Evaluation of Commercial ELISA Assays for the Detection of Egg in Food

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ABSTRACT

It is estimated that 6% of children under the age of 3 are allergic to some form of food with 1.3% allergic to eggs. Currently, the only method available for individuals allergic to a specific food to avoid an allergic reaction is avoidance of the allergenic food. To address the need for validated methods to test food products for the presence of allergens, the FDA has initiated studies to evaluate commercial immunology based assays for the detection of allergenic foods. Seven commercial ELISA-based assays for the detection of egg proteins were evaluated with six different matrices representative of various forms of processing. The matrices examined included baked goods, pasta - analyzed before and after boiling, vanilla ice cream, salad dressing (no processing), and phosphate buffered saline. Each of the matrices were spiked with either 0, 2, 5, 10, 25, or 100 ug/g of the NIST whole egg powder standard reference material (SRM) # 8415. Six of the ELISA kits relied on washing/partitioning to extract the antigenic biomarker into an aqueous buffer. The seventh kit was unique in that it employed reducing–denaturing conditions to extract the antigenic proteins. All seven kits readily detected egg spiked into foods which were subjected to none or minimal heating. However, the assays displayed significant differences in ability to detect egg in foods that were exposed to heat. Only the kit that employed reducing-denaturing conditions to extract the antigenic biomarkers detected egg in all matrices spiked with $\geq$ 2 ug/g of the NIST SRM.

MATERIALS AND METHODS

Egg standard Whole egg powder, NIST standard reference material (SRM) # 8415, was the gift of Stephen G. Capar (FDA).

Spiking and Processing of Commodities:

Food samples were spiked with 0 - 100mg per gram food of the NIST SRM #8415.

Bread samples were baked at 178 ºC for 10 min with the egg solution on top as a glaze.

Muffins containing various amounts of the egg standard mixed into the batter were baked at 218 ºC for 10 min.

Cooked pasta samples were prepared by placing a sealed tube containing 1 g of spiked pasta in 5 ml PBS into a boiling water bath for 10 minutes.

French vanilla ice cream was prepared according to the procedure of Corriher (1997) which entailed spiking the egg solution into vanilla ice cream and heating for 5 minutes in a water bath at 80 ºC. The samples were stored at -20 ºC.

Caesar salad dressing samples were prepared using Ken's Steak House Lite Caesar Dressing which does not contain any eggs or egg products.

Immunology based Assays used in this study:

Egg Residue Microwell ELISA manufactured by ELISA Systems (Windsor, QLD, Australia; distributed by ELISA Technologies, Inc., Gainesville, FL).

Egg Protein ELISA Kit manufactured by Morinaga Institute of Biological Sciences (Yokohama, Japan; distributed by Crystal Chem, Inc., Downers Grove, IL)

Veratox® Quantitative Egg Allergen Test manufactured by Neogen Corp. (Lansing, MI),

The Prolisa™ EggPAK™ Enzyme Immunoassay for the Quantitative Determination of Egg Protein in Food Products by ProLab Diagnostics (Ontario, Canada)

RIDASCREEN® Enzyme Immunoassay for the Quantitative Analysis of Egg White Protein manufactured by R-Biopharm AG (Darmstadt. Germany),

BioKits Egg Assay Kit manufactured by Tepnel BioSystems Ltd. (Flintshire, UK)

TECRA® Egg Visual Immunoassay manufactured by TECRA International Pty Ltd (Frenchs Forest NSW, Australia)

The kits were used as prescribed by the manufacturer with the only modification being the scaling of all reagents to accommodate samples of one gram in place of five. Prior experimentation established comparable properties between the muffins prepared according to the above procedure and standard, 20 gram muffins.

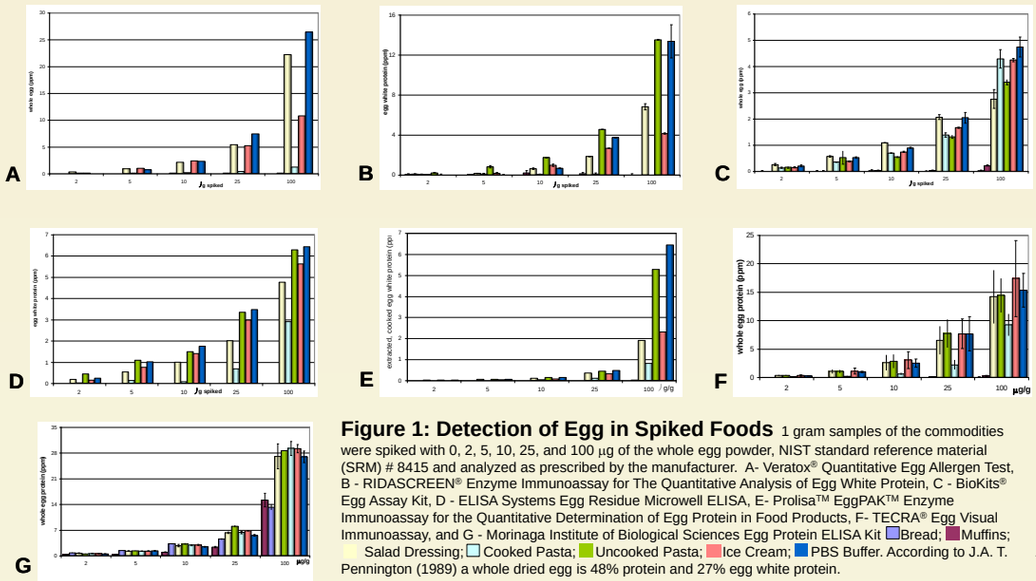

Figure 1: Detection of Egg in Spiked Foods 1 gram samples of the commodities were spiked with 0, 2, 5, 10, 25, and 100 µg of the whole egg powder, NIST standard reference material (SRM) # 8415 and analyzed as prescribed by the manufacturer. A- Veratox® Quantitative Egg Allergen Test, B - RIDASCREEN® Enzyme Immunoassay for The Quantitative Analysis of Egg White Protein, C - BioKits® Egg Assay Kit, D - ELISA Systems Egg Residue Microwell ELISA, E- Prolisa™ EggPAK™ Enzyme Immunoassay for the Quantitative Determination of Egg Protein in Food Products, F- TECRA® Egg Visual Immunoassay, and G - Morinaga Institute of Biological Sciences Egg Protein ELISA Kit Bread; Muffins; Salad Dressing; Cooked Pasta; Uncooked Pasta; Ice Cream; PBS Buffer. According to J.A. T. Pennington (1989) a whole dried egg is 48% protein and 27% egg white protein.

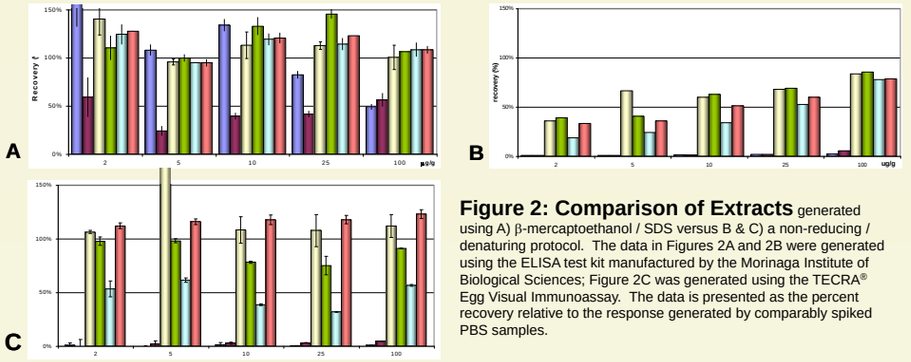

Figure 2: Comparison of Extracts generated using A) β-mercaptoethanol / SDS versus B & C) a non-reducing / denaturing protocol. The data in Figures 2A and 2B were generated using the ELISA test kit manufactured by the Morinaga Institute of Biological Sciences; Figure 2C was generated using the TECRA® Egg Visual Immunoassay. The data is presented as the percent recovery relative to the response generated by comparably spiked PBS samples.

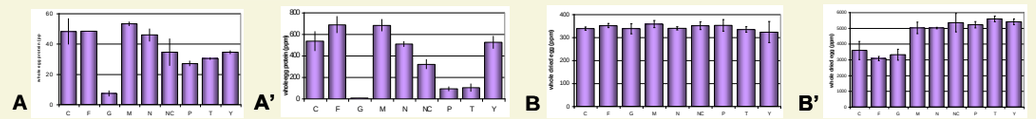

Figure 3: Detection of Egg in Commodities. C - Caesar Salad Dressing (Wishbone); F - French Vanilla Ice Cream (Lucerne); G - Egg Bagels (Lender's); M - Hellmann's Real Mayonnaise; N - Extra Wide Egg Noodles, uncooked (Safeway Inc.); NC - Extra Wide Egg Noodles, cooked (Safeway.); P – Pecan Sandies; T - Breakfast Treats (Stella D'oro); Y - Muffin (Safeway). A - TECRA® Egg Visual Immunoassay analysis of samples as recommended and (A') following a 15-fold dilution; B – Morinaga Institute of Biological Sciences Egg Protein ELISA Kit analysis of samples as recommended and (B') following a 15-fold dilution. Analysis of similar samples using the other ELISA test kits generated data comparable to that observed with the TECRA® Egg Visual Immunoassay.

CONCLUSIONS

The ability of the various assays to detect egg in foods subjected to various forms of preparation are summarized in the following table:

Table 1: Lowest Level of NIST Whole Egg Powder Detected[a] in Spiked/Prepared Food

Test Kit	Bread	Muffins	Dressing	Pasta		Fr. Vanilla Ice Cream	PBS
				cooked	uncooked		
	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)
BioKits	>100	100	2	5	2	2	5
ELISA Systems	>100	>100	2	5	5	5	5
Morinaga Institute	2	2	2	2	2	2	2
Prolisa™ EggPAK™	>100	100	2	10	2	5	2
RIDASCREEN®	>100	>100	5	>100	5	5	10
TECRA® Egg VIA	>100	100	5	5	2	2	2
Veratox®	>100	>100	5	100	5	5	10

[a] µg NIST SRM #8415 per gram needed to generate a response 1.9-times the response observed with the 0 µg/g sample

All seven assays were able to detect egg spiked in food subjected to limited amounts of heat during preparation. Prolonged heating, associated with the preparation of pasta, muffins, or bread, resulted in significant differences in the effectiveness of the assays. Only the ELISA developed by Morinaga Institute of Biological Science, in which reducing – denaturing conditions were used to extract the samples, was able to detect egg in all samples prepared. The ELISA developed by the Morinaga Institute of Biological Science was also more effective in detecting egg in commercially prepared foods.

Comparison of extraction procedures by ELISA test kits entailed normalizing the data to comparably spiked PBS samples. This circumvented problems associated with the lack of conversion factors for the different standards employed by the test kits and the observation that the NIST SRM #8415 generated responses 34% of what was expected based on non-NIST reference material supplied with the test kits.

ACKNOWLEDGEMENTS

Gratitude is expressed to George Ziobro (FDA) and Lynn L.B. Rust (NIH) for stimulating scientific discussions that helped facilitate the research. Gratitude is also expressed to Mohamed Abouzied (Neogen Corp.), Kurt Johnson (R-Biopharm, Inc.), Phil Goodwin & Thomas A. Grace (Tepnel BioSystems Limited), Bruce Ritter (ELISA Technologies, Inc.), Mike Ryan (ELISA Systems PTY LTD) Hans Moederzoon & Robert Rae (ProLab Diagnostics), Ian Garthwaite (TECRA International Pty Ltd), Masahiro Shoji (Morinaga Institute of Biological Science) and Hema Shah (Crystal Chem, Inc.) for providing the ELISA kits used in this study.

FDA

Department of Health and Human Services