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 is 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 ppm of the NIST whole egg powder standard reference material (SRM) # 8415. Six of the ELISA kits failed on washing/partitioning to extract the antigenic biomarkers in 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 2 ppm 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 - 100 ppm 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 paste 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 entails spiking the egg solution into vanilla ice cream and freezing for 5 minutes in a water bath at 80 ºC. The samples were stored at -20 ºC. Caesar salad dressing samples were prepared using Kew's Steak House Light Caesar Dressing which does not contain any eggs or egg products.

Immunology based Assays used in this study:
Egg Resista McKown ELISA manufactured by ELISA Systems (Windnar, QLD, Australia), distributed by ELISA Technologies Inc., Gainesville, FL.

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

Verato® 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).

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

Biosystems Egg Assay K4 manufactured by Tepnel Biological Systems Ltd. (Fremont, UK).

TECRA Egg Visual Immunoassay manufactured by TECRA international Pty Ltd (Fremont 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 muffin.

Figure 1: Detection of Egg in Spiked Foods 1 gram samples of the commodities were spiked with 0, 2, 5, 10, 25, and 100 ppm of the whole egg powder. NIST standard reference material (SRM) #8415 and analyzed as prescribed by the manufacturer. A: Verato® Quantitative Egg Allergen Test. B: RIDASCREE® Enzyme Immunoassay for The Quantitative Analysis of Egg White Protein, C: Biosystems Egg Assay K4. D: 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. Note: Muffins, Salad Dressing, Cooked Pasta, Uncooked Pasta, Ice Cream, PBS Buffer. According to J.A. Peterson (1986) a Whole dried egg is 48% protein and 37% egg white protein.

Figure 2: Comparison of Extracts generated using A) (Enzyme Immunoassay) / 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 (Wiltshire); F: French Vanilla Ice Cream (Lucerne); G: Egg Breakfast Treats (Dancook); H: Extra Whole Egg Noodles, cooked (Safeway Inc.); I: NC - Extra Whole Egg Noodles, cooked (Safeway Inc.); J: NC - Pecan Sandies; T: Breakfast Treats (Stella D'oro); Y: Muffin (Safeway). A: TECRA® Egg Visual Immunoassay analysis of samples as recommended and (J) following a 15-fold dilution; B: Morinaga Institute of Biological Sciences Egg Protein ELISA Kit analysis of samples as recommended and (J) 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>
<thead>
<tr>
<th>Test Kit</th>
<th>Bread</th>
<th>Muffins</th>
<th>Pork</th>
<th>Fish</th>
<th>Veg</th>
</tr>
</thead>
<tbody>
<tr>
<td>assay A</td>
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<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
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<tr>
<td>assay C</td>
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<td>&lt;1</td>
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</tr>
</tbody>
</table>

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 pastas, muffins, or bread, resulted in significant differences in the effectiveness of the assays. Only the ELISA developed by the Morinaga Institute of Biological Sciences, in which reducing - denaturing conditions were used to extract the sample, 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 >100% of what was expected based on non-NIST reference material supplied with the test kits.

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