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Bureau of Chemical Safety
Food Directorate

Bureau d'innocuité des produits chimiques
Direction des aliments

Elisa Systems Sesame Kit (ESSESRD-48): Performance Evaluation

A PAHO/WHO Collaborating Center for Food Contamination Monitoring

World Health Organization

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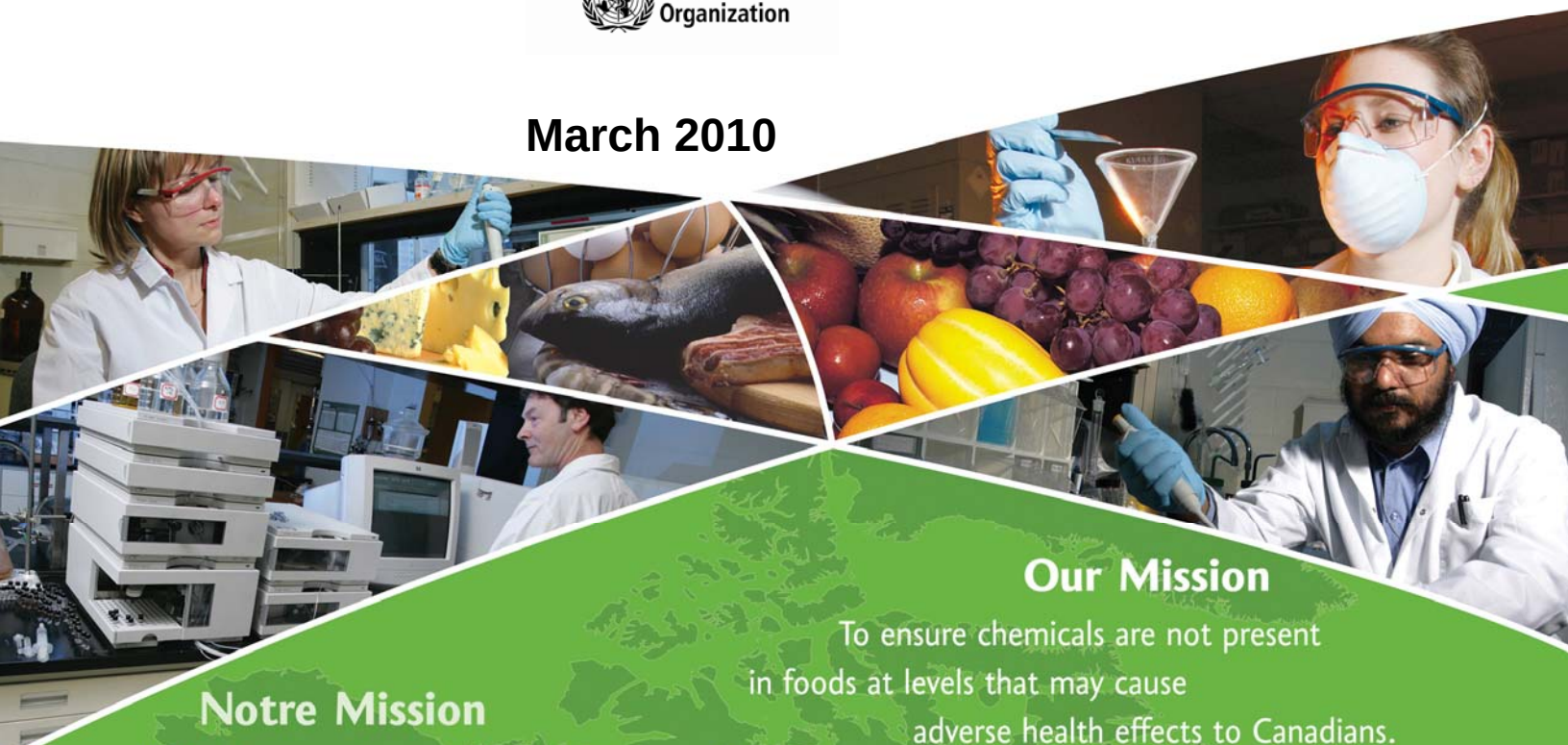

Our Mission

To ensure chemicals are not present in foods at levels that may cause adverse health effects to Canadians.

Notre Mission

Veiller à ce que les produits chimiques ne soient pas présents dans les aliments à des niveaux pouvant entraîner des effets néfastes sur la santé des canadiennes et des canadiens.

Canada

Elisa Systems Sesame Kit (ESSESRD-48): Performance Evaluation

Disclaimer: Inclusion of this method in the compendium does not imply endorsement or approval by Health Canada.

Introduction

The purpose of this study was to generate performance data on the Elisa Systems Sesame test kit for the detection and quantification of sesame seed (*Sesamum indicum*) protein (2S Albumin) as part of ongoing efforts to evaluate food allergen detection methodologies and further their introduction into the Compendium of Methodologies. A representative sesame paste was chosen to be the designated reference material, according to the definition established by the AMC. This evaluation involved the analysis of selected food matrices, which had been artificially fortified (spiked samples) with the sesame paste.

Method Evaluated:

Elisa Systems Sesame ELISA kit (Product Code: ESSESRD-48).

Evaluation Level:

Full evaluation under the guidelines developed for the Compendium of Food Allergen Methodologies.

Designated Reference Material:

There is no reference material currently available for sesame seed protein so one was developed and correlated to the positive controls of the test kit.

Participating Laboratories:

Health Canada Food Allergen Research Laboratory
Banting Building, Ottawa, ON

Health Canada Western Regional Laboratory
Burnaby, BC

CFIA Québec Regional Laboratory
Longueuil, QC

CFIA Western Regional Laboratory
Burnaby, BC

Spiking Levels:

The compendium calls for spiking levels to give a response from the kit of approximately 0, 2 and 5 times the limit of quantitation (LOQ). The calibration range for the Elisa Systems Sesame kit is from 1.0 to 5.0 ppm, which would require the non-zero spiking levels be 2 and 5 ppm. In order to keep the levels within the limits of the calibration curve, without additional dilutions, the spiking levels were changed to 1.5 times (1.5 ppm) and 3.5 times (3.5 ppm) the LOQ when applied to the sesame reference material.

Spiking Conditions:

Sesame seeds were finely ground under liquid nitrogen in a mortar and pestle to obtain a powdered material. This material was suspended in a carboxymethyl cellulose (CMC) solution (1mg/g) based on a method by Trucksess et al, *Preparation of Peanut Butter Suspension for Determination of Peanuts Using Enzyme-Linked Immunoassay Kits*, Journal of AOAC International, vol.87, 2, 2004. This CMC sesame suspension was then used to spike the different matrices at 1.5 and 3.5 ppm. The samples were blind coded and sent to the participating labs.

Matrices of Interest:

Three different matrices were included in the evaluation: bread crumbs, baked crackers and a vegetable dip. These commodities were included as representatives of some of the matrices likely to contain undeclared sesame protein.

Materials and Resources

Each participating lab was provided with enough Elisa Systems Sesame kits (6) to complete the analysis of the blind-coded samples (30 of each matrix and 4 matrix free spikes - 94 samples in total).

Procedure

Spiking Procedure:

The spiking levels were chosen in order to obtain a response of approximately 1.5 and 3.5 times the specified LOQ of the Elisa Systems Sesame kit (1.0ppm). There is no standard reference material for sesame protein so it was necessary to produce a correlation between the sesame seed material developed for this study and the positive controls supplied with the ELISA kit. It was determined that 20.0 – 80.0 ppm of the whole sesame seed CMC suspension developed for this study provided a response of 1.0 – 4.0ppm from the kit controls, which represents 12 – 48 ppm whole sesame seeds used by the kit manufacturer. The small difference in the correlation between the kit controls from Elisa Systems and our reference material is likely due to the different protein content between our material and that used to develop the assay from Elisa Systems.

A freshly prepared stock sesame paste suspension in CMC was prepared on the day of spiking at a concentration of 1mg/g (1000 ppm). This material was diluted in PBS to obtain the 1.5 ppm (7.5mL in 250mL) and the 3.5 ppm (17.5mL in 250mL) spiking solutions. In order to provide normalization among the 5g samples of the three different matrices all were spiked with a 1mL aliquot of the appropriate spiking solution in 250 mL polypropylene bottles. The blank samples were spiked with 1mL of a solution made by diluting a blank CMC suspension in PBS.

Preparation of Samples:

The 360 samples required (10 replicates at each of 3 levels in 3 matrices for 4 laboratories) were prepared at the Health Canada's Food Allergen Research Laboratory in Burnaby. Samples (5 g) were weighed out into 250 ml screw cap bottles (120 samples for each matrix). The samples were separated into groups and spiked at one of the three levels. Each sample was given a blind code number and then the samples were grouped together for each of the four participating laboratories and shipped by courier along with the required kits.

Sample Extraction and Analysis:

Each laboratory extracted and analyzed the samples according to the procedure outlined in the Elisa System’s Sesame kit instructions. The raw data from each lab was sent to Health Canada's Food Allergen Research Laboratory in Ottawa for consolidation and statistical analysis.

Results / Discussion

After compiling the data it was noticed that quite a few spiked cracker samples and some cookie samples from Lab#1 provided very low recoveries, even for the higher spike level. It was discovered from the comments provided by the participating labs that the cracker samples and, to a lesser extent, the cookie samples formed large clumps with the spiking material, which needed to be thoroughly homogenized during the extraction step. It is likely that the difficulty in homogenizing these samples was the cause for the very low recoveries obtained by this laboratory and this data was excluded from further analysis. The remaining data was analyzed for outliers using the Grubb’s test at a 95% confidence level resulting in 5 outliers, which were removed from further analysis.

The results of the remaining 335 samples showed good inter and intra laboratory consistency. There were no false positives to report and 9 of the 210 spiked samples were below the 1.0 ppm positive control resulting in a 4.3% false negative rate. It should be noted that these false negative values all came from the 1.5 ppm spike level and the majority of these had optical densities just below the cut-off, which is considerably higher than the blank sample. One of the difficulties of this study was determining the spiking levels, given that the positive controls range from 1.0-5.0 ppm. In previous studies the first spiking level was two times the lowest calibration standard. This was done to account for variability in the measurements and still keep the result above the lowest calibration standard. In one example from this study Lab #2 reported 4 of the 9 false negative samples, which were observed for the cracker 1.5 ppm spiking level. Analysis of the plate results showed that the optical density for the 1.0 ppm positive control (0.92 ± 0.01) was considerably higher than that for the zero control (0.231 ± 0.002) and even the average for

the blank cracker samples (0.25 ± 0.07, n=8) from this lab. One method of estimating the LOQ is to use 10 times the standard deviation around the blank samples, which would produce a LOQ of 0.5 ppm for this plate, resulting in no false negatives. This information has been discussed with Elisa Systems who have indicated they will be adding a 0.5 ppm control to the calibration curve to extend the range of analysis. If the 0.5 ppm positive control were available for this study there would have been no false positives or false negatives.

The percent recovery determined from the average values obtained is very consistent between the labs and among the different matrices. If the theoretical spiking levels are used in the calculations then the percent recoveries range from 73-93% and 69-80% for the 1.5 ppm and 3.5 ppm spiking levels, respectively. These recoveries are relatively good and are consistent with the other studies in the compendium. The lowest recoveries were obtained from the cracker matrix, which had shown clumping of the material and is the most likely reason for the low recoveries. Other reasons for the low recoveries could be deterioration of the spiking material over time or matrix interferences in the analysis. To get an idea if either of these are occurring matrix free samples were sent to all of the labs for analysis. The levels determined for these samples were 1.2 ± 0.1ppm and 3.0 ± 0.6ppm for the theoretical 1.5 ppm and 3.5 ppm spiking levels, respectively. It appears there has been a very small deterioration of the spiking material response over time, which would result in recoveries of 92-117% and 80-93%, for the 1.5 and 3.5 ppm spiking levels, respectively.

An indication of how good the agreement is between labs and how well one lab did in comparison to the other labs is indicated from the Z-score. The Z score is simply an indication of how far, and in what direction, the mean of one lab deviates from the overall mean of the other labs in the study. For example a Z-score of 2.0 indicates a result that is two standard deviations above the mean and this result is sometimes used as a cut-off to indicate a lab that has obtained results that are statistically different from the others. The Z-scores were calculated for each lab, for each commodity and for each spiking level. All of the Z-scores were between -0.9 and 1.0 and shows that there is very good agreement between all of the participating labs.

A summary of the results from each of the participating labs is presented here. **Please see the end of this document for tables of data**.

Conclusion:

The Elisa Systems Sesame kit has delivered satisfactory results for the matrices and at the levels tested in this evaluation. The cracker samples provided some difficulty in the extraction due to clumping of the material, but the results were comparable with the other matrices once sufficient homogenization was obtained. The bread crumbs and the vegetable dip were less of an issue in terms of extractability problems and produced very similar results.

Cracker Results

	Cracker 0 ppm*			Cracker 1.5 ppm			Cracker 3.5 ppm		
	Range	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**	**Mean**	**SD**
Lab 1	0.0-0.1	0.04	0.02	NA	NA	NA	NA	NA	NA
Lab 2	0.0-0.0	0.01	0.01	1.0-1.2	1.1	0.1	1.6-2.6	2.2	0.3
Lab 3	0.0-0.1	0.06	0.04	1.1-1.2	1.2	0.1	1.0-3.7	2.6	0.8
Lab 4	0.0-0.1	0.04	0.03	1.0-1.3	1.1	0.1	1.6-3.1	2.3	0.4

* The levels are referenced to the kit calibrators and have been normalized to the reference material for this study.

Bread Crumb Results

	Bread Crumbs 0 ppm*			Bread Crumbs 1.5 ppm			Bread Crumbs 3.5 ppm		
	Range	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**	**Mean**	**SD**
Lab 1	0.0-0.1	0.07	0.04	1.0-1.3	1.2	0.1	1.3-3.2	2.3	0.6
Lab 2	0.0-0.1	0.05	0.01	1.2-1.5	1.4	0.1	2.7-4.0	3.3	0.5
Lab 3	0.0-0.1	0.05	0.04	1.0-2.0	1.4	0.4	2.1-3.9	3.0	0.6
Lab 4	0.0-0.2	0.09	0.04	1.2-1.5	1.4	0.1	2.2-3.2	2.8	0.3

* The levels are referenced to the kit calibrators and have been normalized to the reference material for this study.

Vegetable Dip Results

	Dip 0 ppm*			Dip 1.5 ppm			Dip 3.5 ppm		
	Range	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**	**Mean**	**SD**
Lab 1	0.0-0.1	0.04	0.02	1.0-1.4	1.2	0.1	2.3-3.0	2.8	0.3
Lab 2	0.0-0.1	0.05	0.02	1.0-1.2	1.1	0.1	2.3-2.9	2.6	0.2
Lab 3	0.0-0.1	0.06	0.04	1.0-1.4	1.2	0.2	2.1-3.3	2.8	0.4
Lab 4	0.0-0.2	0.10	0.04	1.0-1.4	1.1	0.1	2.3-3.3	2.7	0.4

* The levels are referenced to the kit calibrators and have been normalized to the reference material for this study.

Z-Scores for the participating labs*

	Cracker			Bread Crumbs			Dip		
	0.04 ppm	**1.14 ppm**	**2.36 ppm**	**0.06 ppm**	**1.35 ppm**	**2.84 ppm**	**0.06 ppm**	**1.14 ppm**	**2.72 ppm**
Lab 1	0.0	NA	NA	0.1	-0.8	-0.9	-0.6	0.1	0.1
Lab 2	-0.9	-0.6	-0.3	-0.4	0.1	0.8	-0.3	-0.2	-0.4
Lab 3	0.7	0.6	0.4	-0.4	0.3	0.2	-0.1	0.7	0.2
Lab 4	0.1	-0.2	-0.1	0.7	0.2	0.0	1.0	-0.1	0.0

* The Z-scores are based on the actual level of sesame protein determined rather than the theoretical spiking level.