Evaluation and Validation of a Commercial ELISA-Based Method for the Determination of Egg Protein in Foods

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ABSTRACT

The detection of food allergens like egg protein in heat processed foods using ELISA-based methods has always been challenging. The Canadian Food Inspection Agency has evaluated the performance of the Morinaga Institute of Biological Science ELISA Egg Kit through an inter-laboratory study. Use of the method improves the probability to detect egg allergen in processed products, which will provide further protection to egg-allergic consumers. After a successful single laboratory validation where a conversion factor was established for the NIST 8445 material, a total of 124 samples were distributed to four laboratories. Each participant received 10 ice cream, 10 bread and 11 pasta samples. Each food commodity was either egg free, already contained egg at low (about 4 ppm), or high (about 1800 ppm) level or was spiked at a level of 1.7, 6.7 or 16.8 ppm using the carboxymethylcellulose suspension technique. Z-Scores were calculated for the incurred positive samples and were all well within the acceptable range. The precision (RSD_R) ranged from 5.1 to 12.4% for these materials. The results for spiked samples were close to the expected values and the recoveries varied by commodity, with the ice cream averaging 99%, the bread 101% and the pasta 107% for an overall recovery of 103%. No false negatives were found while a single false positive result was reported. Overall the validation data were deemed satisfactory and confirmed the fitness-for-purpose of the method.

SELECTION OF EGG MATERIAL AND SPIKING PROCEDURE

NIST 8445 Spray-dried Whole Egg for Allergen Detection is the first NIST reference material specifically intended for use in food allergen testing. It was therefore selected for the evaluation and validation of the Morinaga Institute of Biological Science ELISA Egg Kit as well as for quality control purposes. Spiking assays were conducted using the carboxymethylcellulose (CMC) suspension technique: NIST 8445 material (100 mg) was added to 50 g of CMC solution and mixed with POLYTRON® homogenizer. A portion (5 g) of the resulting suspension was then diluted in 100 mL of PBS buffer. The addition of 200 µL of this spiking solution to 1 g of sample (final concentration of 20 ppm of NIST material) produced a response of 19.3 ± 1.4 ng/mL on the calibration curve of the the Morinaga Institute of Biological Science ELISA Egg Kit which is equivalent to 8.1 ppm egg protein.

SELECTION OF FOOD COMMODITIES

Test materials were chosen considering the following criteria. First, matrices of interest for ELISA methods targeting egg listed in the publication "Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices" (Abbott et al, J.AOAC International, 2010, Vol. 93, no 2, 442-450) were considered. Second, in order to challenge the method as much as possible, incurred positive samples had to be included in addition to the typical spiked samples. Based on these criteria and availability of incurred samples, ice cream, bread and pasta were selected.

PRELIMINARY RESULTS

Each food was screened for residual egg protein content, then spiked to evaluate the performance of the method and the applicability of the spiking technique.

Table 1: Egg protein content in selected food commodities and spiking results

Food commodity	Result [1] ppm	Recovery %
Ice cream	< 0.3	96.9 ± 12.4
Bread (egg free)	< 0.3	99.9 ± 8.5
Pasta (egg free)	< 0.3	100.7 ± 14.8
Bread (contain egg, commercially available)	3.6 ± 0.5	N/A
Pasta (contain egg, commercially available)	1780 ± 190	N/A

[1]: Expressed as kit unit (ppm egg protein) Kit LOQ = 0.3 ppm

Based on the preliminary results, the selected food matrices were deemed fit for the purpose of this study. Additionally, incurred bread and pasta samples were tested with other ELISA-based methods:

Table 2: Qualitative results for the detection of egg protein in incurred bread and pasta samples

Test kit (product number)	Bread	Pasta
Morinaga ELISA Egg Kit (141OA)	Positive	Positive
RIDASCREEN® FAST Egg Protein (R6402)	Negative	Positive
Enhanced Egg Residue (ESEGG-48)	Negative	Positive
BioKits Egg Assay Kit (902072T)	Negative	Not tested
Veratox® for Egg Allergen (8450)	Negative	Positive

Detection of a high level of egg protein in incurred pasta sample was achieved by all the kits tested. However, only the Morinaga method was able to detect the low level of egg protein in incurred bread sample.

EXTRACTION OPTIMIZATION

The extraction as described in the method protocol is done by an overnight agitation of the sample after addition of the extraction solution. To speed up the process, a modified extraction procedure was evaluated: after addition of the extraction solution, the sample was homogenized using a vortex mixer for 3 minutes, then boiled in a water bath for 10 minutes. Comparison was carried out for cake mix (proficiency test sample), ice cream (spiked), bread and pasta (spiked and incurred) samples. No statistical difference was observed between the results generated by the two extraction procedures.

INTER-LABORATORY STUDY

An inter-laboratory study involving four laboratories was organized to further validate the method. Each participant received 31 samples consisting of 10 ice cream, 10 bread and 11 pasta samples. Each sample was either egg free, spiked at a level of 1.7, 6.7 or 16.8 ppm or commercially prepared and known to contain egg. The stability of the spiked samples had been confirmed prior to sample shipment by an assay where spiked samples were tested after being left at room temperature for 3 days.

RESULTS AND DISCUSSION

The spike recovery results were satisfactory and varied by commodity, with ice cream averaging 99%, bread 101% and pasta 107% for an overall recovery of 103%. A single false positive result out of 24 negative samples was found and no false negative results out of 69 positive samples were found. Laboratory #4 experienced some problems with an instrument and therefore their results had to be rejected.

Table 3: Inter-laboratory results for spiked samples

Food product	Average recovery %	SD_r %	SD_R %	RSD_r %	RSD_R %
Ice cream	99.4	12.1	12.1	12.2	12.1
Bread	101.2	10.6	12.1	10.5	12.0
Pasta	107.3	16.3	16.5	15.2	15.4

z-scores were only calculated for incurred positive bread and pasta samples. The reproducibility RSD_R was 5.1 and 12.4% for bread and pasta respectively. A relative standard deviation for proficiency of 25% was used to calculate z-scores according to proficiency testing best practices. All the participants obtained acceptable z-Scores well within acceptable range.

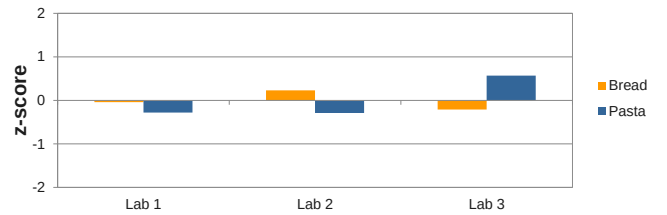

Figure 2: z-scores for egg protein in incurred bread and pasta samples

CONCLUSION

The results generated by the single laboratory validation and inter-laboratory study confirmed the fitness for purpose and excellent performance of the method. The results are particularly interesting considering the fact that the method was benchmarked with both incurred and spiked samples. It was demonstrated that the Morinaga Institute of Biological Science ELISA Egg Kit has the capability to detect egg in processed foods at relatively low level.

ACKNOWLEDGEMENTS

The authors acknowledge the collaborators from the following laboratories for their participation in the inter-laboratory study:

- Canadian Food Inspection Agency Longueuil Laboratory
- Canadian Food Inspection Agency Burnaby Laboratory
- Health Canada Food Allergen Research Laboratory, Ottawa
- Health Canada Western Region Food Laboratory, Burnaby

The authors also want to thank the Morinaga Institute of Biological Science, Inc. and Crystal Chem, Inc. for advice and technical support throughout the project.