AlerTox® ELISA Fish
KIT3060

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1. Scope

The AlerTox® ELISA Fish Kit is designed for the determination of fish residue in food. This report describes the validation process and its results.

2. Precision

2.1 Intra-Assay Variation

The intra-assay variation (Repeatability) was determined by testing three controls of various concentration levels in 20-fold replicates.

Table 1. Intra-Assay Variation of the AlerTox ELISA Fish Kit in Sample Diluent.

Replicate	Concentration Level 1	Concentration Level 2	Concentration Level 3	
1	3.82	9.62	38.64	
2	4.17	11.07	40.27	
3	4.10	11.07	38.60	
4	3.79	11.86	39.96	
5	3.30	10.54	37.89	
6	3.49	9.70	39.88	
7	3.69	11.57	37.89	
8	3.66	11.53	39.17	
9	3.98	11.28	37.85	
10	4.51	11.49	40.54	
11	3.56	10.74	37.51	
12	3.76	11.20	41.18	
13	3.53	10.99	34.17	
14	4.25	10.08	34.01	
15	4.97	10.87	33.68	
16	4.82	10.21	36.82	
17	3.46	10.25	33.60	
18	3.95	9.35	34.55	
19	4.21	9.70	34.75	
20	3.40	10.21	35.04	
Mean	3.92	10.67	35.46	
SD*	0.46	0.74	2.54	Mean
CV† [%]	**11.8**	**7.0**	**7.2**	**8.6**

* SD = standard deviation † CV = coefficient of variation

The coefficient of variation (CV) ranged from 7.2 to 11.8%, depending on the concentration tested.

2.2 Inter-Assay Variation

The inter-assay variation (Reproducibility) was determined by testing three controls of various concentration levels in four different test runs of the same kit lot.

Table 2. Inter-Assay Variation of the AlerTox ELISA Fish Kit in Sample Diluent.

Assay Number	Concentration Level 1	Concentration Level 2	Concentration Level 3	
1	8.28	33.43	75.84	
2	9.01	34.51	93.53	
3	10.34	33.70	78.96	
4	8.71	36.31	81.92	
Mean	9.09	34.48	82.56	
SD*	0.89	1.30	7.72	Mean
CV† [%]	**9.8**	**3.8**	**9.4**	**7.6**

* SD = standard deviation † CV = coefficient of variation

The coefficient of variation (CV) ranged from 3.8 to 9.8%, depending on the concentration tested.

3. Analytical Sensitivity

For determination of the analytical sensitivity, sample diluent and fish-free wine, soup, Worcestershire sauce, Asia sauce, cracker, surimi and spring roll samples were assayed in 24-fold replicates. After identification of possible outliers, the mean and standard deviation of the OD were calculated. The corresponding concentration of the mean OD + 3x standard deviation was defined as the limit of detection (LOD) (Tables 3a and 3b).

Table 3a. Matrix-Independent and Matrix-Dependent Analytical Sensitivity of the AlerTox ELISA Fish Kit.

Replicate	Sample Diluent [OD]	Wine Matrix [OD]	Soup Matrix [OD]	Worcestershire Sauce Matrix [OD]
1	0.124	0.124	0.124	0.103
2	0.112	0.145	0.140	0.096
3	0.124	0.104	0.107	0.098
4	0.135	0.127	0.132	0.096
5	0.147	0.117	0.114	0.099
6	0.150	0.114	0.104	0.096
7	0.122	0.125	0.111	0.099
8	0.139	0.110	0.117	0.089
9	0.133	0.117	0.118	0.102
10	0.116	0.135	0.121	0.108
11	0.136	0.104	0.100	0.091
12	0.140	0.112	0.116	0.100
13	0.167	0.114	0.116	0.098
14	0.143	0.107	0.109	0.098
15	0.130	0.117	0.107	0.091
16	0.122	0.112	0.115	0.093
17	0.135	0.121	0.126	0.099
18	0.135	0.118	0.097	0.092
19	0.144	0.136	0.136	0.109
20	0.141	0.116	0.106	0.102
21	0.121	0.107	0.110	0.098
22	0.121	0.103	0.100	0.095
23	0.118	0.108	0.105	0.108
24	0.118	0.128	0.142	0.100
Mean	0.132	0.118	0.116	0.098
SD*	0.0130	0.0109	0.0125	0.0053
LOD† [ppm]	**1.4 ppm**	**1.5 ppm**	**1.3 ppm**	**0.3 ppm**

* SD = standard deviation † LOD = limit of detection

Table 3b. Matrix-Dependent Analytical Sensitivity of the AlerTox ELISA Fish Kit.

Replicate	Asia Sauce Matrix [OD]	Cracker Matrix [OD]	Surimi Matrix [OD]	Spring Roll Matrix [OD]
1	0.109	0.104	0.217	0.198
2	0.097	0.100	0.178	0.196
3	0.100	0.139	0.182	0.182
4	0.106	0.126	0.198	0.190
5	0.095	0.105	0.191	0.182
6	0.088	0.099	0.185	0.178
7	0.095	0.106	0.174	0.173
8	0.090	0.100	0.186	0.179
9	0.104	0.116	0.172	0.176
10	0.112	0.101	0.182	0.178
11	0.096	0.107	0.180	0.172
12	0.096	0.114	0.239	0.180
13	0.091	0.103	0.186	0.178
14	0.086	0.095	0.172	0.172
15	0.087	0.093	0.182	0.196
16	0.109	0.097	0.177	0.191
17	0.106	0.112	0.187	0.177
18	0.107	0.103	0.174	0.192
19	0.143	0.100	0.173	0.213
20	0.096	0.110	0.187	0.182
21	0.122	0.114	0.170	0.179
22	0.090	0.113	0.205	0.184
23	0.094	0.100	0.173	0.181
24	0.101	0.100	0.177	0.192
Mean	0.101	0.107	0.185	0.184
SD*	0.0126	0.0103	0.0159	0.0099
LOD† [ppm]	**2.1 ppm**	**0.5 ppm**	**1.8 ppm**	**1.3 ppm**

* SD = standard deviation † LOD = limit of detection

The limits of detection varied from 0.3 to 2.1 ppm based on the sample matrix. Note that the derived limits of detection were strictly dependent on the coefficient of variation and thus, may vary in each individual test. The data for sample diluent and matrices were not determined in the same test runs.

The lowest positive standard (4 ppm) was defined as the limit of quantification (LOQ) to ensure that all uncontaminated matrices result in concentrations lower than this value.

4. Recovery

Food Samples

For recovery experiments, different sample matrices were spiked with cod to obtain a range of final concentrations after performing all sample pre-treatment steps. Tested samples and results are shown in Table 4.

Table 4. Recovery of Various Samples Tested with the AlerTox ELISA Fish Kit.

Wine (red)

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	10.7	107
40 ppm	39.4	98
100 ppm	104.4	104
	Mean	103

Soup

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	11.9	119
40 ppm	46.4	116
100 ppm	115.9	116
	Mean	117

Worcestershire Sauce

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	11.5	115
40 ppm	41.3	103
100 ppm	116.8	117
	Mean	112

Asia Sauce

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	11.2	112
40 ppm	37.5	94
100 ppm	103.3	103
	Mean	103

Cracker

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	10.6	106
40 ppm	36.0	90
100 ppm	102.2	102
	Mean	99

Surimi

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	11.1	111
40 ppm	48.4	121
100 ppm	110.2	110
	Mean	114

Spring Roll

Spiked Value	Measured Concentration [ppm]	Recovery [%]
10 ppm	9.7	97
40 ppm	36.0	90
100 ppm	92.4	92
	Mean	93

Mean recoveries ranged from 93 to 117%, depending on the sample matrix.

5. Linearity

Linearity was determined by spiking wine, soup, Worcestershire sauce, Asia sauce, cracker, surimi and spring roll samples with cod and testing subsequent dilutions of the resulting extracts (Table 5). For calculation of the linearity, the highest concentration was defined as the reference value (100%), and further dilutions were expressed as the percentage of this reference after considering the dilution factor.

Table 5. Matrix-Dependent Linearity of the AlerTox ELISA Fish Kit.

Wine (red)

Spiked Value	Measured Concentration [ppm]	Recovery [%]
100 ppm	104.4	100
50 ppm	51.2	98
25 ppm	31.0	119
12.5 ppm	13.0	100
6.25 ppm	7.0	107
	Mean	105

Soup

Spiked Value	Measured Concentration [ppm]	Recovery [%]
100 ppm	115.9	100
50 ppm	72.3	125
25 ppm	29.1	100
12.5 ppm	15.0	104
6.25 ppm	6.3	87
	Mean	103

Worcestershire Sauce

Spiked Value	Measured Concentration [ppm]	Recovery [%]
100 ppm	116.8	100
50 ppm	53.7	92
25 ppm	26.8	92
12.5 ppm	12.9	89
6.25 ppm	6.9	95
	Mean	92

Asia Sauce

Spiked Value	Measured Concentration [ppm]	Recovery [%]
100 ppm	103.3	100
50 ppm	54.2	105
25 ppm	26.3	102
12.5 ppm	10.6	82
6.25 ppm	5.3	81
	Mean	93

Cracker

Spiked Value	Measured Concentration [ppm]	Recovery [%]
100 ppm	102.2	100
50 ppm	55.6	109
25 ppm	24.0	94
12.5 ppm	10.3	81
6.25 ppm	4.6	72
	Mean	89

Surimi

Spiked Value	Measured Concentration [ppm]	Recovery [%]
100 ppm	110.2	100
50 ppm	60.1	109
25 ppm	26.5	96
12.5 ppm	10.4	75
6.25 ppm	5.5	79
	Mean	90

Spring Roll

Spiked Value	Measured Concentration [ppm]	Recovery [%]
100 ppm	92.4	100
50 ppm	50.2	109
25 ppm	24.6	106
12.5 ppm	10.5	91
6.25 ppm	5.5	96
	Mean	100

For different matrices, the mean linearity ranged from 89 to 105%. The linearity was independent of the specific fish concentration and may have been affected by the intra-assay and inter-assay variation, as stated in Section 2.

6. Cross-Reactivity

During testing, no cross-reactivity was detected in the following foods:

Table 6. Non-Cross-Reactive Food Matrices in the AlerTox ELISA Fish Kit.

Raw material	Concentration [ppm]	Raw material	Concentration [ppm]	Raw material	Concentration [ppm]
Almond	0.0	Hazelnut	0.0	Pork	0.3*
Barley	0.0	Lamb	0.0	Potato	0.0
Bean	0.3*	Macadamia	0.0	Pumpkin seed	0.0
Beef	0.0	Milk	0.0	Rice	0.7*
Brazil nut	0.0	Millet	0.0	Rye	0.0
Buckwheat	0.0	Mustard	0.0	Scallop	0.0
Carrot	0.0	Oat	0.0	Sesame	0.0
Cashew	0.2*	Onion	0.0	Shrimp	0.0
Celery	0.0	Pea	0.0	Soy	0.0
Corn	0.0	Peanut	0.0	Sunflower seed	0.0
Egg	0.0	Pecan	0.0	Walnut	0.0
Fish gelatin	0.0	Pistachio	0.0	Wheat	0.0

* This matrix is not considered cross-reactive based on LOQ <4 ppm. (Note: the LOD of this kit is 1.4 ppm.)

The following cross-reactivities were determined:

Table 7. Cross-Reactive Food Matrices in the AlerTox ELISA Fish Kit.

Raw material	Cross-reactivity [%]
Squid	0.001
Chicken	0.0004
Isinglass (fish gelatin)	0.0005

7. Robustness

Robustness was determined by varying some of the handling parameters defined in the instruction manual. The results obtained under various conditions were compared to the results obtained by following the instruction manual. An unspiked wine sample and a sample spiked with 50 ppm cod were analyzed.

7.1 Variation of Extraction Temperature

The extraction temperature, defined as 60 °C, was changed to 25 °C, 40 °C and 70 °C.

Table 8. Variation of Extraction Temperature in the AlerTox ELISA Fish Kit.

Sample	Result 60 °C	Result 25 °C	Result 40 °C	Result 70 °C
Wine, 0 ppm	0.0 ppm	0.3 ppm	0.1 ppm	0.0 ppm
Wine, 50 ppm	54.6 ppm	58.1 ppm	55.4 ppm	47.6 ppm

Considering the intra-assay, inter-assay and inter-extraction variations, the results did not differ significantly.

7.2 Variation of Extraction Time

The extraction time, defined as 15 min, was changed to 10 min and 20 min.

Table 9. Variation of Extraction Time in the AlerTox ELISA Fish Kit.

Sample	Result 15 min	Result 10 min	Result 20 min
Wine, 0 ppm	0.0 ppm	0.2 ppm	0.3 ppm
Wine, 50 ppm	54.5 ppm	46.5 ppm	50.2 ppm

Considering the intra-assay and inter-assay variation, the results did not differ significantly.

7.3 Drift

In contrast to the test procedure defined in the instruction manual, the incubation time of the samples was extended and reduced by 5 minutes compared to the calibrators (20 min).

Table 10. Drift in the AlerTox ELISA Fish Kit.

Sample	Result 20 min	Result 25 min	Result 15 min
White wine, 0 ppm	0.0 ppm	0.0 ppm	0.3 ppm
White wine, 50 ppm	48.8 ppm	53.7 ppm	40.7 ppm

The results differed significantly. Drift in extensive test runs should be avoided by pipetting calibrators once before and once after the samples and using the mean value for the calculation.

8. Application for Other Fish Species

The standards were prepared from fresh cod. To calculate the corresponding concentrations of other fish species, the amount of determined cod must be multiplied by a fish-specific conversion factor. The cross-reactivities of other fish species were determined relative to fresh cod. Fish-specific conversion factors were determined by extraction, serial dilution and determination of the corresponding fresh cod concentration. The following conversion factors have been determined:

Table 11. Cross Conversion factors in the AlerTox ELISA Fish Kit.

Fish	Conversion Factor	Fish	Conversion Factor	Fish	Conversion Factor
Bass	5.0	Herring (smoked)	13	Samlet	1.7
Carp	2.6	Mackerel (smoked)	50	Sardine	101
Catfish	1.7	Perch	5.2	Shark catfish	4.2
Coalfish	3.0	Pike	0.3	Spined loach	32
Devilfish	274	Plaice	2.4	Swordfish	1250
Eel	29	Red mullet	7.5	Trout	2.0
Flounder	7.1	Redfish	103	Tuna	370
Haddock	21	Red Snapper	29	Turbot	27
Hake	12	Salmon	1.7	Zander	11