CHAPTER 4

Japan Food Allergen Labeling Regulation—History and Evaluation

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Contents

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Advances in Food and Nutrition Research, Volume 62
ISSN 1043-4526, DOI: 10.1016/B978-0-12-385989-1.00004-1

Abstract According to a national survey of food allergy cases, the food-labeling system for specific allergenic ingredients (i.e., egg, milk, wheat, buckwheat, and peanut) in Japan was mandated under law on April 1, 2002. By Japanese law, labeling of allergens is designated as mandatory or recommended based on the number of cases of actual illness and the degree of seriousness. Mandatory labeling is enforced by the ministerial ordinance, and the ministerial notification recommends that foods containing walnut and soybean be labeled with subspecific allergenic ingredients. Additional labeling of shrimp/prawn and crab has also become mandatory since 2008. To monitor the validity of the labeling system, the Japanese government announced the official methods for detection of allergens in a November 2002 ministry notification. These official methods, including two kinds of enzyme-linked immunosorbent assay kits for screening, Western blotting analyses for egg and milk, and polymerase chain reaction analyses for wheat, buckwheat, peanut, shrimp/prawn and crab as confirmation tests, have provided a means to monitor the labeling system. To standardize the official methods, the Japanese government described the validation protocol criteria in the 2006 official guidelines. The guidelines stipulate that any food containing allergen proteins at greater than 10 mg/kg must be labeled under the Law. This review covers the selection of the specific allergenic ingredients by the Japanese government, the implementation of regulatory action levels and the detection methods to support them, and the assessment of the effectiveness of this approach.

I. ASSESSMENT OF IMMEDIATE-TYPE FOOD ALLERGIES IN JAPAN

Food allergies that cause immediate reactions had already been under investigation prior to any discussion of ''allergy food labeling'' under the food sanitary law for prepackaged processed foods and food additives. Before implementation of the allergy food-labeling system in Japan, a research group supported by the Ministry of Health and Welfare of Japan had collected epidemiological data on immediate-type food allergies during both childhood and adulthood in Japan in 1998 and 1999. This retrospective study asked hospitals with more than 200 beds to report all immediate-type food allergy cases treated by the emergency department. The questionnaire included information on age, sex, cause of the food allergy, symptoms, IgE CAP RAST, and type of treatment. To focus on the

immediate-type, only cases in which symptoms occurred within 60 min after ingestion of the suspected food were included. Of the 2623 hospitals surveyed, 1623 hospitals responded and 1420 cases were analyzed. As shown in Table 4.1, hen's eggs were the most common allergen, followed by cow's milk, wheat, buckwheat, fishes, fruits, and shrimp. The top three major food allergens were most prevalent among the pediatric population, whereas fishes, buckwheat, and shrimp were mainly reported in adults. Based on these data, the Ministry of Health and Welfare selected 24 candidates that caused more than four cases of adverse reaction for the allergy food-labeling system. Following roundtable discussions among specialists and regulatory officers of the Ministry of Health and Welfare, hen's eggs, cow's milk, wheat, buckwheat, and peanuts were selected as items for mandatory labeling by the 2000 ministerial ordinance; the remaining 19 allergens were designated as items for recommended labeling by a ministerial notification.

To further understand the real-time condition of food allergies in Japan, we investigated prospectively the immediate-type food allergy cases in collaboration with more than 2000 doctors between 2001 and 2002 to account for recall bias in the previous study. The contributing doctors included those working in hospitals with more than 200 beds as well as allergy specialists working in clinics. Contributing doctors were asked to respond to a questionnaire every 3 months for 2 years from 2001 to 2002 and report immediate-type food allergy cases by mail. The same questionnaire as that in the previous studies was used, and only immediate-type food allergies as defined in the previous study were included. A total of 3882 cases were reported within the 2 years (Table 4.2). The cases ranged from 0 to 80 years of age, with 50% (1969) of them below 2 years of age. The most common cause of food allergy was hen's eggs (38.3%), followed by cow's milk (15.9%), wheat (8%), shellfish (6.2%), fruits (6%), buckwheat (4.6%), fishes (4.4%), and peanuts (2.8%). Notably, the cause of food allergy differed greatly among age groups. Food-induced anaphylaxis was seen in 10.9% of the reported cases. As shown in Table 4.3, hen's eggs, cow's milk plus its products, wheat, buckwheat, and peanuts were the major causes of food-induced anaphylaxis in Japan. Compared to our previous investigation, fruit allergies against kiwi and banana seemed to be an increasing trend. Thus, the present Ministry of Health, Labor, and Welfare of Japan (MHLW) has been implementing countermeasures against food allergies to improve the quality of life of afflicted patients. This prospective investigation on immediate-type food allergies has been repeated every 3 years as a means to monitor the condition of food allergies in Japan. The results of these investigations have improved the allergy food-labeling system by including banana as a recommended item by a ministerial notification and shrimp and crab as mandatory items for labeling by a ministerial ordinance.

TABLE 4.1 Immediate type of food allergy cases reported from 1998 to 1999

Offending food, *n* (%)	Total	>1 year	1 year	2–3 years	4–6 years	7–19 years	20+ years
Egg	420 (29.6)	197 (47.4)	72 (30.4)	89 (30.8)	35 (25.0)	19 (9.2)	8 (6.1)
Milk product	324 (22.8)	128 (30.8)	66 (27.8)	70 (24.2)	34 (24.3)	21 (10.1)	5 (3.8)
Wheat	147 (10.4)	40 (9.6)	20 (8.4)	35 (12.1)	12 (8.6)	27 (13.0)	13 (9.9)
Buckwheat	82 (5.8)	1 (0.2)	10 (4.2)	16 (5.5)	10 (7.1)	29 (14.0)	16 (12.2)
Fish	73 (5.1)	15 (3.6)	9 (3.8)	10 (3.5)	5 (3.6)	13 (6.3)	21 (16.0)
Fruits	66 (4.6)	6 (1.4)	13 (5.5)	13 (4.5)	8 (5.7)	19 (9.2)	7 (5.3)
Shrimp	51 (3.6)	0 (0.0)	2 (0.8)	4 (1.4)	4 (2.9)	22 (10.6)	19 (14.5)
Meat	44 (3.1)	9 (2.2)	2 (0.8)	4 (1.4)	4 (2.9)	14 (6.8)	11 (8.4)
Peanut	34 (2.4)	3 (0.7)	12 (5.1)	5 (1.7)	6 (4.3)	5 (2.4)	3 (2.3)
Soybean	27 (1.9)	5 (1.2)	8 (3.4)	4 (1.4)	3 (2.1)	4 (1.9)	3 (2.3)
Other	152 (10.7)	12 (2.9)	23 (9.7)	39 (13.5)	19 (13.6)	34 (16.4)	25 (19.1)
Total	1420	416	237	289	140	207	131

TABLE 4.2 Immediate type of food allergy cases reported from 2001 to 2002

Offending food, *n* (%)	Total	>1 year	1 year	2–3 years	4–6 years	7–19 years	+20 years
Egg	1486 (38.3)	789 (62.1)	312 (44.6)	179 (30.1)	106 (23.3)	76 (15.2)	24 (6.6)
Milk product	616 (15.9)	255 (20.1)	111 (15.9)	117 (19.7)	84 (18.5)	41 (8.2)	8 (2.2)
Wheat	311 (8.0)	90 (7.1)	49 (7.0)	46 (7.7)	24 (5.3)	48 (9.6)	54 (14.8)
Fruits	232 (6.0)	40 (3.1)	30 (4.3)	30 (5.1)	40 (8.8)	45 (9.0)	47 (12.8)
Buckwheat	179 (4.6)	4 (0.3)	23 (3.3)	45 (7.6)	27 (5.9)	54 (10.8)	26 (7.1)
Fish	171 (4.4)	21 (1.7)	32 (4.6)	22 (3.7)	18 (4.0)	37 (7.4)	41 (11.2)
Shrimp	161 (4.1)	4 (0.3)	10 (1.4)	20 (3.4)	29 (6.4)	59 (11.8)	39 (10.7)
Peanut	110 (2.8)	4 (0.3)	22 (3.1)	31 (5.2)	28 (6.2)	22 (4.4)	3 (0.8)
Soybean	76 (2.0)	22 (1.7)	16 (2.3)	9 (1.5)	8 (1.8)	9 (1.8)	12 (3.3)
Meat	71 (1.8)	13 (1.0)	6 (0.9)	7 (1.2)	7 (1.5)	19 (3.8)	19 (5.2)
Other	469 (12.1)	28 (2.2)	88 (12.6)	88 (14.8)	83 (18.3)	89 (17.8)	93 (25.4)
Total	3882	1270	699	594	454	499	366

TABLE 4.3 Anaphylaxis cases reported from 2001 to 2002

No.	Offending food	*n* (%)
1	Egg	109 (27.6)
2	Milk product	93 (23.5)
3	wheat	70 (17.7)
4	Buckwheat	28 (7.1)
5	Peanuts	18 (4.6)
6	Shrimp	14 (3.5)
7	Salmon roe	8 (2.0)
	Peach	8 (2.0)
9	Soybean	7 (1.8)
	Kiwi	7 (1.8)
11	Banana	4 (1.0)
	Yam	4 (1.0)
–	Other	25 (6.3)
	Total	395

II. JAPANESE FOOD ALLERGY-LABELING SYSTEM

Food allergies represent an important health problem in industrialized countries. In Japan, the number of people with food allergies is increasing, especially among young children, due to major changes in dietary habits with the introduction of western foods after World War II.

In 1999, the Joint FAO/WHO Codex Alimentary Commission Session agreed to recommend labeling of eight kinds of food which contain ingredients known to be allergens. This movement has led the Japanese government to take new measures to tackle food allergies in Japan.

A. Japanese regulations for labeling of food allergenic ingredients

The special subcommittee of MHLW held a meeting on the labeling of the Food Sanitation Investigation Council and stated that, ''From the viewpoint of preventing the occurrence of health hazards, mandatory labeling of foods containing specific allergenic ingredients should be required.'' Accordingly, the MHLW decided that the Food Sanitation Law should provide for the mandatory labeling of foods containing allergenic ingredients designated in the 2000 ministerial ordinance.

Since the only therapy for a food allergy is avoidance of the responsible food, it is essential for food allergy patients to eliminate food allergens from their diet. Therefore, the Japanese MHLW decided to improve the

allergen-labeling system by amending the Food Sanitation Law in 2001 (Ebisawa *et al.*, 2003). They organized a labeling study group consisting of clinical experts, patients, researchers, retailers, and food industrialists. The group discussed different labeling system methods. The results were announced as a report. In the report outline, labeling was divided into two stages, mandatory and recommended, based on the number of cases of actual illnesses and the degree of seriousness (Table 4.4). Consequently, eggs, milk, wheat, buckwheat and peanuts, and most recently shrimp and crab require mandatory labeling by the ministerial ordinance; hereinafter, we refer to these seven ingredients as ''specific allergenic ingredients.'' In addition, the ministerial notification recommends labeling of any food that contains the following 18 ingredients: abalone, squid, salmon roe, orange, kiwifruit, beef, walnut, salmon, mackerel, soybean, chicken, banana, pork, Matsutake mushroom, peach, yam, apple, and gelatin. Hereinafter, we refer to these ingredients as ''subspecific allergenic ingredients.'' To the best of our knowledge, Japan is the first country to set up mandatory food allergy labeling and to regulate it under national law in 2002. The additional labeling requirement for shrimp/prawn and crab was introduced by the amendment of the food Sanitation Law under the MHLW in June 2008 due to the almost unlimited use of crustaceans in processed foods in Japan and the frequency of adverse food reactions in allergic patients.

Among shrimp allergy cases, 64.7% of patients showed positive reaction to crabs. The clinical evidence suggests that many shrimp allergy patients react to crabs. On the contrary, as the remaining 35.3% of patients showed no reaction to crabs, some patients with shrimp allergy can eat crabs. Thus, it would be important to label ''shrimp'' and ''crab'' separately, rather than as ''crustacean'' to give consumers more information. Accordingly, the MHLW has revised the mandatory labeling for shrimp

TABLE 4.4 Allergenic ingredients designated by the MHLW of Japan*

Specific allergenic ingredients
Mandatory by ministerial ordinance (seven ingredients)
Egg, milk, wheat, buckwheat, peanut, shrimp/prawn, and crab
Subspecific allergenic ingredients
Recommended by ministerial notification (18 ingredients)
Abalone, squid, salmon roe, orange, kiwifruit, beef, walnut, salmon, mackerel, soybean, chicken, banana, pork, Matsutake mushroom, peach, yam, apple, and gelatin

*Based on the Notification of March 15, 2001 and the newest Notification of June 3, 2008 from the Department of Food Safety, Ministry of Health, Labor, and Welfare (MHLW) of Japan.

and crab to be labeled separately. Since the management of the food-labeling policy was transferred from the MHLW to the Consumer Affairs Agency (CAA) in 2010, CAA announces the Japanese food-labeling system through ministry notifications.

The content scope of allergens for labeling was established based on the Japan Standard Commodity Classification. Japan is the first country to set up mandatory food allergy labeling and to regulate it under national law.

The characteristics of the Japanese labeling system are as follows.

1. Small quantity labeling

The specific allergenic ingredients must be labeled even in cases of carry-over conditions or when used as processing aids. Labeling of the 18 subspecific allergenic ingredients in Table 4.1 is recommended as much as possible.

2. "May contain" labeling

''May contain (name of allergenic ingredients)'' type labeling is prohibited.

3. Combination of specified ingredients

With a few exceptions, the use of major item classifications (declaration of meats, cereals, etc.) is prohibited.

4. Declaration of high-grade food ingredients

In cases with high-grade food ingredients such as abalone, salmon roe, and mushroom mixed in very small quantities, a declaration such as ''contains xxx extract'' is required so as not to mislead consumers.

5. Method of declaring additives

For food additives, labeling shall, in principle, declare the ''name of the substance (derived from).''

6. Declaration of flavorings

Aromatic ingredients have not yet been subjected to labeling, but should be labeled as much as possible.

7. Alcoholic beverages and related products

Alcoholic beverages are not currently subject to regulated labeling.

8. Alternative declaration

Alternative vocabulary usage in declaration is allowed for certain items, if the declaration can be considered allergen labeling in that the general (practical) expression used suggests that an allergenic ingredient is being used.

9. Specified processed foods

Specified processed foods generally known to be made from allergenic ingredients do not require declaration of such ingredients. For example, a sandwich using mayonnaise may mention ''mayonnaise'' instead of ''egg.''

III. REGULATION OF DETECTION METHODS FOR FOOD ALLERGENIC INGREDIENTS

A. Consideration of Japanese allergen-labeling thresholds

A system of labeling for food allergies is necessary for people with allergies. However, in general, proteins and nucleotides from allergens are not necessarily toxins. The threshold dose for an allergic reaction 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 of food allergy labeling and developed the official detection methods for specific allergenic ingredients. To do this, they organized a detection method study group consisting of manufacturing companies, retailers, public research institutes, universities, and private inspection institutes. Thereinafter, we have been developing detection methods for specific allergenic ingredients in foods.

The detection method study group considered how to set the threshold for labeling (Fig. 4.1). They presumed 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 up the threshold for labeling in the range of LOD for ELISA would be difficult due to the large deviation in repeatability and reproducibility. In addition, LODs of lateral flow and polymerase chain reaction (PCR) methods would be approximately 5 μg protein/g food.

The labeling study group determined the threshold for the labeling system, that is, the definition of a trace amount. The group stated that, ''If more than a few micrograms of protein weight per milliliter of food or a few micrograms of protein per gram of food are contained in a food, labeling of that allergen is necessary.''

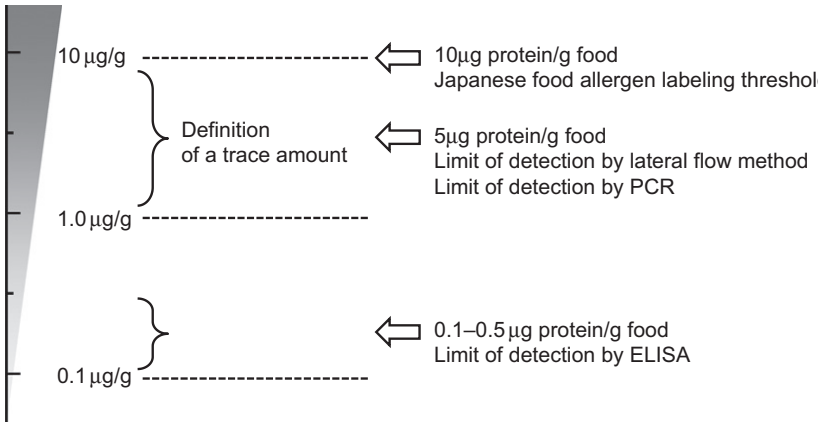

FIGURE 4.1 Consideration of Japanese food allergen-labeling threshold.

Considering these factors, we designated 10 μg protein/g food (the corresponding allergen soluble protein weight/food weight) as a threshold to monitor the labeling using ELISA. We believe that this level is the minimum for controlling the contamination of allergic ingredients using the detection method on an industrial scale.

Therefore, we developed detection methods for determining the presence of proteins on the level of a few micrograms per milliliter or gram of food based on the definition of a trace amount.

Accurate determination of the allergen proteins is difficult, however, as they undergo denaturation and degradation. Further, the standard allergen protein reference could change, as identical allergen proteins cannot always be obtained for every test. In Japan, the labeling of egg, milk, wheat, buckwheat, and peanut ingredients in any processed foods became mandatory in April 2002, while shrimp and crab became mandatory in June 2008. The Japanese official methods consisted of screenings of two different ELISA kits, the Western blot method for egg or milk and the PCR method for wheat, buckwheat, peanut, shrimp/prawn, and crab as the confirmation tests under the ministerial notification (Notification No. 1106001, 2002). The MHLW added the specification and standardization of the extraction buffer, reference material, and the standard solution for the testing of these five allergenic ingredients in 2004 (described in Section III.B). Further, the validation protocol criteria were included in the official guidelines in 2006 to standardize the Japanese official method for allergen detection (Notification No. 1106001, 2002), followed by addition of the ELISA, PCR methods and reference material, and the standard solution for the testing of crustaceans for detection of shrimp/prawn and crab in 2008.

B. Reference material and calibrator

To assess compliance to the mandatory labeling system of allergenic ingredients (eggs, milk, wheat, buckwheat, peanuts, and shrimp/prawn (crustaceans)) in processed foods in Japan established in April 2002, followed by shrimp and crab in June 2008, we have established two types of ELISA. However, some discrepancies exist between the results from the two kits, partly due to the use of different antibodies. Another possibility for the discrepancies could be the differences between the standard solutions provided in the kits. Since the test kits are used for regulatory purposes, we considered that the extraction buffer and reference standard for measurement should be unified and standardized between the test kits. Therefore, the MHLW set the specifications and standardization of the extraction buffer, reference material, and the standard solution for testing the five allergenic ingredients (Notification No. 1106001, 2002).

The specifications and standardization include raw materials, preparation method of the standard solution, concentration of proteins, and the main band on SDS-PAGE. The outline of the procedure for preparation of the calibrators is shown in Fig. 4.2. Table 4.5 shows the raw materials and the preparation method of the initial extract. To prepare the calibrators, the raw materials are extracted by the standard solution containing SDS and mercaptoethanol. The initial extract is prepared by centrifugation and filtration of the extract. The diluted extract is then prepared by 10-fold dilution of the initial extract with phosphate-buffered saline (PBS; pH 7.4). The protein concentration of the diluted extract is assayed using the 2-D Quant kit (Amersham Bio Sciences). The standard solution is then

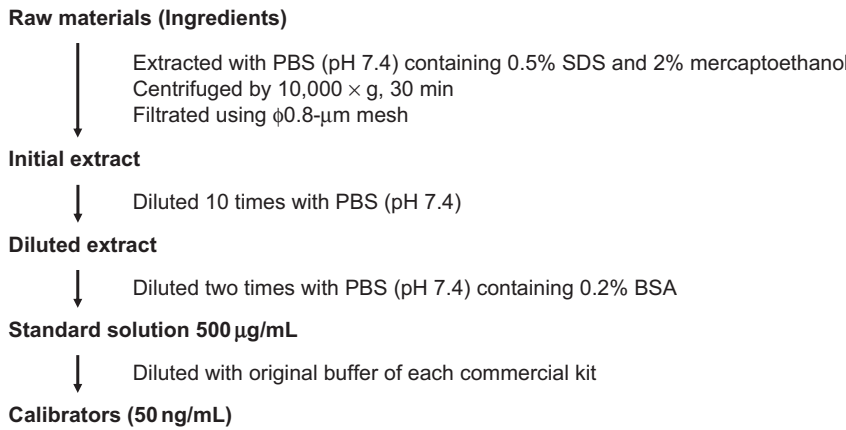

FIGURE 4.2 Procedure for preparing the calibrators.

TABLE 4.5 Raw materials and initial extraction methods

Allergenic food	Raw material (ingredients)	Extraction method (preparation)[a]
Egg	Fresh eggs of white leghorn hen, homogenized, and freeze-dried	0.2 g in 20 mL extraction solution[b] shaken overnight
Milk	Fresh milk of cows, freeze-dried after defatting by churning	0.2 g in 20 mL extraction solution shaken overnight
Wheat	Mixture of 14 species of wheat, pulverized	1.0 g in 20 mL extraction solution shaken overnight
Buckwheat	Mixture of buckwheat produced in Ibaraki Prefecture and China, pulverized	1.0 g in 20 mL extraction solution shaken overnight
Peanut	Virginia species produced in Chiba Prefecture, ground in a mortar	0.4 g in 20 mL extraction solution defatted by acetone and shaken overnight
Shrimp/prawn (Crustacean)	Fresh muscle of black tiger, homogenized, and freeze-dried	0.1 g in 20 mL extraction solution shaken overnight

[a] The protein content of the initial extract was determined using the 2-D Quant kit (Amersham Bio Sciences). The initial extract was diluted 20 times to make up the calibration standard solution.
[b] Extraction solution: buffer containing 0.5% SDS and 2% mercaptoethanol.

prepared by a twofold dilution with PBS (pH 7.4) containing 0.2% BSA. The calibrator included in each commercial kit is prepared by dilution of the standards (concentrated standard solution) to 50 ng/mL with each company kit's original buffer containing the carrier protein.

Three lots of initial extracts for each allergic ingredient were prepared following this procedure to assess the conformity to the specifications. The reproducibility of the protein concentration and the SDS-PAGE pattern of the initial extract solution were also checked (Table 4.6, Fig. 4.3). The initial extract solutions were stored at −80 °C for 6 months to evaluate their stability. The protein concentration and the SDS-PAGE pattern of the 3 lots were equivalent, and no significant variability occurred during the storage period. The calibration standard solution was stored at 4 and 37 °C. The calibration standard solutions were tested by the relevant ELISA kits once a month during storage, and the stability was checked by the obtained absorbance.

TABLE 4.6 Reproducibility of protein concentration determination

	Lot				
	1	2	3	Average	RSD%
Egg	4.55	4.69	4.88	4.71	3.52
Milk	2.57	2.63	2.52	2.57	2.14
Wheat	4.95	4.96	5.10	5.00	1.68
Buckwheat	3.37	3.47	3.59	3.48	3.17
Peanut	3.99	4.47	4.86	4.44	9.81
Shrimp/prawn	3.42	3.46	3.37	3.42	2.00

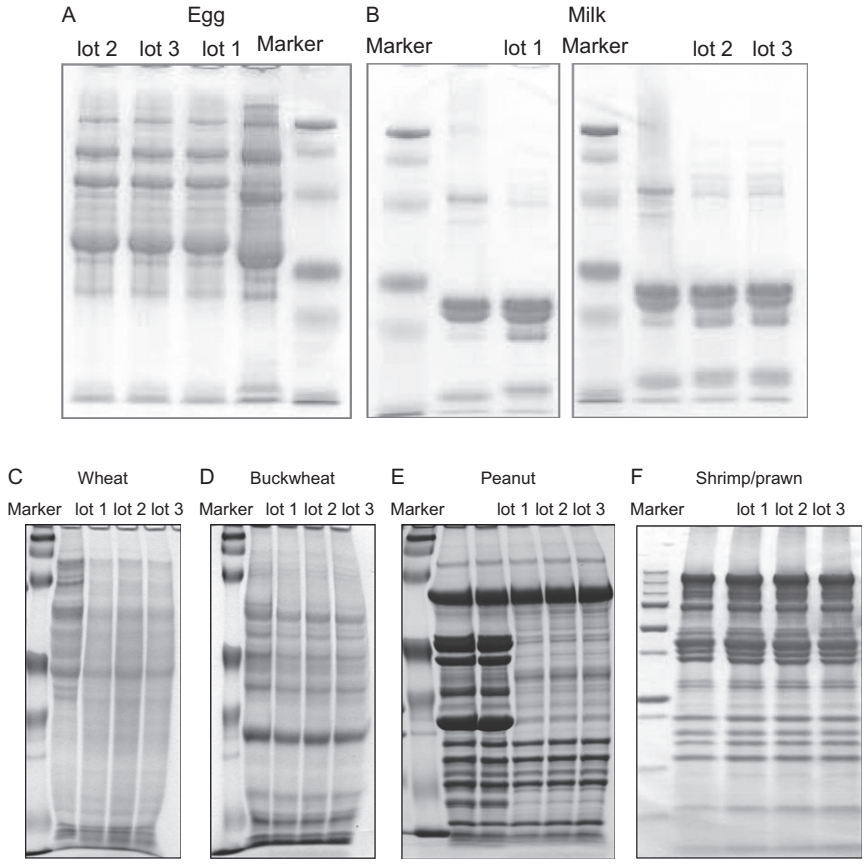

FIGURE 4.3 Reproducibility of SDS-PAGE results.

C. Japanese guideline criteria for validation protocol of specific allergenic ingredient detection method

The MHLW described the validation protocol criteria in the 2006 official guidelines to standardize the Japanese official method for specific allergenic ingredient detection. The outlines of the validation protocol criteria for the food allergenic ingredient quantitative and qualitative detection methods are shown in Tables 4.7 and 4.8, respectively.

The validation protocol criteria for the food allergenic ingredient quantitative detection method are as follows: (1) Eight or more laboratories (independent from the ELISA developer). (2) Five or more food samples (matrices). (3) A concentration of 10 μg/g food specific allergenic ingredient in the food sample (the corresponding allergenic ingredient soluble protein weight/food weight), the concentration defined as the ''trace amount of contamination'' (Any food containing the specific allergenic ingredient protein greater than 10 μg/g must be labeled for the relevant food specific allergenic ingredients under the Food Sanitation Law; if the specific allergenic ingredient protein level is less than 10 μg/g, labeling is not required). The food sample should be prepared by common processing methods, such as heating, baking, frying, acidifying, and

TABLE 4.7 Japanese guideline criteria for validation protocol of quantitative detection methods for food allergenic ingredients[a]

Number of laboratories	≥8
Number of incurred samples	≥5
Number of dose levels	≥1 including 10 μg/g[b]
Recovery	50–150%
RSDr	≤25%

[a] Based on Notification Nos. 1106001 of November 6, 2002, and 0622003 of June 22, 2006, from the Department of Food Safety of the MHLW of Japan.
[b] The corresponding allergenic ingredient soluble protein weight/food weight.

TABLE 4.8 Japanese guideline criteria for validation protocol of qualitative detection methods for food allergenic ingredients[a]

Number of laboratories	≥6
Number of incurred samples	≥5
Number of dose levels	≥2 including negative control (blank) and positive control (10 μg/g[b])
Precision	≥90%

[a] Based on Notification Nos. 1106001 of November 6, 2002, and 0622003 of June 22, 2006, from the Department of Food Safety of the MHLW of Japan.
[b] The corresponding allergenic ingredient soluble protein weight/food weight.

pressurizing processes, hereinafter termed ''model processed (incurred) food.'' It is recommended that food samples comprising animal product, plant product, highly processed food (long heating, high-pressure preparation), or acidic foods be evaluated during the validation to ensure that the ELISA method is applicable to various types of processed foods. (4) The recovery rate from the model processed food should be in the range of 50% and 150%, and the interlaboratory precision (RSD_r) should be less than 25%. (5) The matrix effect data by adding the target specific allergenic ingredient protein to the matrix extract, that of foods showing a false-positive (cross-reactivity) or false-negative result and that of matrices for which the ELISA method hardly applies, should be fully examined and disclosed. (6) ''Reference Material for Monitoring Foods Containing Specific Allergenic Substances'' should be applied for preparing kit standards as well as model processed food samples (Notification No. 1106001, 2002).

In the guidelines and reference materials, the initial extract solution and the extraction procedure from specific allergenic ingredients are also specified and standardized. For developing a food specific allergenic ingredient ELISA, the ELISA performance should fulfill the following interlaboratory validation criteria of the ''Collaborative Study'' protocol based on ISO5725 (JIS Z8402), which is basically the same as that of AOAC, and the obtained performance data must be available to the public.

D. Detection methods for specific allergenic ingredients (Notification No. 1106001, 2002)

1. ELISA

ELISA is the most commonly used method in the food industry and official food control agency laboratories for detecting and quantifying trace specific allergenic ingredients in foods. We introduced two assays using ELISA as the Japanese official method (Matsuda *et al.*, 2006). The best antibody for detecting specific allergenic ingredients in foods was previously determined. Antibodies can be classified into two groups: monoclonal and polyclonal. A polyclonal antibody was chosen for detecting a variety of allergen proteins.

One of the kits for the five allergenic ingredients (eggs, milk, wheat, buckwheat, and peanuts) is the FASTKIT ELISA Ver. II® (Food Allergen Screening Test Kit). This kit uses polyclonal antibodies against multiplex antigens and is produced and commercialized by Nippon Meat Packers, Inc. The concept of this kit is to use polyclonal antibodies to detect whole allergen proteins. Basically, many allergenic ingredients contain multiple allergenic proteins, for example, eggs contain ovalbumin, ovomucoid, and lysozyme, and these proteins can be denatured, degraded, and

combined with other proteins by food processing. To solve this problem, this kit uses multiple antibodies for the native protein, in addition to antibodies for the denatured proteins. The series of FASTKIT ELISA Ver. II® for each allergenic ingredient has been commercialized.

The other ELISA kit for these five allergenic ingredients is the FASPEK KIT®. The concept of this kit uses polyclonal antibodies to detect purified specific proteins or single specific proteins of specific allergenic ingredients. This kit is produced and commercialized by Morinaga Institute of Biological Sciences Co., Ltd. For ELISA, target proteins can be divided into whole proteins and proteins specific to the allergenic ingredient. For the FASPEK KIT®, these specific proteins are used as the target proteins. The target proteins are ovalbumin and ovomucoid for egg, casein, and β-lactoglobulin for milk, gliadin for wheat, the main protein complex for buckwheat, and the protein complex including Ara h2 for peanut. The series of FASPEK KIT® for ovalbumin, ovomucoid, casein, β-lactoglobulin, gliadin, buckwheat main protein complex, and peanut protein complex including Ara h2 has been commercialized. The ovalbumin kit for egg and the casein kit for milk are used as the Japanese official methods because the proportion of these proteins in egg and milk are significant.

In September 2010, CAA announced the addition of ALLERGENEYE® ELISA series of kits for egg, milk, wheat, buckwheat, and peanut as Japanese official methods based on their validation determined by the Japanese validation protocol.

Detection of every kind of protein with consistent sensitivity within a foodstuff is impossible using one kind of ELISA system, as the contents and denaturation of proteins vary greatly. Determination by ELISA is affected by denaturation and extraction efficiency of the target protein. Conventional methods cannot be easily applied to heat- and pressure-processed foods such as retorted and canned foods. Therefore, we developed a unique buffer for extracting insoluble antigens produced during heat and pressure processing (Watanabe *et al.*, 2005) as well as new polyclonal antibodies of the extracted allergen proteins using the new extraction buffer for the Japanese official method kits.

Since the MHLW designated shrimp/prawn and crab for mandatory labeling in June 2008 due to the almost unlimited use of crustacean in the processed foods in Japan and the status as a frequent cause of adverse food reactions in allergic patients, two ELISA methods for the determination of crustacean protein in processed foods have been developed (Seiki *et al.*, 2007; Shibahara *et al.*, 2007): FA test EIA–Crustacean [Nissui]® produced by Nissui Pharmaceutical Co., Ltd. and Crustacean Kit [Maruha®] produced by Maruha Nichiro Foods, Inc. Both kits have been validated according to the Japanese validation protocol (Sakai *et al.*, 2008) and are commercially available. All the commercial ELISA kits are shown in Table 4.9.

TABLE 4.9 Commercial ELISA kits for specific allergenic ingredients

Specific allergenic ingredient	ELISA kits	Target protein
Egg	FASTKIT ELISA Ver.II® for egg	Egg soluble protein
	FASPEK KIT® for egg	Ovalbumin
	ALLERGENEYE® ELISA for egg	Ovalbumin
Milk	FASTKIT ELISA Ver.II® for milk	Milk soluble protein
	FASPEK KIT® for milk	β-lactoglobulin
	ALLERGENEYE® ELISA for milk	Casein
Wheat	FASTKIT ELISA Ver.II® for wheat	Wheat soluble protein
	FASPEK KIT® for wheat	Gliadin
	ALLERGENEYE® ELISA for wheat	Gliadin
Buckwheat	FASTKIT ELISA Ver.II® for buckwheat	Buckwheat soluble protein
	FASPEK KIT® for buckwheat	Soluble peanut protein mixture
	ALLERGENEYE® ELISA for buckwheat	24-kDa protein
Peanut	FASTKIT ELISA Ver.II® for peanut	Peanut soluble protein
	FASPEK KIT® for peanut	Soluble peanut protein mixture
	ALLERGENEYE® ELISA for peanut	Ara h1 protein
Crustacean	Crustacean Kit [Maruha®]	Tropomyosin
	FA test EIA–Crustacean [Nissui]®	Tropomyosin

2. Western blotting method for egg and milk

Western blotting is another protein-based qualitative method. This method has high specificity, because specific proteins are separated according to their molecular mass, irrespective of their original electrochemical charge. Figure 4.4 shows a flowchart of the procedures for Western blotting. First, samples are prepared for polyacrylamide gel electrophoresis (PAGE) and then subjected to blotting and blocking. Next, it is reacted with the primary antibody, followed by the secondary antibody, and then reacted with the avidin-labeled alkaline phosphatase-biotin conjugate, followed by the substrate. The final step is detection of the protein-derived allergens. Western blotting method is prescribed as the confirmation test for egg and milk in the Japanese official methods. The Western blotting kits for egg and milk, FASPEK Western Blot KIT® for egg and milk, are produced and commercialized by Morinaga Institute of Biological Sciences Co.

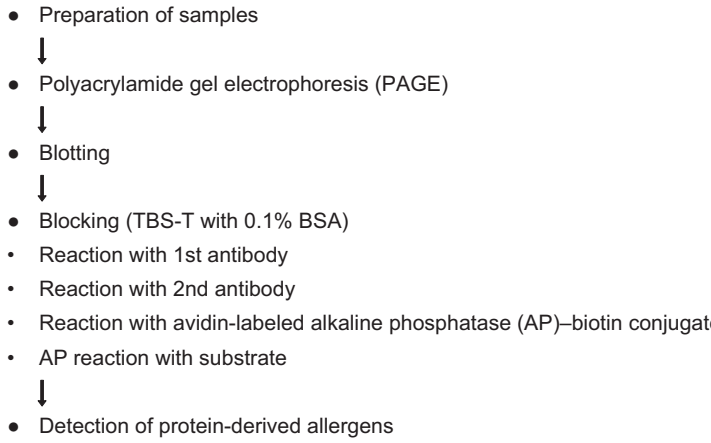

FIGURE 4.4 Flowchart of procedures for Western blotting.

3. PCR method for wheat, buckwheat, peanut, shrimp, and crab

PCR is a DNA-based method that is very specific and sensitive for detection of specific allergenic ingredients in processed foods. The PCR method was established as the confirmation test for wheat, buckwheat, and peanut in the Japanese official methods. Three DNA extraction methods (silica-membrane column-type kit, anion-exchange column-type kit, and CTAB method) are prescribed in the Japanese official methods. The PCR target genes for detection of wheat (Yamakawa *et al.*, 2007a), buckwheat (Yamakawa *et al.*, 2008), and peanut (Watanabe *et al.*, 2006) are shown in Table 4.10. The primer pairs were designed to detect these gene sequences. To check the validity of the extracted DNA for PCR quality, primers recognizing the noncoding region of the chloroplast DNA were designed as the analytical control (Watanabe *et al.*, 2006). To avoid a false-negative result, it is important to check the validity of the extracted DNA for PCR.

Since the MHLW designated shrimp/prawn and crab for mandatory labeling in June 2008, respective PCR methods to discriminate between shrimp/prawn and crabs in processed foods have been developed.

Both methods have been validated according to the Japanese validation protocol (Sakai *et al.*, 2008), and both primers are commercially available. All the Western blotting and PCR kits are shown in Table 4.11.

E. Validation study

We performed collaborative studies using the ELISA methods with model processed foods (sausage, boiled beef in an aluminum pouch, tomato sauce, biscuit, juice, and jam) containing allergen proteins. The six

TABLE 4.10 PCR for wheat, buckwheat, peanut, shrimp and crab

Methods for DNA extraction	
Silica-membrane column-type kit	
Qiagen DNeasy Plant Mini kit	
Anion-exchange column-type kit	
Qiagen Genomic-tip kit	
CTAB method	
PCR target gene sequences	
Wheat	Triticin precursor gene
Buckwheat	Gene encoding soba allergenic protein
Peanut	Agglutinin precursor gene
Shimp/prawn	16S rRNA gene of mitochondrial DNA
Crab	16S rRNA gene of mitochondrial DNA
Plant	Noncoding region of chloroplast DNA
Animal	16S rRNA gene of mitochondrial DNA

model processed foodstuffs were spiked with specific allergenic ingredients to final levels of 10 μg/g in the ingredient stage (Matsuda *et al*., 2006; Sakai *et al*., 2008). We considered that using the model processed foods would be the best way to assess the established ELISA methods by interlaboratory validation. First of all, we conducted a homogeneity test for the model processed foods. Basically, the procedure was performed following the AOAC homogeneity test protocol with some modifications, as described in Table 4.12. The sausage, boiled beef in an aluminum pouch, and tomato sauce were evaluated using a Nippon Meat Packer kit. The biscuit, orange juice, and jam were evaluated using the Morinaga kit.

Table 4.13 shows the method for interlaboratory validation. The first step is the preparation of a standard curve (4-parameter logistic curve) using the absorbance value collected from each participating laboratory. Second, the first and second sets of data are subjected to repeatability using the average values from three wells. Third, Cochran's test and Grubbs's test are used for the removal of outliers (both tests were performed at a significance level of 5%). The final step was estimation of one-way analysis of variance (ANOVA). The 10 participating laboratories included manufacturing companies, public research institutes, local public inspection institutes, and private inspection institutes. Tables 4.14–4.19 show the validation results for egg, milk, wheat, buckwheat, peanut, and shrimp/prawn (crustacean), respectively (Matsuda *et al*., 2006; Sakai *et al*., 2008).

These results were evaluated according to the AOAC protocol and ISO 5725-5 robust statistics. Both kits meet the Japanese acceptance criteria.

TABLE 4.11 Commercial Western blot and PCR kits for specific allergenic ingredients

Specific allergenic ingredient	Western blot or PCR kits	Target protein or gene	Relative molecular weight (Da) or PCR product length (bp)
Egg	Morinaga FASPEK Egg Western Blot Kit (ovalbumin) Morinaga Institute of Biological Sciences Co.	Ovalbumin	50,000
	Morinaga FASPEK Egg Western Blot Kit (ovomucoid) Morinaga Institute of Biological Sciences Co.	Ovomucoid	38,000
Milk	Morinaga FASPEK Milk Western Blot Kit (β-lactoglobulin) Morinaga Institute of Biological Sciences Co.	β-lactoglobulin	18,400
	Morinaga FASPEK Milk Western Blot Kit (Casein) Morinaga Institute of Biological Sciences Co.	Casein	33,000–35,000
Wheat	Allergen checker [Wheat] Oriental Yeast Co., Ltd.	Triticin precursor gene	141
Buckwheat	Allergen checker [Buckwheat] Oriental Yeast Co., Ltd.	Gene encoding soba allergenic protein	127
Peanut	Allergen checker [Peanut] Oriental Yeast Co., Ltd.	Agglutinin precursor gene	95
Shrimp	Primer for shrimp detection, FASMAC Co., Ltd.	16S rRNA gene of mitochondrial DNA	187
Crab	Primer for crab detection, FASMAC Co., Ltd.	16S rRNA gene of mitochondrial DNA	62
Plant DNA	Allergen checker [Plant] Oriental Yeast Co., Ltd.	Noncoding region of chloroplast DNA	Approximately 124
Animal DNA	Allergen checker [Animal] Oriental Yeast Co., Ltd.	16S rRNA gene of mitochondrial DNA	370–470

TABLE 4.12 Homogeneity test for model processed foods[a]

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 portion ($12p$) in random order under repeatable conditions (two wells).
4. Estimate the sampling variance (S^2s) 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^2a) by one-way analysis of variance ($2 \times 12p$) using each well value (estimation variance between each well and each portion).

[a] The procedure basically follows the AOAC homogeneity test protocol with some modification.

TABLE 4.13 Evaluation method for the interlaboratory study

Step 1
The standard (four-parameter logistic) curve was prepared by the simplex method using absorbance values collected from each participating laboratory.
Step 2
The first and second portion data were subjected to a repeatability test using the average values from three wells.
Step 3
Cochran test and *Grubbs test* were performed for the removal of outliers with a significance level of 5%.
Step 4
Estimation of the analytical variance by one-way ANOVA (2 portions × 10 laboratories).

F. Practical test for monitoring the allergy-labeling system.

Figure 4.5 shows the outline of the practical test for monitoring the allergy-labeling system at a local government inspection center. First, we investigated food allergy labeling. As a screening test, quantitative analyses using two different ELISA kits for specific allergenic ingredients were performed to double-check each allergen. We determined the threshold for a positive value to be 10 μg/g in the screening test according to the definition of trace amounts described in Section III.A. Next, we

TABLE 4.14 Recovery, repeatability, and reproducibility for egg detection[a]

Sample	Number of labs	Recovery (%)	Repeatability (%)	Reproducibility (%)
FASTKIT ELISA Ver. II				
Rice gruel	13	85.1	4.3	9.4
Sweet adzuki-bean soup	13	96.0	3.4	9.2
Steamed fish paste	13	83.7	3.6	9.0
Meatball	13	86.1	3.7	8.8
Coffee jelly	13	98.3	3.1	8.5
Fermented soybean soup	13	88.7	3.1	8.8
FASPEK ELISA				
Sausage	10	70.3	4.8	17.4
Boiled beef	9	76.0	3.7	8.0
Cookie	9	51.5	3.5	10.8
Orange juice	10	81.2	3.6	14.2
Jam	9	86.7	4.8	9.4

[a] To confirm the validity of DNA extracted from plants for the PCR and for specific detection of egg.

TABLE 4.15 Recovery, repeatability, and reproducibility for milk detection[a]

Sample	Number of labs	Recovery (%)	Repeatability (%)	Reproducibility (%)
FASTKIT ELISA Ver. II				
Rice gruel	12	89.2	3.4	4.4
Sweet adzuki-bean soup	12	100.3	3.4	5.6
Steamed fish paste	11	74.4	3.7	4.0
Meatball	13	80.8	3.2	8.3
Coffee jelly	12	96.7	4.1	4.5
Fermented soybean soup	13	73.6	4.0	9.9
FASPEK ELISA				
Sausage	10	109.3	5.4	14.9
Boiled beef	10	115.1	7.1	12.2
Cookie	10	96.8	4.9	17.4
Orange juice	10	89.2	4.7	13.3
Jam	10	137.0	2.7	12.1

[a] To confirm the validity of DNA extracted from plants for the PCR and for specific detection of milk.

TABLE 4.16 Recovery, repeatability, and reproducibility for wheat detection[a]

Sample	Number of labs	Recovery (%)	Repeatability (%)	Reproducibility (%)
FASTKIT ELISA Ver. II				
Rice gruel	13	138.9	4.5	9.0
Sweet adzuki-bean soup	13	126.9	3.4	9.9
Steamed fish paste	11	124.4	4.2	5.3
Meatball	13	111.4	5.0	9.0
Coffee jelly	13	129.0	5.1	9.2
Fermented soybean soup	13	110.5	5.7	10.4
FASPEK ELISA				
Chicken meatball	10	92.2	6.2	16.2
Steamed fish paste	10	115.0	10.9	12.9
Orange juice	10	111.7	5.4	11.7
Pudding	10	129.6	6.4	10.6
Mixed stew	10	128.3	6.7	12.0
Tomato sauce	10	122.4	7.0	10.2

[a] To confirm the validity of DNA extracted from plants for the PCR and for specific detection of buckwheat.

TABLE 4.17 Recovery, repeatability, and reproducibility for buckwheat detection[a]

Sample	Number of labs	Recovery (%)	Repeatability (%)	Reproducibility (%)
FASTKIT ELISA Ver. II				
Rice gruel	13	117.5	5.8	18.0
Sweet adzuki-bean soup	13	137.2	6.7	13.3
Steamed fish paste	13	123.0	3.5	10.0
Meatball	13	91.1	7.8	12.7
Coffee jelly	13	112.2	6.6	10.8
Fermented soybean soup	13	93.8	5.4	12.9
FASPEK ELISA				
Sausage	9	101.1	4.5	7.6
Boiled beef	10	121.8	6.5	20.2
Tomato sauce	10	146.1	14.5	17.6
Cookie	10	149.1	8.4	13.4
Orange juice	10	145.7	12.3	17.2

[a] To confirm the validity of DNA extracted from plants for the PCR and for specific detection of buckwheat.

TABLE 4.18 Recovery, repeatability, and reproducibility for peanut detection[a]

Sample	Number of labs	Recovery (%)	Repeatability (%)	Reproducibility (%)
FASTKIT ELISA Ver. II				
Rice gruel	13	74.9	2.5	7.9
Sweet adzuki-bean soup	12	88.9	3.4	7.3
Steamed fish paste	13	100.5	2.5	12.9
Meatball	13	104.1	3.2	12.6
Coffee jelly	13	75.6	3.5	9.7
Fermented soybean soup	13	52.1	2.8	7.8
FASPEK ELISA				
Fermented soybean soup	12	86.8	2.8	4.8
Chicken meat ball	11	87.5	2.0	4.9
Jerry	12	89.1	4.2	5.5
Orange juice	11	84.6	3.0	5.7
Corn soup	12	104.7	2.4	5.7
Tomato sauce	12	109.6	3.5	6.2

[a] To confirm the validity of DNA extracted from plants for the PCR and for specific detection of peanuts.

TABLE 4.19 Recovery, repeatability, and reproducibility for shrimp/prawn detection[a]

Sample	Number of labs	Recovery (%)	Repeatability (%)	Reproducibility (%)
Crustacean kit [Maruha]				
Fish sausage	10	102.8	5.1	23.2
Freeze-dried egg soup	9	98.3	4.1	19.4
Tomato sauce	10	95.8	9.7	19.7
Creamy croquette	10	82.1	8.2	20.6
Chicken ball	10	100.0	6.6	21.6
FA test EIA–Crustacean [Nissui]				
Fish sausage	8	63.5	4.0	6.1
Freeze-dried egg soup	10	73.6	3.9	9.4
Tomato sauce	10	85.7	4.6	5.5
Creamy croquette	10	77.7	4.8	6.2
Chicken ball	10	72.2	5.1	8.9

[a] To confirm the validity of DNA extracted from plants for PCR and for specific detection of shrimp/prawn.

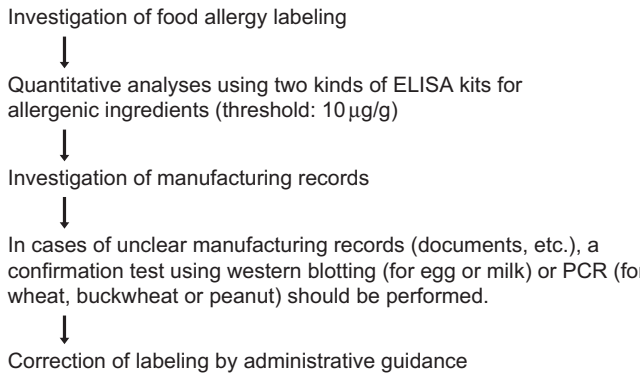

FIGURE 4.5 Outline of the practical test used to monitor the allergy-labeling system.

examined the manufacturing records. If the presence of an allergen cannot be elucidated, a confirmation test using Western blotting for egg or milk or PCR for wheat, buckwheat, peanut, shrimp/prawn, or crab should be performed. If an allergen can be positively detected using the confirmation test, labeling should be corrected according to Ministry guidance. If a company does not follow the guidelines, it can be penalized under the law. Figure 4.6 shows the decision tree for the practical test used to monitor the allergy-labeling system. Local governments and health centers monitor labeling according to this decision tree. Incorrect labeling of specific allergenic ingredients on the processed food products has occurred. Such errors should be corrected using Ministry guidance.

G. Development of detection methods for subspecific allergenic ingredients

1. Soybean

In 1999, the Joint FAO/WHO Codex Alimentarius Commission agreed to recommend the labeling of eight food ingredients, including soybean, which are known to be allergens (FAO, 1995; Hefle *et al.*, 1996). As soybean is one of the ''big eight'' ingredients believed to be responsible for 90% of all food allergies (Zarkadas *et al.*, 1999), it is recommended that labeling is an important issue, more so because of the almost unlimited use of soybean and the increasing number of patients who are allergic soybeans (Bock and Atkins, 1990; Foucard and Malmheden Yman, 1999; Sampson, 2001; Sicherer *et al.*, 2000).

We therefore developed an ELISA for the detection of soybean protein in processed foods using polyclonal antibodies raised against p34 as a soybean marker protein and using a specific extraction buffer (Morishita *et al.*, 2008). The p34 protein, originally characterized as an oil

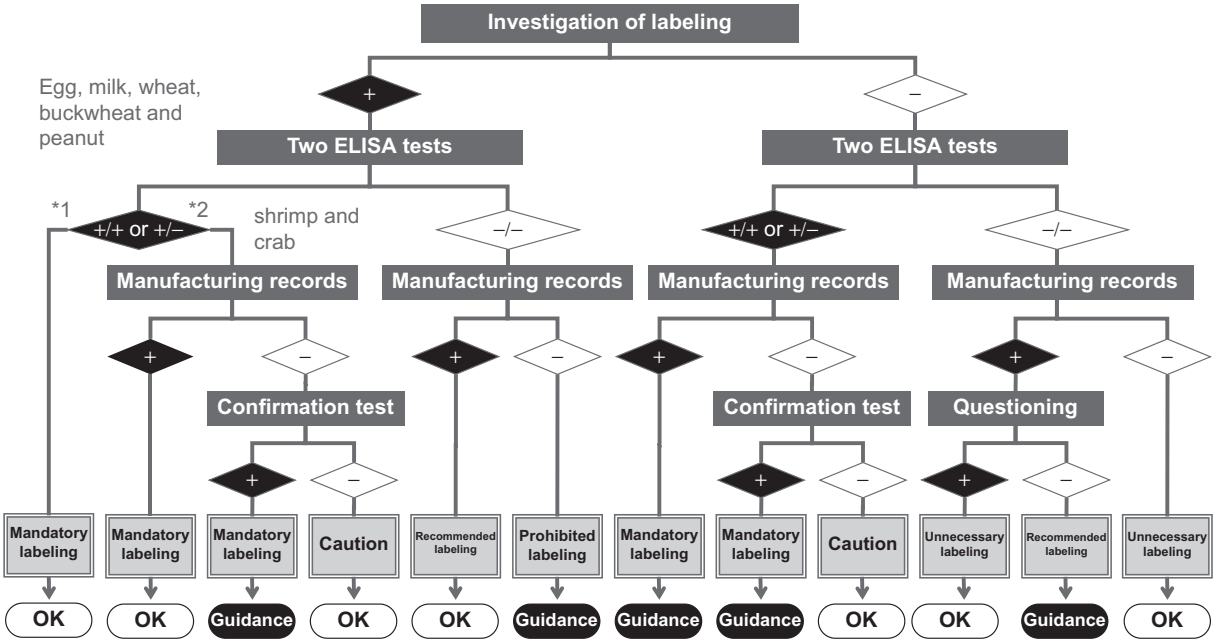

FIGURE 4.6 Decision tree for the practical test used to monitor the allergy-labeling system.

body-associated protein in soybean, has been identified as one of the major allergenic proteins and named Gly m Bd 30K. Our ELISA method is highly specific for this soybean protein, with the LOD of 0.47 ng/mL (equivalent to 0.19 μg/g food) and limit of quantification (LOQ) of 0.94 ng/mL (equivalent to 0.38 μg/g food). Recovery ranged from 87.7% to 98.7%, while the intra- and interassay coefficients of variation were less than 4.2% and 7.5%, respectively. These results show that this ELISA method is specific, precise, and reliable for quantitative analysis of the soybean protein in processed foods. Five types of incurred samples (model processed foods: rice gruel, sausage, sweet adzuki-bean soup, sweet potato cake, and tomato sauce) containing 10 μg soybean soluble protein/g food were prepared for use in interlaboratory evaluations of the soybean ELISA kit (Sakai *et al.*, 2009). The kit displayed a sufficient RSD_r value (interlaboratory precision: 9.3–13.4% RSD_r) and a high recovery (97–114%) for all incurred samples. The RSD_r value for the incurred samples was mostly $<4.8\%$. The results of this interlaboratory evaluation suggest that the soybean kit can be used as a precise and reliable tool for determination of soybean proteins in processed foods.

A sensitive qualitative detection method for soybeans in foods using PCR was also developed (Yamakawa *et al.*, 2007b). For specific detection of soybeans with high specificity, the primer pair was designed using the gene encoding the *Glycine max* repetitive sequence. Trace amounts of soybeans in commercial food products could be qualitatively detected by this method.

2. Walnut

Tree nuts are regarded as one of the most potent of all known food allergens and are often attributed as the cause of severe food anaphylaxis and death. Walnut (*Juglans regia*) is the most common allergenic tree nut and this allergy can be observed in all age groups (Bock *et al.*, 2001). In addition, the walnut allergy is extremely potent, inducing life threatening allergic reactions similar to peanut allergy (Clark and Ewan, 2003; Pumphrey, 2000; Pumphrey and Roberts, 2000). According to Japanese regulations, the labeling of food products containing walnut is recommended. To ensure proper labeling, a novel sandwich ELISA kit for the determination of walnut protein in processed foods has been developed (Doi *et al.*, 2008). The sandwich ELISA method is highly specific for walnut soluble proteins. The recovery ranged from 83.4% to 123%, while the intra- and interassay coefficients of variation were less than 8.8% and 7.2%, respectively. We prepared seven types of incurred samples (model processed foods: biscuit, bread, sponge cake, orange juice, jelly, chicken meatball, and rice gruel) containing 10 μg walnut soluble protein/g food for use in interlaboratory evaluations of the walnut ELISA method (Sakai *et al.*, 2010a). The walnut kit displayed a sufficient RSD_r

(interlaboratory precision: 5.8–9.9% RSD_r) and a high level of recovery (81–119%) for all the incurred samples. All RSD_r values for the incurred samples examined were less than 6.0%. The results of this interlaboratory evaluation suggest that the walnut ELISA method can be used as a precise and reliable tool for determination of walnut proteins in processed foods.

A sensitive qualitative detection method for walnut using PCR was also developed (Yano *et al.*, 2007). For detection of walnuts with high specificity, the primer pair was designed based on walnut *matK* genes. Trace amounts of walnuts in commercial food products can be qualitatively detected using this method.

3. Kiwifruit

Kiwifruit (*Actinidia deliciosa* and *A. chinensis*) is a major fruit allergen that produces severe symptoms and is responsible for a large number of clinical cases worldwide (Lucas *et al.*, 2003; Lucas *et al.*, 2004; Möller *et al.*, 1998a). Under Japanese regulations, it is recommended for labeling as much as possible. To develop PCR-based methods for detection of trace amounts of kiwifruit in foods, we designed two primer pairs targeting the ITS-1 region of the *Actinidia* spp. using PCR simulation software (Taguchi *et al.*, 2007). On the basis of the known distribution of a major kiwifruit allergen (actinidin) within the *Actinidia* spp., in addition to reports on clinical and immunological cross-reactivities, one of the primer pairs was designed to detect all *Actinidia* spp. and the other to detect commercially grown *Actinidia* spp. (i.e., *A. arguta* and is interspecific hybrids) except for *A. polygama*. The specificity of these methods using designed primer pairs was verified by PCR on eight *Actinidia* spp. and 26 other plants, including fruits. The methods were considered to be specific enough to yield products of the target-size only from *Actinidia* spp. and sensitive enough to detect 5–50 fg of *Actinidia* spp. DNA spiked in 50 ng salmon testis DNA used as a carrier (1–10 ppm of kiwifruit DNA) and 1700 ppm (wt/wt) of fresh kiwifruit puree spiked in a commercial plain yogurt (corresponded to ca. 10 ppm of kiwifruit protein). These methods are expected to be useful in the detection of unidentified kiwifruit and its related species in processed foods.

4. Banana

Banana contains food allergens that are common to those in latex or pollens (Ito *et al.*, 2006; Sanchez-Monge *et al.*, 1999). Many clinical studies have reported cross-reactivity of banana and latex, referred to as the latex-fruit syndrome (Blanco *et al.*, 1999; Ikezawa and Osuna, 2002; Möller *et al.*, 1998b). These studies monitored the number of patients with food allergy in Japan and found that patients with banana allergy comprised the second largest population (below only kiwifruit allergy) among those with fruit allergies. We developed specific PCR methods for detection of

banana residue in processed foods. For high banana specificity, the primer pair was designed based on the large subunit of ribulose-1, 5-bisphosphate carboxylase (*rbcL*) genes of chloroplasts and used to obtain amplified products specific to banana by both conventional and real-time PCR (Sakai *et al.*, 2010b). To confirm the specificity of these methods, genomic DNA samples from 31 other species were examined; no amplification products were detected. Subsequently, eight kinds of processed foods containing banana were investigated using these methods to confirm the presence of banana DNA. Conventional PCR had a detection limit of 1 ppm (wt/wt) banana DNA spiked in 50 ng of salmon testis DNA, while SYBR Green I real-time semi-quantitative PCR had a detection limit as low as 10 ppm banana DNA. Thus, both methods show high sensitivity and may be applicable as specific tools for the detection of trace amounts of banana in commercial food products.

5. Pork, beef, chicken, mutton, and horseflesh

As the modern diet often comprises processed foods, especially minced meats, manufacturers are obligated to properly label raw materials. Hence, a rapid method of detecting meat ingredients in processed foods is needed to verify proper labeling. A rapid real-time quantitative PCR method to detect trace amounts of pork, beef, chicken, mutton, and horse meat in foods was developed (Tanabe *et al.*, 2007). The primers and TaqMan minor groove binder (MGB) probes were designed using the gene encoding cytochrome *b* for specific detection of each species. The LOQ of this method was 100 fg/μL of each mitochondrial DNA in 10 ng/μL of wheat mitochondrial DNA matrix. The calculated R^2 values of the standard curves for the five species ranged between 0.994 and 0.999. This method is particularly useful in the detection of unidentified minced meat in processed foods for verification of food labeling.

IV. PATIENT EVALUATION OF ALLERGY FOOD LABELING

To clarify the usefulness and reliability of the food-labeling system, food allergy patients (or their parents) at Sagamihara National Hospital were asked to evaluate it by questionnaire. We received responses from 169 patients. As shown in Table 4.20, patients' profiles were an average age of 49.3 ± 35.6 months, age of the first onset of symptoms of 10.1 ± 14.1 months, and average of 2.9 ± 2.5 eliminated foods. Eliminated foods included hen's eggs (135), cow's milk (79), and wheat (47), as well as peanuts and fish eggs. Of these patients, 44.2% had a past history of anaphylaxis, and 80.2% had experienced symptoms following exposure to even extremely small amounts of the causative foods.

TABLE 4.20 Characteristics of surveyed subjects

169 parents of food allergy patients at Sagamihara National Hospital	
Age of patients	49.3 ± 35.6 months M/F = 1.9
Age of first onset of symptom	10.1 ± 14.1 months
Number of eliminated foods	2.9 ± 2.5
Eliminated foods	
Hen's eggs	135
Cow's milk	79
Wheat	47
Peanuts	51
Fish eggs	28
Past history of anaphylaxis	44.2%
Incidence of symptom by extremely small amount	80.2%

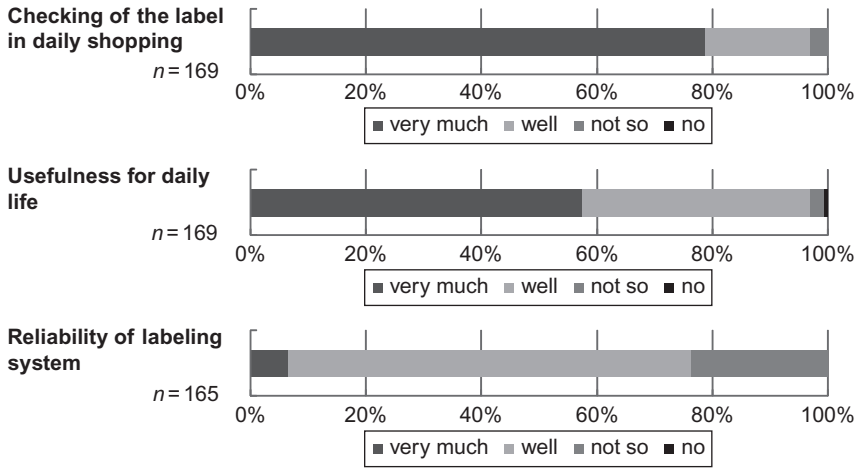

FIGURE 4.7 Evaluation of allergy food labeling.

As shown in Fig. 4.7, 97% of patients routinely checked the allergy food label during daily shopping, and 97% evaluated the allergy food labeling as ''very useful'' or ''useful.'' In addition, 76.4% of the respondents relied on the allergy food-labeling system, and 79.3% had a correct understanding of the food-labeling system based on self-evaluation. On the other hand, 48.8% of respondents answered that the labeling system was ''very easy'' or ''easy'' to understand (Fig. 4.8). Patients who had experienced accidental intake by misreading a label or by mislabeling comprised 30.9% and 13.9%, respectively (Fig. 4.9).

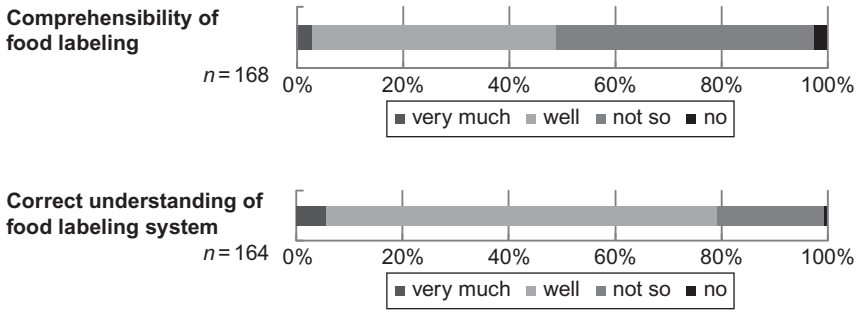

FIGURE 4.8 Comprehension and understanding of allergy food labeling.

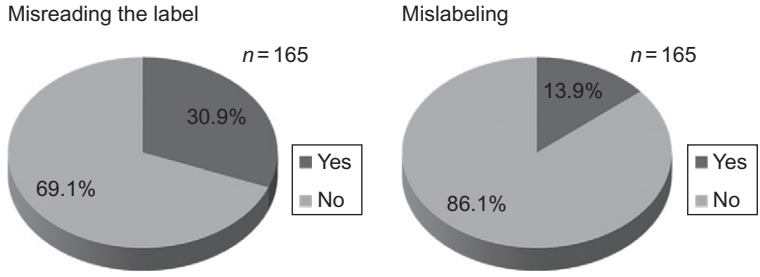

FIGURE 4.9 Incidences of accidental intake by misreading and mislabeling of food labels.

Overall, the Japanese food allergy-labeling system was highly evaluated by food allergy patients and parents. Almost all patients felt that the food-labeling system was very useful, although there were cases of accidental intake either by misreading the label or by mislabeling by food companies.

ACKNOWLEDGMENTS

The authors acknowledge the help and advice of Ms. Chizuko Sugizaki and Ms. Noriko Hayashi, and the advice and support from Professor Atsuo Urisu, Dr. Reiko Teshima, Dr. Reiko Adachi, and the staff of Division of Novel Foods and Immunochemistry, National Institute of Health Sciences. This study was supported by Health and Labor Science Research Grants for Research from the Ministry of Health, Labor, and Welfare of Japan.

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