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Validation of Quantitative and Qualitative Methods for Detecting Allergenic Ingredients in Processed Foods in Japan

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ABSTRACT: A labeling system for food allergenic ingredients was established in Japan in April 2002. To monitor the labeling, the Japanese government announced official methods for detecting allergens in processed foods in November 2002. The official methods consist of quantitative screening tests using enzyme-linked immunosorbent assays (ELISAs) and qualitative confirmation tests using Western blotting or polymerase chain reactions (PCR). In addition, the Japanese government designated 10 μg protein/g food (the corresponding allergenic ingredient soluble protein weight/food weight), determined by ELISA, as the labeling threshold. To standardize the official methods, the criteria for the validation protocol were described in the official guidelines. This paper, which was presented at the Advances in Food Allergen Detection Symposium, ACS National Meeting and Expo, San Diego, CA, Spring 2012, describes the validation protocol outlined in the official Japanese guidelines, the results of interlaboratory studies for the quantitative detection method (ELISA for crustacean proteins) and the qualitative detection method (PCR for shrimp and crab DNAs), and the reliability of the detection methods.

KEYWORDS: *food allergy, interlaboratory validation, enzyme-linked immunosorbent assay, polymerase chain reaction, crustacean*

INTRODUCTION

Food allergies, defined as an immune response to food proteins, affect as many as 4−8% of young children and 2% of adults in developed countries, and their prevalence appears to be rising.[1] Food allergies affect 12.8% of infants, 5.1% of toddlers, and 1.3−2.6% of school-aged children in Japan.[2] In 1999, the Joint FAO/WHO Codex Alimentarius Commission agreed to recommend the labeling of eight kinds of foods (milk, eggs, peanuts, tree nuts, seafood, shellfish, soy, and wheat) that contain ingredients known to be allergens.[3,4] This prompted the Japanese government to take new measures to address the issue of food allergies in Japan.

Because it is essential for food allergy patients to eliminate food allergens from their diet, the Ministry of Health, Labour and Welfare of Japan (MHLW) decided to improve the food labeling system by amending the Food Sanitation Law in 2001.[5] They organized a labeling study group consisting of clinical experts, patients, researchers, retailers, and food manufacturers. This group discussed various methodologies for labeling systems, and the results of their discussions were compiled in a report in November 2000. In this report, labeling was divided into two stages, mandatory and recommended, according to the actual number of allergy cases and the degree of the severity.

The labeling was made mandatory by ministerial ordinance for seven ingredients: egg, milk, wheat, buckwheat, peanut, crab, and shrimp (prawn). Furthermore, for 18 ingredients, the labeling was recommended by ministerial notification: abalone, squid, salmon roe, orange, kiwifruit, beef, walnut, salmon, mackerel, soybean, chicken, banana, pork, *matsutake* mushroom, peach, yam, apple, and gelatin.

Consequently, egg, milk, wheat, buckwheat, peanut, shrimp, and crab require mandatory labeling, and we refer to these seven ingredients as "*specific allergenic ingredients*." To the best of our knowledge, Japan was the first country to set up mandatory food allergy labeling and to regulate it under national law (2002).[6]

Among shrimp allergy cases, 64.7% of patients also showed a positive reaction to crabs.[7] However, the fact that the remaining 35.3% of patients showed no reaction to crabs suggests that some patients with a shrimp allergy can eat crabs. Thus, in terms of providing consumers with accurate information, it is important to label "shrimp" and "crab" separately, rather than grouping them together as "crustacean." Accordingly, the MHLW has instructed that shrimp and crab should be labeled separately.

In general, proteins and nucleotides from allergens are not necessarily toxins. The threshold for the prevention of allergic reactions is often considered to be zero. However, a zero tolerance for the offending food would create enormous practical problems for the food industry. Therefore, the MHLW established a threshold for the labeling and the official detection methods for the *specific allergenic ingredients*. To do this, the MHLW organized a detection method study group consisting of food manufacturing companies, retailers, public research institutes, universities, and private inspection institutes. This study group concluded that the limits of detection (LOD) for enzyme-linked immunosorbent assay (ELISA) are generally in the range of 0.1−1.0 μg protein/g food. However, setting a labeling threshold for the ELISA LOD range was quite difficult because of problems with repeatability and reproducibility. In addition, the LODs of the lateral flow and polymerase chain reaction (PCR) methods would be approximately 5 μg protein/g food.

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On the other hand, the labeling study group proposed the threshold for the labeling system, that is, the definition of a trace amount. The group stated that "if more than a few μg/mL protein or a few μg/g protein of an allergen are contained in a food, labeling of that allergen is necessary". Considering these factors, the MHLW designated 10 μg soluble protein/g food ingredient, determined by the quantitative detection method, as the threshold for the labeling of food allergens. We believe that this level is the minimum required to control contamination by allergenic ingredients using detection methods on an industrial scale. Therefore, we developed methods to determine the presence of food allergenic ingredient proteins at levels of a few micrrograms per milliliter or a few micrograms per gram of food, based on the definition of a trace amount.[8−15]

In Japan, the labeling of five food ingredients (egg, milk, wheat, buckwheat, and peanut) in any processed food has been mandatory since April 2002, and the labeling of shrimp and crab has been mandatory since June 2008. The MHLW announced the Japanese official methods for the detection of the *specific allergenic ingredients* in a ministry notification, based on the methods developed by the detection method study group. These official methods consist of two types of ELISA kit screenings, the Western blotting method for egg and milk, and the PCR method for wheat, buckwheat, peanut, shrimp, and crab as the confirmation tests listed in the ministerial notification.[16] In 2004, the MHLW added the specification and standardization of the extraction buffer, reference material, and standard solution for testing of the *specific allergenic ingredients*. Furthermore, in 2006 the MHLW announced the validation protocol criteria to standardize the Japanese official methods for allergen detection.[17] The quantitative and qualitative detection methods have been validated according to the Japanese validation protocol.[9,14,18−25]

In this paper, we show the results of some actual interlaboratory validations, conducted with robustness and accuracy according to the Japanese official guidelines: the crustacean ELISA kits as quantitative detection method (M kit, Crustacean Kit (Maruha);[10] N kit, FA Test Crustacea (Nissui)[11]) and the shrimp and crab PCRs as qualitative detection method.[15]

■ MATERIALS AND METHODS

Validation Protocol Criteria for Detection Methods for the *Specific Allergenic Ingredients*. The MHLW established validation protocol criteria to standardize the Japanese official methods for the detection of the *specific allergenic ingredients*, as described in the 2006 official guidelines.[17] The outlines of the validation protocol criteria for the quantitative and qualitative detection methods for the *specific allergenic ingredients* are shown in Table 1.

Table 1. Japanese Guideline Criteria for Validation Protocol for Food Allergenic Ingredients

	Quantitative Detection Method
no. of laboratories	≥8
no. of incurred samples	≥5
no. of dose levels	≥1, including 10 μg/g[a]
recovery	50−150%
reproducibility (RSD_R)	≤25%
	Qualitative Detection Method
no. of laboratories	≥6
no. of incurred samples	≥5
no. of dose levels	≥2, including negative control (blank) and positive control (10 μg/g[a])
precision	≥90%

[a]Soluble protein weight/food weight of the corresponding allergenic ingredient. Notification 286 (Consumer Affairs Agency).

The validation protocol criteria for the quantitative detection method of *specific allergenic ingredients* are as follows:

(1) Number of laboratories: eight or more.
(2) Number of incurred samples (matrices): five or more.
(3) *Specific allergenic ingredients* in the incurred sample should include a concentration of 10 μg/g (the corresponding allergenic ingredient soluble protein weight/food weight), which is the concentration defined as "trace amount of contamination" (under the Food Sanitation Law, any food containing protein from the *specific allergenic ingredients* at a concentration >10 μg/g must be labeled). The incurred sample should be prepared by common processing methods, such as heating, baking, frying, acidifying, and pressurizing. It is recommended that the incurred samples evaluated during validation be selected from foods such as animal products, plant products, highly processed food (extended heating, high-pressure preparation), or acidic foods, because the corresponding ELISA should be applicable to various types of processed products.
(4) The recovery rate from each incurred sample should be in the range of 50−150%. In addition, reproducibility (RSD_R) should be ≤25%.

In the guidelines and reference material, the initial extract solution and the extraction procedure for the *specific allergenic ingredients* were also specified and standardized. For developing an ELISA for the detection of a food-specific allergenic ingredient, the ELISA performance should fulfill the interlaboratory validation criteria of the "Collaborative Study" protocol based on ISO5725 (JIS Z8402), which is basically the same as that of the Association of Official Analytical Chemists (AOAC), and the obtained performance data must be available to the public.[26,27] AOAC International is one of the few organizations providing expertise in the validation of ELISA methods for allergen detection, having validated several methods under the auspices of the AOAC Research Institute.

The validation protocol criteria for the qualitative detection method of food allergenic ingredients are as follows:

(1) Number of laboratories: six or more.
(2) Number of incurred samples (matrices): five or more.
(3) Number of dose levels ≥2, including the negative control (blank sample) and 10 μg/g of the positive control sample. The preparation of the incurred sample is the same as with the quantitative detection method mentioned above.
(4) Precision should be ≥90% (at this level, the incurred sample is positive and the blank is negative).

Because an international validation protocol of the qualitative detection method for *specific allergenic ingredients* has not yet been established, the essential elements designated on the provisional current criteria might be modified in a future evaluation of the qualitative detection method.

Preparation of Incurred Samples for Interlaboratory Validation of Quantitative and Qualitative Detection Methods. Black tiger shrimp (*Penaeus monodon*) and king crab (*Paralithodes camtschticus*) muscles were homogenized and freeze-dried to prepare shrimp powder and crab powder, respectively. The shrimp soluble protein (SP) and crab soluble protein (CP) were extracted from the powders, using phosphate-buffered saline (PBS, pH 7.4) containing 0.5% sodium dodecyl sulfate (SDS) and 2% β-mercaptoethanol. The protein contents were calculated using a 2-D Quant Kit (GE Healthcare, Buckinghamshire, UK). Shrimp and crab powders contained approximately 684 and 549 mg/g of soluble protein, respectively.

According to the guideline described above, various types of the incurred samples were selected for the interlaboratory validations. Five incurred samples (fish sausage, freeze-dried egg soup, tomato sauce, croquette, and chicken meatball) containing SP were prepared for the validation of crustacean ELISA kits. Six incurred samples (rice gruel, chicken meatball, freeze-dried vegetable soup, croquette, soybean soup, and konjac noodle) containing SP were prepared for the validation of shrimp PCR. Six incurred samples (rice gruel, chicken meatball, freeze-dried vegetable soup, croquette, soybean soup, and seasoning powder) containing CP were prepared for the validation of crab PCR.

All of the incurred samples were prepared using general procedures described by the manufacturers (e.g., heating, baking, boiling, and pressurizing). To obtain a final soluble protein content of 10 μg/g in these foods, the amount of shrimp/crab powder added was calculated by taking into account the protein content of the shrimp/crab powder and the change in weight of the foods during preparation.

For each type of sample, blanks to which SP or CP was not added were prepared to confirm potential contamination, false positives, and matrix effects. All of the values for the blank samples using crustacean ELISA kits were lower than the LOD, and these blank samples did not yield products by shrimp/crab PCR during the in-house validation (data not shown).

The incurred samples were homogenized with a food processor (DLC-XG, Cuisinart, Stamford, CT, USA) and sent to the participants as test materials for the interlaboratory validation of the quantitative method. For the interlaboratory validation of the qualitative method, the DNA extracted from each incurred sample and each blank sample was used as test material. Genomic DNA was extracted from 2 g of each sample with 20 mL of buffer G2 (Qiagen, Hilden, Germany) and purified using Genomic-tip 20/G (Qiagen) according to the manufacturer's instructions with slight modifications. This extraction procedure was carried out by the organizer to exclude operators' skill in preparing the samples. Because the detection methods have to be applicable to various types of commercial foods, we assumed that the use of incurred samples would be the best option for the interlaboratory validation of the detection methods.

Homogeneity Test of the Incurred Samples. The homogeneity of the incurred samples was verified by the organizer before distribution, following the procedure described in the International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories,[28] except that the number of tested materials was six.

Basically, the procedure was performed following the AOAC homogeneity test protocol, with some modifications:

(1) Randomly select 3 g × 6 samples (n).
(2) Take 1 g × 2 test portions (p) from each 3 g sample.
(3) Analyze the $2n$ test portions (12 p) in random order under repeatability conditions (two wells).
(4) Estimate the sampling variance (S^2_s) by one-way analysis of variance ($2 \times 6n$), using the average value of each well (estimation variance between each portion and each sample).
(5) Estimate the analytical variance (S^2_a) by one-way analysis of variance ($2 \times 12p$), using the value from each well (estimation variance between each well and each portion).

Interlaboratory Validation of the Quantitative Detection Method (Crustacean ELISA).[23] Ten laboratories participated in the interlaboratory evaluation organized by the National Institute of Health Sciences (NIHS; Tokyo, Japan). The participants included food manufacturing companies, public research institutes, local public inspection institutes, and private inspection institutes. The organizer sent each laboratory the five test materials (3 g each) and two ELISA kits (the M and N kits), together with the extraction solution and the calibration standard solutions. The participants took two portions from each test material, extracted the protein, and assayed each extract using the ELISA kits. The calibration standard solution was diluted and assayed simultaneously with the sample extracts. Each sample extract was analyzed in triplicate (3 wells/sample extraction), and their average absorbance was used for the calculation. The absorbance data of the calibration solutions and test materials were reported to the organizer.

The evaluation method for interlaboratory validation was as follows:

(1) A standard curve (a four-parameter logistic curve) was prepared using the absorbance value collected from each participant.
(2) The data from the first and second portions were subjected to a repeatability test using the average values from three wells.
(3) Cochran's test and Grubbs' test for the removal of outliers were performed (both tests at a significance level of 2.5%).
(4) Analytical variance was estimated by one-way ANOVA.

The results obtained were evaluated according to the AOAC protocol and ISO5725-5 algorithm.[26,27] The values reported by the participants (20 data items, as two portions from ten laboratories) were fed to the calculation.

Interlaboratory Validation of the Qualitative Detection Method (Shrimp and Crab PCR). Eleven laboratories participated in the interlaboratory validations, also organized by the NIHS. The participants included food manufacturing companies, public research institutes, local public inspection institutes, and private inspection institutes. The organizer sent each participating laboratory the primer pair sets for shrimp PCR and crab PCR, the PCR master mix (PCR buffer, dNTPs, $MgCl_2$, and *Taq* DNA polymerase), and positive control plasmids. To assess the validity of the extracted DNA, PCR to detect universal DNA (animal and/or plant) was carried out. This step is very important to avoid false negatives. As test samples, we prepared a total of 24 DNA extracts consisting of six incurred samples, two dose levels (10 μg/g for incurred sample, and blank sample), and the duplicates of each sample. The organizer summarized the data and calculated the accuracy of the detection methods (the percentages of positive results for incurred samples and those of negative results for blank samples). If a laboratory could not obtain the band derived from the positive control plasmid, it was removed as an outlier.

RESULTS AND DISCUSSION

Homogeneity of the Incurred Samples. Table 2 shows the results of the homogeneity test of the incurred samples,

Table 2. Homogeneity Test Results of Incurred Samples for Interlaboratory Validation Studies

	mean (μg/g)	RSD (%[a])	n	F ratio	F_{crit}[b]
Samples for Validation of Quantitative Detection Method (Crustacean ELISA)[23]					
fish sausage	6.48[c]	4.49	6	1.62	4.39
freeze-dried egg soup	8.45[c]	2.42	6	0.91	4.39
tomato sauce	9.02[c]	4.93	6	1.52	4.39
croquette	8.50[c]	4.30	6	1.01	4.39
chicken meatball	7.07[c]	5.48	6	3.83	4.39
Samples for Validation of Qualitative Detection Method (Shrimp PCR)					
rice gruel	8.49[c]	4.47	6	4.18	4.39
chicken meatball	7.07[c]	5.48	6	3.83	4.39
freeze-dried vegetable soup	9.74[c]	7.80	6	0.73	4.39
croquette	8.50[c]	4.30	6	1.01	4.39
soybean soup	7.41[c]	8.45	6	1.53	4.39
konjac noodle	8.00[c]	6.02	6	2.53	4.39
Samples for Validation of Qualitative Detection Method (Crab PCR)					
rice gruel	3.24[d]	7.66	6	1.85	4.39
chicken meatball	2.99[d]	2.35	6	0.39	4.39
freeze-dried vegetable soup	3.83[d]	8.84	6	1.35	4.39
croquette	4.33[d]	5.99	6	2.24	4.39
soybean soup	3.52[d]	3.36	6	1.45	4.39
seasoning powder	5.66[d]	6.12	6	1.36	4.39

[a]RSD% calculated from s_s(SD of sampling) and s_a (SD of analysis). [b]F_{crit} = critical F value. [c]Concentration of SP. [d]Concentration of CP.

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including average concentrations of SP and CP, RSD percentages calculated from S_s and S_a, F ratios, and critical F values.

Because the F ratios for all incurred samples were below the critical F value (4.39), the homogeneity of all the incurred samples was regarded as sufficient for each validation. For most test materials, the RSD values were <8.84%. In the case of the incurred samples for crab PCR, the average concentrations of each sample were determined to be approximately half of those for shrimp PCR. Because the polyclonal and monoclonal antibodies to shrimp tropomyosin of the crustacean ELISA kit have the same reactivity against crab tropomyosin,[11] these results indicate that the tropomyosin content in CP may be smaller than that in SP. It is well-known that the best source of information on method performance for allergen detection is an incurred sample, which is defined as one in which a known amount of the food allergen has been incorporated during processing, mimicking as closely as possible the actual conditions under which the sample matrix would normally be manufactured. Therefore, we consider that all of the interlaboratory validations of detection method for food allergen should be carried out using incurred samples, rather than the samples with food allergen added to them after processing (spiked samples).

Interlaboratory Validation of the Quantitative Detection Method (Crustacean ELISA).[23] The recovery of SP, repeatability (RSD_r), and reproducibility (RSD_R) calculated using ANOVA after removal of outliers are shown in Table 3.

Table 3. Results of the Interlaoorarory Validation for Two Crustacean ELISA Kits (Adapted from Sakai et al.[23])

sample	no. of labs	recovery of SP (%)	repeatability (RSD_r, %)	reproducibility (RSD_R, %)
		Crustacean Kit Maruha (M Kit)		
fish sausage	10	102.8	4.9	20.5
freeze-dried egg soup	9	96.8	3.6	17.6
tomato sauce	10	95.8	9.3	17.6
croquette	10	82.1	9.9	18.8
chicken meatball	10	100.0	6.1	19.2
		FA Test Crustacea Nissui (N Kit)		
fish sausage	8	64.8	4.0	4.0
freeze-dried egg soup	10	73.6	4.1	8.4
tomato sauce	10	85.7	4.7	6.8
croquette	10	77.7	4.6	5.9
chicken meatball	10	72.2	5.1	8.4

Twenty data items—the results of 2 samples from each of 10 laboratories—were used for the calculation. Freeze-dried egg soup from one laboratory was removed following Cochran's test for the M kit, and fish sausage from two laboratories was removed following Grubbs' test for the N kit.

As shown in Table 3, the recoveries of SP obtained with the M kit from five types of incurred samples were in the range from 82.1 to 102.8%, whereas those obtained with the N kit were in the range from 64.8 to 85.7%. In this interlaboratory validation, the M kit had a higher recovery compared with the N kit. The RSD_R values of five types of incurred sample obtained with the M kit ranged between 17.6 and 20.5%, whereas those obtained with the N kit ranged between 4.0 and 8.4%. The N kit showed higher reproducibility compared with the M kit. RSD_r is a measure of the variance arising from the entire analytical procedure in a particular laboratory. In this study, all of the RSD_r values were found to be <9.9%. According to the Horwitz theory, the RSD_r value is likely to be less than two-thirds of the RSD_R value.[29] In this study, most RSD_r values for both kits were less than two-thirds of the corresponding RSD_R values, although some RSD_r values for the N kit were similar to the corresponding RSD_R values.

In summary, although there were some differences in the recovery and reproducibility achieved with the M kit and the N kit, both satisfied the official Japanese criteria for interlaboratory validations: ≥8 laboratories, ≥5 incurred samples, 50–150% recovery, ≤25% reproducibility. Recently, Abbott et al. reported validation procedures for quantitative detection methods (ELISA) and provided best practice recommendations for validation studies.[30−32] The protocol was applied to the interlaboratory validations of detection methods for two priority allergens (egg and milk). Further guidance for other priority allergens would be developed and harmonized by the AOAC Presidential Task Force on Food Allergens, with the active contribution of the Allergen Working Group. At present (August 2012), the key elements of their acceptance criteria are almost the same as those of the official Japanese criteria.

Interlaboratory Validation of the Qualitative Detection Method (Shrimp and Crab PCR). Our collaborators, Taguchi et al., developed two PCR detection methods for shrimp and crab, with sufficiently high sensitivity to detect 5 pg of DNA from target species.[15] Under the Japanese food allergy labeling regulations, certain allergenic ingredients must be declared on the food label when present at a level of ≥10 μg/g. Because processed commercial foods are made from many kinds of materials and processed by various methods, it is thought that the sensitivity of the qualitative detection method could be affected by PCR inhibition, DNA degradation, and differences in the DNA extraction efficiency between target species and other matrices. Therefore, we evaluated the sensitivity of each PCR method, using incurred samples containing 10 μg/g of soluble protein of the corresponding allergenic ingredient. As a consequence, PCR products with the target size were detected in all positive incurred samples, but not in negative (blank) samples. Hence, we considered that the two PCR methods would be sensitive enough to detect trace amounts of shrimp and crab species in processed commercial foods and could be used as a confirmation method for positive ELISA screening tests.

For each positive incurred sample and blank sample, 20 data items—the results of 2 test samples from 10 participating laboratories—were used for evaluation of the interlaboratory validation of shrimp and crab PCR. The results of the interlaboratory validations of shrimp and crab PCR are summarized in Table 4.

Two laboratories were removed from the shrimp PCR evaluation as outliers, because they could not obtain the band of positive control plasmid. There was only one false-negative result for DNA extract from konjac noodle and only one false-positive result for rice gruel. As shown in Table 4, the precision of these samples was calculated as 94% (15 correct answers/16 total subjects).

For the interlaboratory validation of crab PCR, the organizer collected 18 results for each incurred sample and blank sample from nine participating laboratories, after removal of an outlier for the same reason as with shrimp PCR. As shown in Table 4,

Table 4. Results of the Interlaboratory Validation for Shrimp and Crab PCR

Shrimp PCR (Number of Laboratories: 8)						
	rice gruel	chicken meatball	freeze-dried vegetable soup	croquette	soybean soup	konjac noodle
precision for incurred sample containing shrimp powder (%)	100 (16/16)[a]	100 (16/16)	100 (16/16)	100 (16/16)	100 (16/16)	94 (15/16)
precision for blank (%)	94 (1/16)	100 (0/16)	100 (0/16)	100 (0/16)	100 (0/16)	100 (0/16)
Crab PCR (Number of Laboratories: 9)						
	rice gruel	chicken meatball	freeze-dried vegetable soup	croquette	soybean soup	seasoning powder
precision for incurred sample containing crab powder (%)	100 (18/18)[a]	100 (18/18)	100 (18/18)	100 (18/18)	100 (18/18)	100 (18/18)
precision for blank (%)	100 (0/18)	100 (0/18)	100 (0/18)	100 (0/18)	100 (0/18)	100 (0/18)

[a]The fraction in parentheses indicates the number of detections of specific PCR products (numerator)/total subjects (denominator).

all nine participants reported accurate results for the six incurred samples for crab PCR (18 correct answers/18 total subjects); the PCR products with the target size were detected in all incurred samples, but not in negative (blank) samples.

Both detection methods satisfied the official Japanese acceptance criteria for qualitative detection methods: ≥6 laboratories, ≥5 incurred samples, two dose levels including a negative control and 10 μg/g of incurred sample, and ≥90% precision.

In conclusion, the results of the interlaboratory validations of the quantitative and qualitative detection methods suggest that these methods correctly determined specific allergenic proteins and detected DNA from allergenic ingredients. The present results demonstrate that these methods can detect the allergenic ingredients contained in most commercial processed foods and are capable of accurately monitoring labeling systems in a reliable manner, so they may be useful for inspections performed in accordance with Japanese regulations. The Japanese labeling system for food allergens is highly valued by food allergy patients and their families. Almost all patients feel that the food-labeling system is very useful, although there have been a few cases of accidental intake either through misreading of the label or mislabeling by food companies. Consequently, in this decade, very few accidental allergic reactions have occurred due to appropriate labeling under the effective administrative policy, the contribution of the food industry, and better informed consumers.

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Notes

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ABBREVIATIONS USED

ANOVA, analysis of variance; ELISA, enzyme-linked immunosorbent assay; LOD, limit of detection; MHLW, Ministry of Health Labour and Welfare of Japan; PCR, polymerase chain reaction; RSD, relative standard deviation; SD, standard deviation.

REFERENCES

(1) Cianferoni, A.; Spergel, J. M. Food allergy: reviews, classification and diagnosis. *Allergol. Int.* **2009**, *58*, 457−466.

(2) Ito, K.; Urisu, A. Diagnosis of food allergy based on oral food challenge test. *Allergol. Int.* **2009**, *58*, 467−474.

(3) Food and Agriculture Organization of the United Nations, World Health Organization (FAO). *Report of FAO Technical Consultation on Food Allergens*; Rome, Nov 1995; pp 13−14.

(4) Hefle, S. L.; Nordlee, J. A.; Taylor, S. L. Allergic foods. *Crit. Rev. Food Sci. Nutr.* **1996**, *36*, 69−89.

(5) Ebisawa, M.; Ikematsu, K.; Imai, T.; Tachimoto, H. *J. World Allergy Organization* **2003**, *15*, 214−217.

(6) Akiyama, H.; Imai, T.; Ebisawa, M. Japan food allergen labeling regulation − history and evaluation. *Adv. Food Nutr. Res.* **2011**, *62*, 139−171.

(7) Tomikawa, M.; Suzuki, N.; Urisu, A.; Tsuburai, T.; Ito, S.; Shibata, R.; Ito, K.; Ebisawa, M. Characteristics of shrimp allergy from childhood to adulthood in Japan. *Arerugi* **2006**, *55*, 1536−1542.

(8) Akiyama, H.; Sakai, S.; Adachi, R.; Teshima, R. Japanese regulations and buckwheat allergen detection. In *Molecular Biological and Immunological Techniques and Applications for Food Chemists*; Wiley: Hoboken, NJ, 2010; pp 293−310 (ISBN: 978-0-470-06809-0).

(9) Matsuda, R.; Yoshioka, Y.; Akiyama, H.; Aburatani, K.; Watanabe, Y.; Matsumoto, T.; Morishita, N.; Sato, H.; Mishima, T.; Gamo, R.; Kihira, Y.; Maitani, T. Interlaboratory evaluation of two kinds of ELISA kits for the detection of egg, milk, wheat, buckwheat, and peanut in foods. *J. AOAC Int.* **2006**, *89*, 1600−1608.

(10) Seiki, K.; Oda, H.; Yoshioka, H.; Sakai, S.; Urisu, A.; Akiyama, H.; Ohno, Y. A reliable and sensitive immunoassay for the determination of crustacean protein in processed foods. *J. Agric. Food Chem.* **2007**, *55*, 9345−9350.

(11) Shibahara, Y.; Oka, M.; Tominaga, K.; Ii, T.; Umeda, M.; Uneo, N.; Abe, A.; Ohashi, E.; Ushio, H.; Shiomi, K. Determination of crustacean allergen in food products by sandwich ELISA. *Nippon Shokuhin Kagaku Kogaku Kaishi* **2007**, *54*, 280−286.

(12) Yamakawa, H.; Akiyama, H.; Endo, Y.; Miyatake, K.; Sakata, K.; Sakai, S.; Toyoda, M.; Urisu, A. Specific detection of wheat residues in processed foods by polymerase chain reaction. *Biosci., Biotechnol., Biochem.* **2007**, *71*, 2561−2564.

(13) Yamakawa, H.; Akiyama, H.; Endo, Y.; Miyatake, K.; Sakata, K.; Sakai, S.; Toyoda, M.; Urisu, A. Specific detection of buckwheat residues in Processed foods by polymerase chain reaction. *Biosci., Biotechnol., Biochem.* **2008**, *72*, 2228−2231.

(14) Watanabe, T.; Akiyama, H.; Yamakawa, H.; Iijima, K.; Yamazaki, F.; Matsumoto, T.; Futo, S.; Arakawa, F.; Watai, M.; Maitani, T. A specific qualitative detection method for peanut (*Arachis hypogaea*) in foods using polymerase chain reaction. *J. Food Biochem.* **2006**, *30*, 215−233.

(15) Taguchi, H.; Watanabe, S.; Temmei, Y.; Hirao, T.; Akiyama, H.; Sakai, S.; Adachi, R.; Sakata, K.; Urisu, A.; Teshima, R. Differential detection of shrimp and crab for food labeling using polymerase chain reaction. *J. Agric. Food Chem.* **2011**, *59*, 3510−3519.

(16) Department of Food Safety, Ministry of Health, Labour and Welfare of Japan, Notification 1106001 of Nov 6, 2002.

(17) Department of Food Safety, Ministry of Health, Labour and Welfare of Japan, Revised Notification 0622003 of June 22, 2006.

(18) Akiyama, H.; Nakamura, K.; Harikai, N.; Watanabe, H.; Iijima, K.; Yamakawa, H.; Mizuguchi, Y.; Yoshikawa, R.; Yamamoto, M.; Sato, H.; Watai, M.; Arakawa, F.; Ogasawara, T.; Nishihara, R.; Kato, H.; Yamauchi, A.; Takahata, Y.; Morimatsu, F.; Mamegoshi, S.; Muraoka, S.; Honjoh, T.; Watanabe, T.; Sakata, K.; Imamura, T.; Toyoda, M.; Matsuda, R.; Maitani, T. Inter-laboratory evaluation studies for establishment of notified ELISA methods for allergic substances (peanuts). *Shokuhin Eiseigaku Zasshi* **2004**, *45*, 325−331.

(19) Akiyama, H.; Nakamura, K.; Harikai, N.; Watanabe, H.; Iijima, K.; Yamakawa, H.; Mizuguchi, Y.; Yoshikawa, R.; Yamamoto, M.; Sato, H.; Watai, M.; Arakawa, F.; Ogasawara, T.; Nishihara, R.; Kato, H.; Yamauchi, A.; Takahata, Y.; Morimatsu, F.; Mamegoshi, S.; Muraoka, S.; Honjoh, T.; Watanabe, T.; Sakata, K.; Imamura, T.; Toyoda, M.; Matsuda, R.; Maitani, T. Inter-laboratory evaluation studies for establishment of notified ELISA methods for allergic substances (buckwheat). *Shokuhin Eiseigaku Zasshi* **2004**, *45*, 313−318.

(20) Akiyama, H.; Isuzugawa, K.; Harikai, N.; Watanabe, H.; Iijima, K.; Yamakawa, H.; Mizuguchi, Y.; Yoshikawa, R.; Yamamoto, M.; Sato, H.; Watai, M.; Arakawa, F.; Ogasawara, T.; Nishihara, R.; Kato, H.; Yamauchi, A.; Takahata, Y.; Morimatsu, F.; Mamegoshi, S.; Muraoka, S.; Honjoh, T.; Watanabe, T.; Sakata, K.; Imamura, T.; Toyoda, M.; Matsuda, R.; Maitani, T. Inter-laboratory evaluation studies for development of notified ELISA methods for allergic substances (wheat). *Shokuhin Eiseigaku Zasshi* **2004**, *45*, 128−134.

(21) Akiyama, H.; Isuzugawa, K.; Harikai, N.; Watanabe, H.; Iijima, K.; Yamakawa, H.; Mizuguchi, Y.; Yoshikawa, R.; Yamamoto, M.; Sato, H.; Watai, M.; Arakawa, F.; Ogasawara, T.; Nishihara, R.; Kato, H.; Yamauchi, A.; Takahata, Y.; Morimatsu, F.; Mamegoshi, S.; Muraoka, S.; Honjoh, T.; Watanabe, T.; Sakata, K.; Imamura, T.; Toyoda, M.; Matsuda, R.; Maitani, T. Inter-laboratory evaluation studies for development of notified ELISA methods for allergic substances (milk). *Shokuhin Eiseigaku Zasshi* **2004**, *45*, 120−127.

(22) Akiyama, H.; Isuzugawa, K.; Harikai, N.; Watanabe, H.; Iijima, K.; Yamakawa, H.; Mizuguchi, Y.; Yoshikawa, R.; Yamamoto, M.; Sato, H.; Watai, M.; Arakawa, F.; Ogasawara, T.; Nishihara, R.; Kato, H.; Yamauchi, A.; Takahata, Y.; Morimatsu, F.; Mamegoshi, S.; Muraoka, S.; Honjoh, T.; Watanabe, T.; Wakui, C.; Imamura, T.; Toyoda, M.; Maitani, T. Inter-laboratory evaluation studies of notified ELISA methods for allergic substances (egg). *Shokuhin Eiseigaku Zasshi* **2003**, *44*, 213−219.

(23) Sakai, S.; Matsuda, R.; Adachi, R.; Akiyama, H.; Maitani, T.; Ohno, Y.; Oka, M.; Abe, A.; Seiki, K.; Oda, H.; Shiomi, K.; Urisu, A. Interlaboratory evaluation of two enzyme-linked immunosorbent assay kits for the determination of crustacean protein in processed foods. *J. AOAC Int.* **2008**, *91*, 123−129.

(24) Sakai, S.; Adachi, R.; Akiyama, H.; Teshima, R.; Morishita, N.; Matsumoto, T.; Urisu, A. Interlaboratory evaluation of an enzyme-linked immunosorbent assay kit for the determination of soybean protein in processed foods. *J. AOAC Int.* **2009**, *93*, 243−248.

(25) Sakai, S.; Adachi, R.; Akiyama, H.; Teshima, R.; Doi, H.; Shibata, H.; Urisu, A. Determination of walnut protein in processed foods by enzyme-linked immunosorbent assay interlaboratory study. *J. AOAC Int* **2010**, *93*, 1255−1261.

(26) *Official Methods of Analysis*, 18th ed.; AOAC International: Gaithersburg, MD, 2005.

(27) International Organization for Standardization. ISO5725, sccuracy (trueness and precision) of measurement methods and results; Geneva, Switzerland, 1994.

(28) Thompson, M.; Wood, R. International harmonized protocol for proficiency testing of (chemical) analytical laboratories. *J. AOAC Int.* **1993**, *76*, 926−940.

(29) Horwitz, W. Protocol for the design, conduct and interpretation of method-performance studies. *Pure Appl. Chem.* **1995**, *67*, 331−343.

(30) Popping, B.; Abbott, M. Food allergens in the focus. *J. AOAC Int.* **2010**, *93*, 432−433.

(31) Diaz-Amigo, C.; Popping, B. Analytical testing as a tool for the enforcement of future regulatory thresholds for food allergens. *J. AOAC Int.* **2010**, *93*, 434−441.

(32) Abbott, M.; Hayward, S.; Ross, W.; Godefroy, S. B.; Ulberth, F.; Van Hengel, A. J.; Roberts, J.; Akiyama, H.; Popping, B.; Yeung, J. M.; Wehling, P.; Taylor, S. L.; Poms, R. E.; Delahaut, P. Validation procedure for quantitative food allergen ELISA methods: Community guidance and best practices. *J. AOAC Int.* **2010**, *93*, 442−450.