FOOD COMPOSITION AND ADDITIVES

Interlaboratory Evaluation of Two Enzyme-Linked Immunosorbent Assay Kits for the Detection of Egg, Milk, Wheat, Buckwheat, and Peanut in Foods

RIEKO MATSUDA, YASUO YOSHIOKA, and HIROSHI AKIYAMA
National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan
KENICHI ABURATANI and YUMIKO WATANABE
Morinaga Institute of Biological Science, 2-1-16 Sachiura, Kanazawa-ku, Yokohama 236-0003, Japan
TAKASHI MATSUMOTO and NAOKI MORISHITA
Nippon Meat Packers, Inc., R&D Center, 3-3 Midorigahara, Tsukuba, Ibaraki 300-2646, Japan
HIDETAKA SATO and TAKASHI MISHIMA
Japan Food Research Laboratories, Chitose Laboratory, 2-3, Bunkyo, Chitose, Hokkaido 066-0052, Japan
REIKO GAMO
Nippon Gene Co., Ltd., 1-1-25, Arakawa, Toyama 930-0982, Japan
YASUNORI KIHIRA
Oriental Yeast Co., Ltd., 50, Kano-cho, Nagahama, Shiga 526-0804, Japan
TAMIO MAITANI
National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

The labeling of 5 major allergenic ingredients (egg, milk, wheat, buckwheat, and peanut) is mandatory in Japan, and 2 series of enzyme-linked immunosorbent assay (ELISA) kits have been established as official screening methods. However, these official methods have not provided the necessary sensitivity, due in part to poor extraction efficiency. To address this need, 2 novel ELISA kits have been developed: the FASTKIT ELISA Ver. II Series and the FASPEK Allergenic Substances Detection Kit. The new kit systems use an improved extraction buffer that can extract insoluble proteins produced by processing and feature new antibodies that bind to the denatured proteins extracted with the new extraction buffer. The analytical performances of the 2 new ELISA kit series were evaluated in an interlaboratory study. Ten laboratories participated in the study and determined the major allergenic ingredients contained in 5 types of model processed food. The 2 ELISAs displayed fairly good reproducibility and sufficient recovery.

The number of patients with food allergies in Japan continues to rise (1). The reason appears to be due to drastic changes in dietary habits in Japan. The most effective means of preventing allergic reactions to food is to avoid foods that contain allergens; it is therefore essential that patients with food allergies be able to obtain accurate information on food allergens contained in processed foods. The Japanese Ministry of Health, Labor and Welfare (MHLW) organized a Labeling Study Group to discuss an appropriate food labeling system. The study group submitted a report on their discussions in 2000, in response to which the MHLW decided that processed foods containing 5 major allergic ingredients (egg, milk, wheat, buckwheat, and peanut) should be labeled (2).

The MHLW has developed detection methods for the 5 major allergic ingredients mentioned above and has established them as the official Japanese methods. These official methods consist of 2 kinds of enzyme-linked immunosorbent assay (ELISA) kits as the screening methods (3, 4), the Western blot method for egg and milk, and the polymerase chain reaction (PCR) method for wheat, buckwheat, and peanut as the confirmation method.

Unfortunately, the official ELISA kits were not adequate in some areas. They could not detect the allergic ingredients in highly processed foods in spite of being able to detect those added to extracts of processed foods. One of the reasons for this low sensitivity was the low extraction efficiency of proteins from processed foods (5–9). Similarly, the antibody used could not recognize proteins that had been denatured during food processing. To remedy these defects, we developed a novel extraction buffer for extracting the insoluble proteins produced by heat and pressure processing, and with new antibodies that recognize the denatured proteins extracted using the new buffer (10). We have established 2 ELISA kits for detecting the 5 major allergic ingredients. Both use the same extraction buffer, leading to reduced

Received November 24, 2005. Accepted by AH February 8, 2006.
Corresponding author's e-mail: matsuda@nihs.go.jp

Table 1. Allergenic foods spiked to the test materials

Test material	Allergenic ingredients
Sausage	Egg, milk, wheat, buckwheat, peanut
Boiled beef	Egg, milk, wheat, buckwheat, peanut
Tomato sauce	Wheat, buckwheat, peanut
Cookie	Egg, milk, buckwheat
Orange juice	Egg, milk, wheat, buckwheat, peanut
Strawberry jam	Egg, milk, wheat, peanut

differences between the results from the kits arising from the sample extraction, and both are less labor-intensive.

This report describes the results of the interlaboratory study on the performance of these improved ELISA kits.

Experimental

Materials and Methods

Test materials.—Six model processed foods (sausage, boiled beef, tomato sauce, cookie, orange juice, and strawberry jam) containing major allergenic ingredients were prepared and used as the test materials. Table 1 shows the allergic ingredients spiked to the model processed foods. Each ingredient was spiked to 5 types of processed foods. The allergic ingredients were spiked at the ingredient stage before processing to obtain a final level of 10 μg/g protein.

Preparation of spiking powders or solutions of the allergic ingredients.—Egg powder for spiking was prepared from the eggs of White Leghorn hens by freeze-drying. Milk from Holstein Friesian cows was also freeze-dried. An equivalent mixture of 14 brands of whole wheat flour, an equivalent mixture of whole buckwheat flours produced in China and Japan, and Virginia peanuts produced in Chiba Prefecture were used to prepare the standard bulk powders. The content of protein in each spiking powder was assayed using a 2-D Quant Kit (Amersham Biosciences, Uppsala, Sweden). The amount of protein present in 1 g spiking powder was about 450 mg for egg, 260 mg for milk, 100 mg for wheat, 70 mg for buckwheat, and 90 mg for peanuts. The amount of each standard bulk powder to obtain a final protein concentration of 10 μg/g was calculated, taking into account the protein content and the change in weight of the model processed foods during preparation. The spiking solution of the allergic ingredient was prepared by dissolving or suspending the bulk powder in purified water.

Preparation of model processed foods.—All the model processed foods were prepared following the usual procedures used by the manufacturers. The prepared model processed foods were homogenized with a food processor (sausage, boiled beef, and tomato sauce) or a homogenizer (cookie, orange juice, and jam), and sent to the participants.

Sausage was made of pork leg meat (minus bones, sinews, blood vessels, and fat), vegetable oil, salt, sugar, ice water, and the spiking solutions. Vegetable oil, salt, sugar, ice water, and the spiking solutions were added to the meat and mixed thoroughly. The mixture was ground using a small cutter, and the kneaded mixture was manually placed into sausage casings. These were heated at 80°C for 20 min, cooled in flowing water for 5 min, and kept in a refrigerator at 5°C overnight.

Boiled beef was made of beef shoulder meat (minus bones, sinews, blood vessels, and fat), agar solution, salt, sugar, and the spiking solutions. The meat was immersed in the agar solution containing salt, sugar, and the spiking solutions, and the mixture was kept in a refrigerator at 5°C for 60 min. The mixture was then placed in an aluminum pouch, heated at 121°C for 1 min, cooled in flowing water for 5 min, and then placed in a refrigerator at 5°C overnight.

Tomato sauce was made of tomato purée, sugar, Worcestershire sauce, apple vinegar, salt, potato starch, water, and the spiking solutions. The measured raw materials were mixed thoroughly and placed in an aluminum pouch. The sauce was heated at 90°C for 30 min, cooled in flowing water for 5 min, and then placed in a refrigerator at 5°C overnight.

Cookies were made of wheat, sugar, shortening, salt, raising agents, lecithin, water, and the standard bulk powders. The raw materials were homogeneously mixed and kneaded for 20 min. The dough was then rolled out, cut with a cookie cutter, and baked at 240°C for 7 min.

Orange juice was made of water, concentrated juice, sugar, citric acid, and the standard bulk powders. The raw materials were mixed homogeneously. Each 190 mL sample of the mixture was canned and heated at 90°C for 10 min.

Strawberry jam was made of strawberries, saccharides, pectin, citric acid, and the standard bulk powders. The raw materials were mixed thoroughly and heated at 94°C for 4 min.

Homogeneity tests of samples.—The homogeneity of the samples was verified following the procedure laid out in the International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories (11), except that the number of test samples was 6. Twelve test portions were analyzed using the ELISA kits. Sausage, boiled beef, and tomato sauce were analyzed with FASTKIT (Nippon Meat Packers, Osaka, Japan), and cookie, orange juice, and jam were analyzed with FASPEK (Morinaga Institute of Biological Science, Yokohama, Japan). The obtained concentrations of allergic protein were submitted to one-way analysis of variance (ANOVA). Table 2 shows the averaged concentration, the relative standard deviation (RSD) values calculated from s_s (SD of sampling) and s_a (SD of analysis), and the *F*-ratios. The *F*-ratios for all samples were below the critical *F* value.

ELISA Kits

FASTKIT ELISA Ver. II Series (FASTKIT).—The detection procedure was prescribed by the manufacturer. Diluted standard solutions and sample solutions were added to an antibody-coated plate and incubated for 1 h at room temperature. The plate was washed with washing buffer, and then a solution of biotinylated polyclonal antibodies that recognizes multiple antigens was added and the mixture was

Table 2. Homogeneity test results of the test materials

	Mean	RSD%[a]	*n*	*F*-ratio	*F*crit[b]
Sausages					
Egg	5.3	4.6	6	0.67	4.39
Milk	5.2	10.2	6	0.70	4.39
Wheat	11.3	4.0	6	1.52	4.39
Buckwheat	5.9	5.3	6	0.41	4.39
Peanuts	8.0	3.6	6	0.51	4.39
Boiled beef					
Egg	7.4	5.4	6	0.15	4.39
Milk	5.2	3.1	6	1.32	4.39
Wheat	12.1	5.0	6	0.27	4.39
Buckwheat	6.1	4.0	6	1.32	4.39
Peanuts	7.4	3.4	6	0.28	4.39
Tomato sauce					
Wheat	13.0	14.0	6	3.70	4.39
Buckwheat	15.7	10.3	6	4.10	4.39
Peanuts	14.0	10.6	6	2.82	4.39
Cookie					
Egg	4.6	2.7	6	0.90	4.39
Milk	8.1	5.8	6	0.05	4.39
Buckwheat	15.4	6.4	6	1.70	4.39
Orange juice					
Egg	7.6	2.9	6	1.46	4.39
Milk	7.5	3.0	6	0.85	4.39
Wheat	13.2	4.7	6	1.75	4.39
Buckwheat	14.0	12.5	6	0.32	4.39
Peanuts	11.8	2.3	6	0.57	4.39
Strawberry jam					
Egg	6.8	4.7	6	0.59	4.39
Milk	11.8	1.8	6	0.85	4.39
Wheat	1.1	7.8	6	3.37	4.39
Peanuts	5.5	3.9	6	0.82	4.39

[a] RSD% calculated from s_s (SD of sampling) and s_a (SD of analysis).
[b] *F*crit = Critical *F* value.

incubated for 1 h at room temperature. The plate was again washed with the washing buffer, after which the streptavidin–peroxidase reagent was added, and the mixture was incubated for 30 min at room temperature. The plate was once more washed, and a solution of 3,3′,5,5′-tetramethylbenzidine, the substrate for the enzyme, was added and incubated for 20 min at room temperature. The reaction was stopped with 0.5 N sulfuric acid, and the absorbances were measured at 450 nm, with 630 nm as the reference wavelength.

FASPEK allergenic substances detection kit (FASPEK).—The polyclonal antibodies used in FASPEK recognized ovalbumin (egg), casein (milk), gliadin (wheat), soluble buckwheat protein (buckwheat), and soluble peanut protein (peanut). The detection procedure as prescribed by the manufacturer was followed. Diluted standard solutions and sample solutions were added to an antibody-coated module and incubated for 1 h at room temperature. After washing the module, a solution of the antibody, labeled with peroxidase, was added and allowed to stand for 30 min. After the second washing, a solution of 3,3′,5,5′-tetramethylbenzidine was added and the module was allowed to stand at 25°C for exactly 10 min. The reaction was stopped by addition of 0.1 N sulfuric acid, and the absorbances were measured at 450 nm, with 630 nm as the reference wavelength.

Extraction

The extraction procedure was common to both ELISA series. A 1 g portion of the sample was extracted with 19 mL extraction buffer containing sodium dodecyl sulfate (SDS) and mercaptoethanol. The extraction buffer was also common to both ELISA series. The mixture was shaken horizontally overnight (>12 h) at room temperature, and then centrifuged at 3000 × *g* for 20 min after adjustment of the pH to 6. The supernatant was filtered or centrifuged, if necessary, diluted 20 times, and subjected to ELISA.

Calibration Standard Solutions

The calibration standard solutions were common to both ELISA kits. The ingredients for the calibration solutions were same as those of the bulk standards powder for spiking to the samples: *Egg.*—Fresh eggs of White Leghorn hen, homogenized and freeze-dried; *Milk.*—Fresh milk of Holstein cows, freeze-dried after defatting by churning; *Wheat.*—A mixture of 14 species of wheat, pulverized; *Buckwheat.*—A mixture of buckwheats from Ibaraki Prefecture (Japan) and from China, pulverized; *Peanuts*.—Virginia species from Chiba Prefecture (Japan), ground in a mortar.

The ingredients were extracted with 20 mL extraction solution containing 0.5% SDS and 2% mercaptoethanol by shaking overnight. The protein content of the initial extract was assayed using a 2-D Quant Kit (Amersham Biosciences).

The initial extract was diluted to make up the calibration standard solution (50 ng/mL extracted protein). The calibration standard solutions of egg and milk were provided by Nippon Gene Co. Ltd. (Toyama, Japan) and those of wheat, buckwheat, and peanut were provided by Oriental Yeast Co. Ltd. (Nagahama, Japan).

Interlaboratory Study

Ten laboratories participated in the interlaboratory study, coordinated by the National Institute of Health Sciences (Tokyo, Japan). The coordinator sent the 6 test materials (3 g each) and 10 ELISA kits plus the extraction solution and the calibration standard solutions. The participants took 2 portions from each test material, extracted the protein using the extraction procedure, and assayed each extract with the ELISA kits. The calibration standard solution was diluted and assayed simultaneously with the sample extracts. The averaged absorbance of 3 wells was used for the calculation. The obtained absorbance data of calibration solutions and test samples were reported to the coordinator.

The coordinator calculated the 4-parameter logistic calibration curve from the absorbance data of the calibration

Table 3. Results of interlaboratory study for FASPEK: protein recovery content, %

Lab	Sausage, μg/g		Boiled beef, μg/g		Cookie, μg/g		Orange juice, μg/g		Jam, μg/g	
					Egg					
A	6.8	68	7.2	72	4.7	47	8.1	81	8.1	81
B	8.7	87	8.2	82	5.7	57	8.8	88	9.3	93
C	8.2	82	8.2	82	5.1	51	8.3	83	8.4	84
D	7.2	72	8.0	80	5.6	56	9.0	90	9.0	90
E	4.8	48	2.7[a]	27[a]	4.0	40	5.4[a]	54[a]	2.8[a]	28[a]
F	8.4	84	8.4	84	6.0	60	9.7	97	9.9	99
G	7.0	70	7.1	71	4.4	44	7.3	73	7.2	72
H	6.5	65	7.2	72	5.2	52	8.3	83	8.9	89
I	6.0	60	7.2	72	4.8	48	8.3	83	8.6	86
J	6.6	66	6.9	69	4.9	49	8.0	80	8.6	86
					Milk					
A	10.6	106	11.8	118	10.1	101	9.1	91	13.1	131
B	11.3	113	11.5	115	9.9	99	10.0	100	14.4	144
C	13.7	137	13.4	134	10.6	106	9.7	97	14.0	140
D	11.4	114	11.2	112	10.0	100	9.3	93	14.4	144
E	9.2	92	9.7	97	6.1	61	6.3	63	9.8	98
F	13.0	130	12.5	125	11.5	115	9.6	96	14.2	142
G	10.8	108	9.3	93	7.6	76	7.5	75	12.2	122
H	8.6	86	12.4	124	11.1	111	9.5	95	15.0	150
I	9.6	96	11.1	111	9.4	94	9.3	93	15.3	153
J	11.2	112	12.3	123	10.4	104	8.8	88	14.6	146
					Wheat					
A	9.3	93	12.4	124	12.3	123	12.6	126	1.6	16
B	11.8	118	14.0	140	11.8	118	11.8	118	1.3	13
C	11.2	112	12.1	121	14.1	141	11.2	112	1.1	11
D	11.0	110	11.0	110	10.7	107	12.0	120	1.8	18
E	11.4	114	13.4	134	12.0	120	12.5	125	1.1	11
F	11.6	116	12.1	121	12.0	120	12.1	121	1.7	17
G	10.6	106	11.7	117	11.2	112	11.3	113	1.7	17
H	1.0[b]	10[b]	12.1	121	14.3	143	13.0	130	2.1	21
I	8.3	83	10.1	101	11.8	118	11.3	113	1.2	12
J	10.9	109	11.7	117	12.2	122	11.9	119	2.6	26
					Buckwheat					
A	10.4	104	12.8	128	15.9	159	14.8	148	15.6	156
B	9.7	97	11.3	113	16.4	164	15.2	152	13.8	138
C	11.5	115	14.3	143	14.9	149	16.9	169	14.8	148
D	9.8	98	12.1	121	15.0	150	15.7	157	17.2	172
E	5.7[b]	57[b]	6.4	64	10.0	100	11.0	110	9.4	94
F	10.1	101	12.2	122	13.7	137	14.8	148	16.4	164
G	10.4	104	13.6	136	15.1	151	15.6	156	14.7	147
H	10.3	103	15.2	152	17.5	175	17.4	174	15.9	159
I	10.0	100	12.8	128	14.8	148	13.9	139	14.1	141
J	8.8	88	11.1	111	12.7	127	13.6	136	13.9	139
					Peanuts					
A	27.4	274	23.4	234	19.8	198	17.7	177	12.7	127
B	20.9	209	15.2	152	18.7	187	17.2	172	11.8	118
C	19.8	198	14.0	140	16.1	161	14.5	145	13.1	131
D	16.6	166	15.5	155	15.4	154	15.3	153	12.4	124
E	3.8	38	3.6	36	5.8[b]	58[b]	8.7	87	4.6[b]	46[b]
F	23.0	230	16.8	168	16.3	163	15.0	150	11.3	113
G	17.6	176	15.2	152	16.9	169	15.3	153	11.1	111
H	5.6	56	15.4	154	17.6	176	15.6	156	11.9	119
I	17.7	177	17.1	171	14.3	143	13.5	135	11.8	118
J	20.5	205	16.2	162	15.1	151	13.5	135	10.3	103

[a] Values removed after the Cochran test.

[b] Values removed after the Grubbs test.

Table 4. Results of interlaboratory study for FASTKIT: protein recovery content, %

Lab	Sausage, μg/g		Boiled beef, μg/g		Cookie, μg/g		Orange juice, μg/g		Jam, μg/g	
					Egg					
A	7.2	72	8.1	81	7.2	72	10.5	105	9.9	99
B	8.1	81	8.0	80	6.8	68	9.4	94	9.4	94
C	7.8	78	8.0	80	6.2	62	9.3	93	9.4	94
D	7.2	72	8.6	86	6.8	68	10.2	102	10.2	102
E	5.9	59	6.7	67	7.0	70	9.7	97	10.0	100
F	7.1	71	7.6	76	6.1	61	9.6	96	9.2	92
G	6.4	64	7.8	78	6.0	60	9.2	92	8.5	85
H	6.9	69	7.9	79	7.8	78	11.1	111	10.2	102
I	5.4	54	7.3	73	30.5[a]	305[a]	9.6	96	9.3	93
J	5.3	53	6.2	62	5.6	56	9.2	92	9.2	92
					Milk					
A	7.3	73	6.9	69	5.4	54	8.9	89	9.0	90
B	6.4	64	6.0	60	4.6	46	8.1	81	8.9	89
C	6.4	64	6.1	61	4.4	44	7.9	79	8.5	85
D	7.4	74	6.8	68	5.1	51	8.5	85	9.4	94
E	6.0	60	5.7	57	5.3	53	8.8	88	9.6	96
F	6.4	64	6.2	62	4.6	46	8.2	82	8.4	84
G	7.0	70	6.4	64	4.5	45	7.2	72	7.2	72
H	4.7	47	7.0	70	5.9	59	9.3	93	9.3	93
I	5.6	56	6.2	62	4.5	45	8.6	86	8.8	88
J	5.3	53	6.2	62	5.1	51	9.4	94	10.0	100
					Wheat					
A	13.4	134	13.2	132	12.5	125	13.7	137	2.5	25
B	12.9	129	13.0	130	13.5	135	13.7	137	3.7	37
C	11.5	115	11.4	114	14.7[b]	147[b]	11.2	112	3.5	35
D	11.3	113	12.2	122	11.3	113	12.7	127	2.6	26
E	12.2[b]	122[b]	9.6	96	11.9	119	13.3	133	2.9	29
F	11.1	111	10.5	105	9.7	97	11.9	119	2.5	25
G	11.2	112	11.2	112	10.3	103	11.0	110	1.9	19
H	0.5[a]	5[a]	11.0	110	13.2	132	11.6	116	3.3	33
I	8.7	87	10.7	107	10.4	104	11.2	112	2.4	24
J	9.9	99	10.6	106	11.7	117	12.4	124	3.2	32
					Buckwheat					
A	8.6	86	6.7	67	14.7	147	9.3	93	14.8	148
B	6.9	69	5.1	51	14.0	140	9.9	99	13.3	133
C	6.8	68	5.7	57	11.9	119	8.6	86	13.0	130
D	5.5	55	5.9	59	12.7	127	8.2	82	15.5	155
E	6.0	60	5.1	51	12.3	123	8.7	87	13.6	136
F	7.9	79	6.0	60	11.5	115	8.4	84	11.9	119
G	4.8	48	5.3	53	10.8	108	7.2	72	12.6	126
H	3.9	39	5.7	57	12.9	129	9.4	94	14.2	142
I	8.8	88	10.2[a]	102[a]	13.3	133	8.8	88	14.7	147
J	6.9	69	6.6	66	10.9	109	9.2	92	12.1	121
					Peanuts					
A	16.7	167	12.8[a]	128[a]	12.3	123	10.2	102	9.7	97
B	10.6	106	8.1	81	13.3	133	12.3	123	10.5	105
C	8.7	87	7.8	78	11.4	114	10.5	105	12.2	122
D	6.7	67	8.1	81	10.6	106	10.9	109	11.3	113
E	8.7	87	7.0	70	9.5	95	9.9	99	10.7	107
F	12.6	126	9.2	92	11.4	114	10.4	104	10.2	102
G	7.1	71	7.6	76	10.9	109	9.6	96	9.1	91
H	5.6	56	8.6	86	12.6	126	11.7	117	10.5	105
I	10.0	100	9.3	93	12.3	123	11.1	111	11.5	115
J	8.8	88	8.5	85	11.7	117	10.4	104	10.5	105

[a] Values removed after the Grubbs test.

[b] Values removed after the Cochran test.

standard solution and calculated the concentration of allergen protein in the sample using the calibration curve. The average of the 3 results was treated as the data for 1 portion.

Statistical Analysis

The reported values from the participants are summarized in Tables 3 and 4. Twenty data items, as 2 portions from 10 laboratories, were fed into the calculation. The Cochran and Grubbs tests were used to remove outlying data (P = 2.5%). The removed values are also shown in Tables 3 and 4. Recovery, repeatability, and reproducibility were calculated by one-way ANOVA using the remaining data after removal of outliers.

Results and Discussion

Sample Homogeneity

The resultant F-ratios of the homogeneity test of sausage, boiled beef, cookie, orange juice, and jam were <2. The critical value of F was 4.69, and the homogeneity of the samples was sufficient. The F-ratios from tomato sauce were higher than the others, but lower than the critical F. For most samples, the RSD values among portions were <10% and smaller than the expected RSD_R values.

Recovery

The recovery, repeatability (RSD_r), and reproducibility (RSD_R) values calculated using ANOVA are shown in Table 5 with the number of remaining laboratories after removing outliers.

The recoveries of egg and milk proteins from 5 types of test materials were >50%, with one exception. The FASTKIT for milk gave a low recovery of milk protein in the cookies. The recoveries of milk protein using FASPEK were higher than those using the FASTKIT for all the samples, whereas recoveries of egg proteins were comparable between the kits.

The recoveries of wheat proteins from sausage, boiled beef, tomato sauce, and orange juice were almost 100% for both kits. Both kits gave low recoveries of <30% for wheat protein in jam. There were large differences between the recoveries of buckwheat between the kits. FASPEK showed recoveries of 100–140% for boiled beef, tomato sauce, cookie, and orange juice, whereas the recoveries using the FASTKIT were 50–100% and lower than those with FASPEK for all the test materials. The recoveries of peanut protein showed similar patterns to those of buckwheat. The recoveries of peanut protein using FASPEK were >100% for all the test materials, whereas FASTKIT gave recoveries >100%.

There were discrepancies in the recoveries of some proteins between the 2 kits. Because the extracts from each test material and the calibration standards were shared between the tests, these discrepancies were due to differences in reactivity to the denatured proteins between the antibodies used. The results from orange juice prepared using short heating showed comparable recovery between the 2 kits.

Repeatability

Repeatability is a measure of the variance arising from the extraction and the determination procedure in a laboratory. In most cases, RSD_r values were <10%. The RSD_r values of FASPEK for wheat protein from tomato sauce and those for buckwheat protein from tomato sauce and jam were >10%. The results of a homogeneity test of tomato sauce and orange juice (Table 2) showed large s_a values for wheat and buckwheat, suggesting that the extraction efficiency of wheat or buckwheat protein from an acidic matrix is susceptible to small variations in extraction conditions. The difference in variability in the extraction efficiency may be attributable to the difference in types of antibodies used.

Reproducibility

Reproducibilities, expressed by RSD_R values, were <20% for egg and milk in all the test materials. The low recovery of wheat protein from jam resulted in high RSD_R values. The large RSD_r of FASPEK for wheat protein in tomato sauce was reflected in the RSD_R values but the value of 19% demonstrated acceptable reproducibility. RSD_R values of wheat protein in other samples were satisfactory. The results for buckwheat and peanuts also gave good RSD_R values, except for sausage. As shown in Table 2, the homogeneity of sausage samples was guaranteed and the RSD_R values for egg or milk protein in sausage were similar to other test materials. The reason for the large RSD_R values of buckwheat and peanut proteins in sausage can be attributed to the variation in extraction efficiency of buckwheat protein and peanut protein.

Sensitivity

Figure 1 shows the calibration curves of the 2 ELISAs. All the curves are almost straight between 5 and 25 ng/mL, and give sufficiently high absorbance at 25 ng/mL that corresponds to 10 μg/g allergen protein in the sample.

Specificity

The cross-reactivity of FASTKIT and FASPEK was investigated by the manufacturers. The cross-reactivity of FASTKIT with 120 kinds of commodities was surveyed. The kit for egg and the kit for buckwheat did not display any cross-reactivity. The kit for milk displayed cross-reactivity with goat and sheep milk, and the kit for wheat reacted with rye, oats, and barley. The kit for peanuts displayed cross-reactivity with macadamia nuts and kelp (2).

The cross-reactivity of the FASPEK kit with 140 kinds of commodities was surveyed. The FASPEK for egg displayed cross-reactivity with quail and duck eggs. The FASPEK for milk reacted with goat and sheep milk. The FASPEK for wheat displayed cross-reactivity with many species of grain, including rye, oats, and barley, and other commodities such as toasted almonds, poppy seeds, and coriander. The FASPEK for peanuts reacted with macadamia nuts. The FASPEK for buckwheat did not cross-react with any of the foods investigated but reacted with many Polygonum plants (2).

Table 5. Recoveries, repeatabilities (RSD_r), and reproducibilities (RSD_R) of the FASPEK and FASTKIT for egg, milk, wheat, buckwheat, and peanut

	No. of laboratories		Mean, μg/g		s_r, μg/g		s_R, μg/g		Recovery, %		RSD_r, %		RSD_R, %	
Sample	FASPEK	FASTKIT	FASPEK	FASTKIT	FASPEK	FASTKIT	FASPEK	FASTKIT	FASPEK	FASTKIT	FASPEK	FASTKIT	FASPEK	FASTKIT
							Egg							
Sausage	10	10	7.0	6.7	0.3	0.3	1.2	1.0	70	67	5	4	17	15
Boiled beef	9	10	7.6	7.6	0.3	0.2	0.6	0.7	76	76	4	2	8	10
Cookie	9	9	5.2	6.6	0.2	0.3	0.6	0.7	52	66	4	5	11	11
Orange juice	10	10	8.1	9.8	0.3	0.2	1.2	0.7	81	98	4	2	14	7
Jam	9	10	8.7	9.5	0.4	0.3	0.8	0.6	87	95	5	3	9	6
							Milk							
Sausage	10	10	10.9	6.3	0.6	0.3	1.6	0.9	109	63	5	5	15	14
Boiled beef	10	10	11.5	6.4	0.86	0.3	1.4	0.5	115	64	7	4	12	7
Cookie	10	10	9.7	4.9	0.5	0.2	1.7	0.5	97	49	5	4	17	10
Orange juice	10	10	8.9	8.5	0.4	0.3	1.2	0.7	89	85	5	4	13	8
Jam	10	10	13.7	8.9	0.4	0.2	1.7	0.8	137	89	3	2	12	9
							Wheat							
Sausage	9	8	10.7	11.3	0.8	0.6	1.3	1.6	107	113	7	5	12	14
Boiled beef	10	10	12.1	11.4	0.9	0.7	1.3	13	121	114	7	7	11	11
Tomato sauce	10	9	12.2	11.6	2.3	1.1	2.3	1.5	122	116	19	9	19	13
Orange juice	10	10	12.0	12.3	0.3	0.5	0.6	1.2	120	123	3	4	5	9
Jam	10	10	1.6	2.8	0.3	0.3	0.5	0.7	16	28	18	18	33	22
							Buckwheat							
Sausage	9	10	10.1	6.6	0.5	0.5	0.8	1.7	101	66	5	8	8	25
Boiled beef	10	9	12.2	5.8	0.8	0.4	2.5	0.7	122	58	7	6	20	11
Tomato sauce	10	10	14.6	12.5	2.1	0.8	2.6	1.4	146	125	15	7	18	11
Cookie	10	10	14.9	8.8	1.3	1.1	2.0	1.1	149	88	8	13	13	12
Orange juice	10	10	14.6	13.6	1.8	1.0	2.5	1.4	146	136	12	7	17	10
							Peanut							
Sausage	10	10	17.3	9.5	2.1	0.4	7.5	3.2	173	95	5	4	45	34
Boiled beef	10	9	15.2	8.2	1.0	0.6	4.9	0.9	152	82	5	7	11	10
Tomato sauce	9	10	16.7	11.6	0.9	0.5	1.9	1.2	167	116	5	4	15	10
Cookie	10	10	14.6	10.7	0.9	0.5	2.6	0.9	146	107	6	5	14	9
Orange juice	9	10	11.8	10.6	0.9	0.6	1.1	1.0	118	106	8	5	11	9

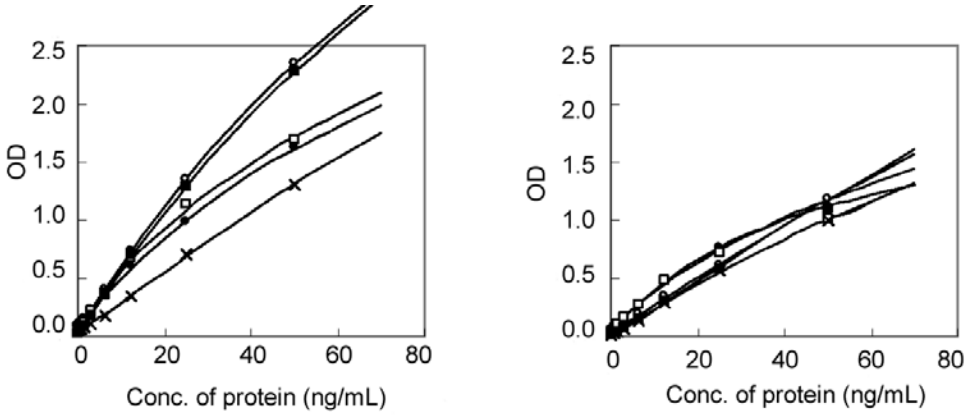

Figure 1. Calibration curves of FASTKIT (left) and FASPEK (right); ○ = egg; ● = milk; □ = wheat; ■ = buckwheat; × = peanuts.

Conclusions

In conclusion, the results from the interlaboratory study suggest that the 2 test kits, FASPEK and FASTKIT, correctly determined egg and milk protein. Wheat, buckwheat, and peanut, even if contained in highly processed foods such as sausage or cookie, were determined by the 2 kits, although the interlaboratory variations were higher than those for egg and milk. Neither kit could determine wheat protein contained in jam. The interlaboratory study was performed using highly processed model foods rather than the standard reference materials that contained less processed protein to ensure that the ELISA kits could detect the allergic substances under actual conditions. The results demonstrated that the kits would detect the allergic ingredients contained in processed foods and support the food labeling system.

The notable feature of these kits is the unified extraction solution. A unified extract from a sample can be used for determination with both kits, and the variation of results between the 2 kits can be significantly reduced. Furthermore, the standard calibration solutions were also unified. This should make it possible to compare the results of the 2 kits using a common measure and make the results traceable to a defined amount of protein.

Acknowledgments

We thank the following laboratories for participating in the interlaboratory study:

FASMAC Co., Ltd. (Atsugi, Japan)

Japan Food Research Laboratories (Chitose, Japan)

Japan Frozen Foods Inspection Corp. (Tokyo, Japan)

Japan Inspection Association of Food and Food Industry Environment (Tokyo, Japan)

Kanagawa Prefectural Public Health Department (Chigasaki, Japan)

Nippon Gene Co., Ltd. (Toyama, Japan)

Nisshin Seifun Group, Inc. (Fujimino, Japan)

Rohto Pharmaceutical Co., Ltd. (Osaka, Japan)

San-Ei Gen F.F.I., Inc. (Osaka, Japan)

Showa Sangyo Co., Ltd. (Funabashi, Japan)

This study was supported by a grant from MHLW of Japan (Tokyo, Japan).

References

(1) Ebisawa, M. (2003) *Report to the Grant from the Ministry of Health, Labor and Welfare of Japan*, Tokyo, Japan, pp 160–162

(2) Ministry of Health, Labor and Welfare of Japan (November 6, 2002) *Notification No. 106001*

(3) Takahata, Y. (2002) *J. Food Hyg. Soc. Jpn.* **43**, J275–J277

(4) Mamegoshi, S. (2002) *J. Food Hyg. Soc. Jpn.* **43**, J277–J279

(5) 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. (2003) *J. Food Hyg. Soc. Jpn.* **44**, 213–219

(6) 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. (2004) *J. Food Hyg. Soc. Jpn.* **45**, 120–127

(7) 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. (2004) *J. Food Hyg. Soc. Jpn.* **45**, 128–134

(8) 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. (2004) *J. Food Hyg. Soc. Jpn.* **45**, 313–318

(9) 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. (2004) *J. Food Hyg. Soc. Jpn.* **45**, 325–331

(10) Watanabe, Y., Aburatani, K., Mizumura, T., Sakai, M., Muraoka, S., Mamegosi, S., & Honjoh, T. (2005) *J. Immunol. Methods* **300**, 115–123

(11) Thompson, M., & Wood, R. (1993) *J. AOAC Int.* **76**, 926–940