex 2 min, shake 30 min	Blend 2 min
Remove particles	Filter or centrifuge 10 min	Filter or centrifuge 10 min	Filter or centrifuge 10 min	Filter or centrifuge 10 min
Dilution of extract	None	1 : 5 w/Extract buffer	1 : 4 or 1 : 10 w/diluent	1 : 20 w/diluent
First set of wells	150 uL of extract	Skip	Skip	Skip
Antibody coated wells	Transfer 100 uL	Transfer 100 uL	200 uL	100 uL
Move wells to mix	Yes	Yes	Yes	Yes
Incubate room temp	10 min	30 min	Shake 40 min	1 h
Discard liquid and wash *t*	5 times	250 uL 3 times	5 times	5 times
Add conjugate	100 uL	100 uL	175 uL	50 uL
Incubate room temp.	10 min	30 min	20 min	1 h
Discard liquid and wash X times	5 times	250 uL 3 times	5 times	5 times
Add Peroxidase	Skip	Skip	Skip	50 uL
Incubate room temp.	Skip	Skip	Skip	15 min
Discard liquid and wash X times	Skip	Skip	Skip	5 times

(continued)

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Table 3. Continued

Parameter	Neogen's Veratox Kit	Ridascreen Peanut Kit	CFTL Prolisa Peanut PAK Kit	ELISA-TEK Peanut Protein Kit
Add substrate	100 uL	50 uL and 50 uL Chromogen	175 uL	100 uL
Incubate room temp.	10 min	30 min	7 min in dark	45 min w/cover
Stop solution	100 uL	100 uL	75 uL	50 uL
Microwell reader	650 nm	450 nm	450 nm	450 nm
Total incubation and extraction time	65 min	120 min	119 min	192 min
Total time plus washings and plating	100 min	142 min	143 min	217 min

Table 4. Summary of ELISA Kit Results

ELISA Kits	Conc. in PPM	Given Value in PPM
Kit #1 Dark Chocolate	ND	0
Kit #1 Milk Chocolate	>25	10
Kit #1 Dark Chocolate	>25	40
Kit #1 Milk Chocolate	>25	200
Kit #2 Dark Chocolate	ND	0
Kit #2 Milk Chocolate	5	10
Kit #2 Dark Chocolate	12	40
Kit #2 Milk Chocolate	>90	200
Kit #3 Dark Chocolate	ND	0
Kit #3 Milk Chocolate	10	10
Kit #3 Dark Chocolate	100	40
Kit #3 Milk Chocolate	60	200
Kit #4 Dark Chocolate	<1	0
Kit #4 Milk Chocolate	<1	10
Kit #4 Dark Chocolate	<1	40
Kit #4 Milk Chocolate	50	200

Table 5. Method/Kit Precision Data

Sample Identification	%CV
Kit #1 Dark Chocolate	12.8
Kit #1 Milk Chocolate	9.0
Kit #1 Dark Chocolate	4.7
Kit #1 Milk Chocolate	4.4
Kit #2 Dark Chocolate	6.1
Kit #2 Milk Chocolate	6.5
Kit #2 Dark Chocolate	3.8
Kit #2 Milk Chocolate	1.2
Kit #3 Dark Chocolate	5.4
Kit #3 Milk Chocolate	2.3
Kit #3 Dark Chocolate	4.5
Kit #3 Milk Chocolate	1.5
Kit #4 Dark Chocolate	11.3
Kit #4 Milk Chocolate	1.9
Kit #4 Dark Chocolate	5.6
Kit #4 Milk Chocolate	3.7

assumption that the samples were totally homogeneous. That would seem to be the case, but one never can be sure. Additionally, each of the kits tends to use a different peanut standard which, likely, may contribute to the variability of the results.

CONCLUSIONS

The data presented in this study provide a comparison of four commercially available kits for the quantitative determination of peanut in chocolate paste. On the qualitative level, all kits performed adequately and, at the extremes of 0 and 200 ppm, the quantitative data were suitable. Where the performance was disappointing was in the concentration range where the kits would seem to have the greatest usage. Some vendors have indicated refinements in kit design that are in progress; others have indicated the current generation of kit will be the only one available from their organization, with a final group taking a ''wait and see'' approach, The response of this group likely may depend on government action or the results of a collaborative study by an independent organization.

Finally, the study had a limited scope and the results should not be extrapolated to any other matrix. Chocolate was chosen for this study since the literature indicates the determination of peanut in chocolate can be problematic. The results reported here would seem to be typical of those obtained in a food analysis laboratory and provide a perspective on this analysis.

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