

FOOD COMPOSITION AND ADDITIVES

Interlaboratory Study of ELISA Kits for the Detection of Egg and Milk Protein in Processed Foods

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The labeling of seven specific allergenic ingredients (egg, milk, wheat, buckwheat, peanut, shrimp, and crab) is mandatory in Japan. To ensure proper labeling, two kinds of ELISA kits using polyclonal antibodies have been developed. However, we developed two novel ELISA kits using monoclonal antibodies with improved specificity, the Allergeneye ELISA Egg (AE-Egg) and Allergeneye ELISA Milk (AE-Milk) Kits, to detect egg and milk proteins in processed foods, respectively. Five types of processed food containing 10 mg/kg of egg or milk soluble protein were prepared for an interlaboratory study of the performance of these kits. The kits showed a relatively high reproducibility level of interlaboratory precision (AE-Egg RSD_R , 3.7–5.7%; AE-Milk RSD_R , 6.8–10.5%) and satisfied the recovery rate stipulated by Japanese guidelines (AE-Egg, 61.6–89.3%; AE-Milk, 52.1–67%) for all processed foods. Our results suggest that the AE-Egg and AE-Milk Kits are precise and reliable tools for detecting egg or milk proteins in processed foods.

In recent years, food allergies have increased in Japan (1–4). Because food containing allergens represents a health hazard, there is an increasing need to inform consumers through labeling. In 2002, the Ministry of Health, Labor and Welfare of Japan (MHLW) introduced a labeling system for allergenic foods to ensure that information regarding their content is available to allergic consumers. According to Japanese regulations, labeling of food products containing egg, milk, wheat, buckwheat, peanuts, shrimp, and crab is mandatory; labeling is also recommended for 20 other food materials. To guarantee the validity of the labeling system, the MHLW announced official methods for the detection of allergens for mandatory labeling in a 2002 notification (5). These official methods, including a screening test using two kinds of ELISA kits, provided a means to monitor the labeling system. The ELISA kits have been tested in interlaboratory studies and showed a relatively high RSD_R and sufficient recovery for all processed foods tested (6, 7).

In Japan, the ELISA kits are used by local government public health centers as the official method to monitor food labeling and by food manufacturers to verify the integrity of product labels. Particularly in the latter case, it is often noted that the

ELISA kits yield few false-positive results. False-positive results necessitate re-examination of the product; consequently, the shipment of products is delayed, which is disadvantageous to food manufacturers. Therefore, high accuracy and fewer false-positive results are requirements for ELISA kits. Hence, the use of antibodies with high specificity is essential.

The two conventional ELISA kits use polyclonal antibodies. The Japanese official guidelines for evaluating methods of detecting food allergens state that antibodies used in ELISA kits can be polyclonal or monoclonal (8). However, to date, no ELISA kits have been developed using monoclonal antibodies alone. The principal advantages of monoclonal antibodies are their homogeneity, consistency, and high specificity compared with polyclonal antibodies (9). Therefore, there is a need to develop ELISA kits with new inspection characteristics that use monoclonal antibodies with high specificity. Focusing on egg and milk, which account for more than 50% of cases of allergic reactions in Japan, we developed two novel sandwich ELISA kits using monoclonal antibodies to detect egg and milk (10, 11). The ELISA kit for detecting egg, named Allergeneye ELISA Egg (AE-Egg; PRIMA Meat Packers, Ltd, Ibaraki, Japan), uses monoclonal antibodies against chicken egg ovalbumin, and the ELISA kit for detecting milk, named Allergeneye ELISA Milk (AE-Milk; PRIMA Meat Packers, Ltd), uses monoclonal antibodies against cow's milk β -lactoglobulin. These antibodies are able to detect denatured proteins extracted with the official extraction buffer, which has been developed for extracting insoluble proteins in processed foods produced with heat and/or high pressure (12). For the pre-evaluation of the AE-Egg and AE-Milk Kits, 10 kinds of commercially processed foods containing egg (such as bread, cup noodle, pasta sauce, pudding, potato salad, Chinese dumpling, fried rice, egg soup, sandwich, and whipped cream rice cake) or milk (such as bread, roast ham, pasta sauce, pudding, coffee jelly, gratin, potato potage soup, ice cream, sandwich, and fried noodles) were analyzed. The results showed >20 mg/kg over the calibration curve range, with the exception of whipped cream rice cake containing egg that showed 7.4 mg/kg; two conventional ELISA kits showed 7.2 and 6.5 mg/kg. AE-Egg Kit detected egg from all the commercially processed foods with labeling of egg. AE-Milk Kit detected milk from all the commercially processed foods with labeling of milk. Thus, the obtained measurement results were consistent with the labels. Therefore, we concluded that various eggs and milks present in commercially processed foods can be successfully measured. In addition, the results of our pre-evaluation showed that there was no cross-reactivity to 128 food ingredients other than egg or milk, indicating that these ELISA kits have extremely high specificity for egg and milk (10, 11).

We evaluated the performance of the AE-Egg and AE-Milk Kits through an interlaboratory study in accordance with the official guidelines (8). In brief, interlaboratory validation protocols must meet the following criteria: number of laboratories ≥ 8 ; number of samples ≥ 5 ; dose level ≥ 1 (10 mg/kg allergenic ingredient soluble protein weight/food weight); recovery 50–150%; and $RSD_R \leq 25\%$. This paper describes the results of the interlaboratory evaluation of the AE-Egg and AE-Milk Kits.

Experimental

Materials and Methods

(a) Preparation of egg and milk powders for spiking.—Egg and milk powders were prepared according to the Japanese official method (8). Egg powder for spiking was prepared by homogenization of White Leghorn eggs followed by freeze-drying. Milk from Holstein Friesian cows was also freeze-dried after defatting by churning. Soluble egg protein (EP) from egg powder and soluble milk protein (MP) from milk powder were extracted using the extraction buffer: phosphate-buffered saline (pH 7.4) containing 0.5% sodium dodecyl sulfate (SDS) and 2% β -mercaptoethanol. EP and MP contents were then calculated using a 2-D Quant Kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK). The amount of protein present in 1 g of each spiking powder was 507 mg for egg and 224 mg for milk.

(b) Preparation of processed foods.—Five kinds of processed food (rice gruel, sweet adzuki bean soup, orange juice, pork sausage, and miso soup) containing EP or MP were prepared and used as test materials. All processed foods were prepared according to the manufacturers' instructions. EP or MP was added at the ingredients stage before processing to establish a final level of 10 mg/kg (EP or MP weight/sample weight). The prepared processed foods were homogenized with a food processor (MK-K80P; Panasonic Corporation, Osaka, Japan) and sent to participants as test materials.

The rice gruel sample comprised rice, salt, and water. Rice, salt, water, and egg or milk spiking powder were mixed in a food processor and homogenized. The homogenate was packed in an aluminum pouch and cooked at 121°C for 4 min, cooled in flowing water, and stored at -40°C .

The sweet adzuki bean sample was made from adzuki beans, sugar, and water. Adzuki beans, sugar, water, and egg or milk spiking powder were mixed in a food processor and homogenized. The homogenate was packed in an aluminum pouch, placed in boiling water (approximately 100°C) for 10 min, cooled in flowing water, and stored at -40°C .

The orange juice sample was made from concentrated orange juice and citric acid. Orange juice, citric acid, and egg or milk spiking powder were mixed in a juicer mixer and homogenized. The homogenate was packed in an aluminum pouch and placed in boiling water (approximately 100°C) for 30 min, cooled in flowing water, and stored at -40°C .

The pork sausage sample was made from pork, fat, salt, sodium phosphate, sodium nitrite, sodium ascorbate, and water. Pork, fat, salt, sodium phosphate, sodium nitrite, sodium ascorbate, water, and egg or milk spiking powder were mixed in a food processor and homogenized. The homogenate was

packed in an aluminum pouch, cooked at 75°C for 30 min, cooled in flowing water, and stored at -40°C .

The miso soup sample was made from miso (soy bean paste) and fish stock powder. Miso, fish stock powder, and egg or milk spiking powder were mixed in a juicer mixer and homogenized. The homogenate was packed in an aluminum pouch and placed in boiling water (approximately 100°C) for 10 min, cooled in flowing water, and stored at -40°C .

(c) Homogeneity tests of samples.—The homogeneity of the samples was verified by the procedure of the International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories (13), except that the number of test samples was six. Twelve test portions of each sample were analyzed using the AE-Egg and AE-Milk Kits. The resulting EP or MP concentrations were then determined using one-way analysis of variance (ANOVA).

ELISA Kits

(a) The AE-Egg Kit.—Monoclonal antibodies to ovalbumin in chicken eggs are used in the AE-Egg Kit (10) with the EP solution as a calibrator. Each of the diluted standard solutions and sample solutions was added to a monoclonal antibody-coated module and incubated for 1 h at room temperature (20–25°C). After the module was washed, a solution of monoclonal antibodies labeled with horseradish peroxidase was added and allowed to stand at room temperature (20–25°C) for 30 min. After further washing, a solution of 3,3',5,5'-tetramethylbenzidine was added, and the module was allowed to stand at room temperature (20–25°C) for 10 min. The reaction was stopped by the addition of 0.5 M sulfuric acid, and the absorbance was measured at 450 nm with 650 nm as the reference wavelength. The AE-Egg Kit was highly specific; it showed cross-reactivity only to duck and Japanese quail eggs and no cross-reactivity to other food ingredients. We determined the LOD and LOQ according to the International Organization for Standardization and International Union of Pure and Applied Chemistry guidelines (14). The LOD was calculated as three times the SD of the dilution buffer mean value after 24 experiments. The LOQ was calculated as 10 times the SD of the dilution buffer mean value after 24 experiments. The LOD and the LOQ of the AE-Egg Kit determined using the dilution buffer were 0.5 ng/mL (equivalent to 0.2 mg/kg food sample) and 1.6 ng/mL (equivalent to 0.6 mg/kg food sample), respectively. Consequently, the practical detection range was between 0.8 and 50 ng/mL (equivalent to between 0.3 and 20 mg/kg food sample), and the quantification range was between 2.5 and 50 ng/mL (equivalent to between 1 and 20 mg/kg food sample).

(b) The AE-Milk Kit.—Monoclonal antibodies to cow's milk β -lactoglobulin are used in the AE-Milk kit (11) with the MP solution as a calibrator. The measurement method with the kit is the same as that with the AE-Egg Kit. The AE-Milk Kit was highly specific; it showed cross-reactivity only to sheep's and goat's milk, and no cross-reactivity to other food ingredients. We determined the LOD and LOQ in the same way as for the AE-Egg Kit. The LOD and LOQ of the AE-Milk Kit determined using the dilution buffer were 0.5 ng/mL (equivalent to 0.2 mg/kg food sample) and 1.5 ng/mL (equivalent to 0.6 mg/kg food sample), respectively. Consequently, the practical detection range was between 0.8 and 50 ng/mL (equivalent to between 0.3 and 20 mg/kg food sample), and the quantification range

Table 1. Homogeneity test^a results of the egg test materials

Test sample	Mean, mg/kg	RSD, % ^b	<i>n</i>	<i>F</i> -ratio	<i>F</i> crit ^c
Rice gruel	6.1	7.8	6	0.97	4.39
Sweet adzuki bean soup	6.8	6.2	6	2.26	4.39
Orange juice	8.6	2.3	6	1.68	4.39
Pork sausage	12.3	8.1	6	1.14	4.39
Miso soup	8.9	5.6	6	0.73	4.39

^a Homogeneity test was carried out with AE-Egg.^b RSD, % calculated from s_s (SD of sampling) and s_a (SD of analysis).^c *F* crit = Critical *F*-value.

was between 2.5 and 50 ng/mL (equivalent to between 1 and 20 mg/kg food sample).

Extraction

The same extraction procedure was used for the AE-Egg and AE-Milk Kits. A 1 g portion of the sample was extracted with 19 mL extraction buffer (Morinaga Institute of Biological Science, Inc., Yokohama, Japan; 12). The mixture was shaken horizontally overnight (>12 h) at room temperature and then centrifuged at $3000 \times g$ for 20 min after adjusting the pH to 6.0–8.0. The supernatant was filtered or centrifuged, if necessary, diluted 20 times, and subjected to ELISA.

Calibration Standard Solutions

The ingredients for the calibration solutions were identical to those of the bulk standard powder for spiking: eggs, fresh White Leghorn eggs, homogenized and freeze-dried; and milk, fresh milk from Holstein Friesian cows, freeze-dried after defatting by churning.

The ingredients were extracted with 20 mL extraction solution containing 0.5% SDS and 2% β -mercaptoethanol by shaking overnight. Protein content was determined using a 2-D Quant Kit. The initial extract was diluted to yield the calibration standard solution (50 ng/mL extracted protein).

Interlaboratory Study

Fourteen laboratories participated in the interlaboratory study, which was coordinated by the Research and Development Department of PRIMA Meat Packers, Ltd. The coordinators sent

the five kinds of test materials (3 g each), the ELISA kits, and the extraction buffer and the calibration standard solutions to the participants. The participants took two portions from each test material, extracted the protein using the extraction procedure, and assayed each extract with the ELISA kits. The calibration standard solution was diluted and assayed simultaneously with the sample extracts. The average absorbance of the three wells was used for calculation. The absorbance data from the calibration solutions and test samples obtained were reported to the coordinator.

The coordinator calculated a four-parameter logistic calibration curve from the absorbance data of the calibration standard solution and calculated the concentration of allergen protein in the sample using the calibration curve. The average of three results was treated as the data for one portion.

Statistical Analysis

Twenty-eight data items, comprising data items for two portions from each of the 14 laboratories, were used for the calculation. The Cochran and Grubbs' tests were used to remove outlying data ($P = 2.5\%$). The use of these statistical tests to identify outliers was in accordance with the AOAC protocol (15). Recovery, repeatability, and reproducibility were calculated by one-way ANOVA using the data remaining after the removal of outliers.

Results and Discussion

Sample Homogeneity

Tables 1 and 2 show the results of the homogeneity tests of samples using the AE-Egg and AE-Milk Kits, respectively,

Table 2. Homogeneity test^a results of the milk test material

Test sample	Mean, mg/kg	RSD, % ^b	<i>n</i>	<i>F</i> -ratio	<i>F</i> crit ^c
Rice gruel	6.1	4.7	6	1.99	4.39
Sweet adzuki bean soup	7.1	3.2	6	0.83	4.39
Orange juice	5.7	4.5	6	2.57	4.39
Pork sausage	6.1	9.3	6	3.26	4.39
Miso soup	7.6	5.2	6	0.92	4.39

^a Homogeneity test was carried out with AE-milk.^b RSD, % calculated from s_s (SD of sampling) and s_a (SD of analysis).^c *F* crit = Critical *F*-value.

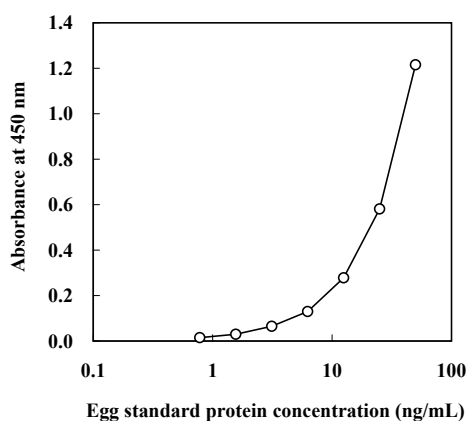


Figure 1. Egg protein calibration curve of the AE-Egg Kit.

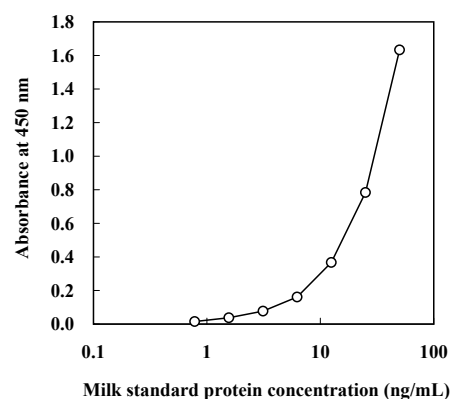


Figure 2. Milk protein calibration curve of the AE-Milk Kit.

including the averaged protein concentrations, RSD_R values calculated from the s_s (SD of sampling) and s_a (SD of analysis), F -ratios, and critical F -values. The resultant F -ratios for the five kinds of sample tested using the AE-Egg Kit shown in Table 1 were <2.26 . Table 2 shows the resultant F -ratios of the homogeneity tests of the samples using the AE-Milk Kit, which were <3.26 . The F -ratios for all samples were below the critical F -value of 4.39; therefore, the homogeneity of the test materials was sufficient for interlaboratory evaluation.

Calibration Curves

Figures 1 and 2 show calibration curves of the AE-Egg and AE-Milk Kits, respectively. Each calibration curve was obtained using a four-parameter logistic method using the mean value after eight experiments. The concentrations of the calibration standards (calculated EP or MP weight/food weight) were 0.8, 1.6, 3.1, 6.3, 12.5, 25, and 50 ng/mL (equivalent to 0.3, 0.6, 1.3,

2.5, 5, 10, and 20 mg/kg of food sample). The curves show that the AE-Egg and AE-Milk Kits have quantitative performance sufficient to measure 25 ng/mL (10 mg/kg) of test material.

Values Reported by Interlaboratory Study Participants

Values reported by the interlaboratory study participants are summarized in Tables 3 (AE-Egg) and 4 (AE-Milk). As shown in Table 3, the data for rice gruel, sweet adzuki bean soup, orange juice, pork sausage, and miso soup from Laboratory F, and the data for sweet adzuki bean soup, orange juice, pork sausage, and miso soup from Laboratory G, were determined to be outliers using the Grubbs' test. As shown in Table 4, the data for miso soup from Laboratory D were determined to be outliers using Grubbs' test. Quantitative values for the results from Laboratory F could not be calculated because the absorbance

Table 3. Results of the interlaboratory study for AE-Egg protein recovery content (values expressed as mg/kg and %)^a

Lab	Rice gruel		Sweet adzuki bean soup		Orange juice		Pork sausage		Miso soup	
	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%
A	5.9	59.2	7.1	71.4	6.9	69.3	8.7	87.1	6.8	67.9
B	6.1	61.1	7.0	69.8	7.0	70.0	9.2	92.1	7.2	72.3
C	6.1	61.4	6.7	66.7	6.7	67.1	9.0	89.6	7.2	71.8
D	6.0	60.3	7.0	70.4	7.0	70.2	9.3	93.3	6.9	69.3
E	6.2	61.6	7.0	70.4	7.0	70.3	9.2	92.5	7.0	69.7
F	7.7 ^b	77.1	8.7 ^b	87.4	8.5 ^b	85.1	10.7 ^b	107.4	9.0 ^b	89.7
G	7.0	69.7	8.4 ^b	83.7	8.3 ^b	82.8	5.0 ^b	50.1	8.6 ^b	86.2
H	6.4	63.9	7.0	69.6	7.1	71.4	8.4	83.8	7.3	73.3
I	5.7	57.0	6.8	68.2	6.6	65.8	8.9	88.6	6.7	67.4
J	6.5	65.5	7.6	76.4	7.2	71.6	9.4	94.0	7.3	72.7
K	5.9	59.1	6.9	68.9	6.7	66.9	8.5	85.2	6.9	68.7
L	6.1	61.1	7.2	72.5	7.3	73.4	9.4	94.5	7.5	75.2
M	6.0	59.6	6.8	68.0	6.6	66.5	9.1	91.1	6.4	64.2
N	6.1	61.1	6.6	66.0	6.8	68.3	7.9	79.5	6.8	67.9

^a The incurred sample contained EP of approximately 10 mg/kg.

^b Values removed after the Grubbs' test.

Table 4. Results of interlaboratory study for AE-milk protein recovery content (values expressed as mg/kg and %)^a

Lab	Rice gruel		Sweet adzuki bean soup		Orange juice		Pork sausage		Miso soup	
	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%
A	5.8	57.6	6.7	66.9	5.6	55.6	6.7	67.3	5.6	55.5
B	5.6	55.7	6.5	64.7	5.2	51.8	6.6	66.3	5.8	58.2
C	5.8	58.3	6.7	67.3	5.5	54.9	6.8	67.7	6.3	62.8
D	5.5	54.9	6.8	67.7	5.2	51.9	6.7	67.1	5.3 ^b	53.0
E	5.7	57.2	6.9	68.5	5.4	53.7	7.2	72.3	5.7	57.4
F	— ^c	— ^c	— ^c	— ^c	— ^c	— ^c	— ^c	— ^c	— ^c	— ^c
G	5.3	53.0	6.1	61.2	5.1	51.5	6.4	63.6	5.4	53.6
H	5.4	53.8	6.0	60.3	4.9	49.2	6.6	65.8	5.9	58.6
I	6.8	67.9	7.8	78.2	6.3	62.6	7.1	70.5	6.7	67.4
J	5.5	55.5	6.4	64.4	5.3	53.4	6.6	65.9	5.7	57.1
K	5.3	52.8	6.1	60.5	4.8	47.9	6.6	66.2	5.5	55.1
L	6.3	62.9	7.2	72.2	5.6	55.5	7.7	76.7	6.3	62.6
M	5.0	49.9	5.8	57.6	4.8	47.5	6.0	59.6	4.7	47.1
N	4.4	44.4	5.7	57.3	4.2	41.7	6.3	62.5	4.8	48.3

^a The incurred sample contained MP of approximately 10 mg/kg.^b Values removed after the Grubbs' test.^c Could not be calculated quantitative value, that absorbance value of the calibration curve was above the detected limit of the microplate reader.

value of the calibration curve was above the detection limit of the microplate reader.

Recovery

The number of laboratories remaining after the removal of outliers, average concentration (mg/kg), s_r (repeatability SD, mg/kg), s_R (reproducibility SD, mg/kg), recovery (%), repeatability (RSD_r , %) and reproducibility (RSD_R , %), calculated using ANOVA are shown in Tables 5 (AE-Egg) and 6 (AE-Milk).

The recovery rates of EP and MP were in the range of 50–150% for all five test materials. As shown in Table 5, EP recovery rates from the five test materials using the AE-Egg Kit were in the range of 61.6–89.3%. The recovery rate was the highest from pork sausage (89.3%) and lowest from rice gruel (61.6%). As shown in Table 6, MP recovery rates from the five test materials using the AE-Milk Kit were in the range of 52.1–67.0%. The recovery rate was the highest from pork sausage (67.0%) and lowest from orange juice (52.1%).

Matsuda et al. (6) reported the results of an interlaboratory study of ELISA kits, FASTKIT ELISA Version II (Nippon Meat Packers, Inc., Osaka, Japan) and FASPEK Allergenic Substances Detection Kit (Morinaga Institute of Biological

Science, Inc.), for the determination of egg, milk, wheat, buckwheat, and peanut proteins in processed foods. There were discrepancies between the two kits in the recovery of some proteins. In the case of egg detection kits, the rates of recovery in five processed foods were in the range of 66–98% using a FASTKIT Kit and in the range of 52–87% using a FASPEK Kit. These discrepancies were thought to be due to differences in the reactivity of the polyclonal antibodies used in these kits to the denatured proteins. In addition, the processed food matrixes or heating conditions of processing may have affected the reactivity of the polyclonal antibodies.

We developed the AE-Egg and AE-Milk Kits using monoclonal antibodies. These monoclonal antibodies are able to detect denatured proteins extracted using the official extraction buffer (10, 11). The different recovery rates from the five processed foods may reflect differences in reactivity of the monoclonal antibodies to the denatured proteins. Food matrixes or heating conditions of processing may also have affected the reactivity of the monoclonal antibodies.

Repeatability

Repeatability is a measure of the variance arising from the extraction and detection procedures in a laboratory. In the

Table 5. Recoveries, repeatabilities (RSD_r), and reproducibilities (RSD_R) of AE-Egg

Sample	No. of labs	Mean, mg/kg	s_r , mg/kg	s_R , mg/kg	Recovery, %	RSD_r , %	RSD_R , %
Rice gruel	13	6.2	0.2	0.4	61.6	3.2	5.7
Sweet adzuki bean soup	12	7.0	0.2	0.3	69.8	3.3	4.6
Orange juice	12	6.9	0.1	0.3	69.2	1.9	3.7
Miso soup	12	7.0	0.3	0.4	70.0	3.6	5.1
Pork sausage	12	8.9	0.2	0.5	89.3	2.2	5.4

Table 6. Recoveries, repeatabilities (RSD_r), and reproducibilities (RSD_R) of AE-Milk

Sample	No. of laboratories	Mean, mg/kg	s _r , mg/kg	s _R , mg/kg	Recovery, %	RSD _r , %	RSD _R , %
Rice gruel	13	5.6	0.1	0.6	55.7	2.6	10.5
Sweet adzuki bean soup	13	6.5	0.2	0.6	65.1	3.1	9.4
Orange juice	13	5.2	0.2	0.5	52.1	3.7	10.0
Miso soup	12	5.7	0.2	0.6	57.0	3.1	10.4
Pork sausage	13	6.7	0.2	0.5	67.0	2.9	6.8

interlaboratory study, all RSD_r values for the AE-Egg and AE-Milk Kits were found to be <3.6 and <3.7, respectively. According to Horwitz's theory, the RSD_r value is generally likely to be less than two-thirds that of the RSD_R value (16). In this study, all RSD_r values for the AE-Egg and AE-Milk Kits were less than two-thirds that of the corresponding RSD_R values with two exceptions: using the AE-Egg Kit, the RSD_r value was 3.3 and the RSD_R value was 4.6 for sweet adzuki bean soup (two-thirds of the RSD_R of 4.6 is approximately 3.1), and the RSD_r value was 3.6 and the RSD_R value was 5.1 for sweet miso soup (two-thirds of the RSD_R of 5.1 is approximately 3.4). These satisfactory RSD_r values were considered to guarantee the reliability of this analytical method.

Reproducibility

Reproducibilities, expressed by RSD_R values, for the five test materials were in the range of 3.7–5.7% for the AE-Egg Kit and 6.8–10.5% for the AE-Milk Kit. The AE-Egg and AE-Milk Kits demonstrated relatively high levels of reproducibility in the interlaboratory study. Therefore, we conclude that these two kits are useful as a general method of monitoring allergenic ingredients to ensure accurate labeling.

Conclusions

In the official guidelines published in 2006 (8), the MHLW established interlaboratory validation protocols to evaluate methods of inspecting foods containing allergens. In brief, interlaboratory validation protocols must meet the following criteria: number of laboratories ≥8; number of samples ≥5; dose level ≥1 (including 10 mg/kg, allergenic ingredient soluble protein weight/food weight); recovery 50–150%; and RSD_R ≤25%. These criteria are based on ISO 5725 (JIS Z8402), which is largely consistent with the AOAC guidelines (15).

We developed the AE-Egg and AE-Milk Kits for the quantitative analysis of chicken egg and cow's milk in specified food ingredients, and the kits satisfied the official Japanese interlaboratory validation criteria for quantitative assays for food allergens.

In conclusion, the results of our interlaboratory study suggest that the AE-Egg and AE-Milk Kits are suitable for use as precise and reliable tools for the detection of egg or milk proteins in processed foods. The proposed system is capable of accurately monitoring the labeling system in a reliable manner, and it may be useful for inspections performed in accordance with Japanese regulations. The AE-Egg and AE-Milk Kits are currently recognized as the official method for the detection of egg and milk proteins in processed foods in Japan.

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