

Validation Report

AlerTox ELISA Sesame

KIT3051/KT-5908

INDEX

1. Scope	2
2. Precision	2
A) Intra-Assay Variation	2
B) Inter-Assay Variation.....	3
3. Recovery	3
4. Analytical Sensitivity.....	4
5. Linearity	6
6. Cross-Reactivity	8
7. Robustness	8

1. Scope

The AlerTox ELISA Sesame is designed for the determination of sesame in food. The present report describes the validation process and its results.

2. Precision

A) Intra-Assay Variation

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

Table 1: Intra-assay variation of the AlerTox ELISA Sesame

Replicate	Level 1	Level 2	Level 3	
1	5.73	17.6	42.4	
2	5.45	17.6	30.8	
3	5.43	16.1	32.9	
4	5.26	15.9	43.5	
5	5.77	16.7	40.2	
6	5.29	19.1	43.1	
7	5.36	18.1	36.4	
8	5.31	17.4	39.2	
9	5.94	16.2	32.4	
10	4.84	16.1	36.1	
11	5.24	17.8	36.0	
12	5.27	18.4	29.7	
13	5.08	16.1	38.0	
14	5.72	17.5	29.0	
15	5.29	15.2	39.6	
16	5.21	14.5	35.4	
17	5.30	17.4	33.1	
18	5.86	17.5	33.9	
19	5.37	16.7	39.2	
20	4.99	16.8	29.0	
Mean	5.38	16.9	36.0	
SD	0.29	1.11	4.60	Mean
CV [%]	5.4	6.5	12.8	8.2

The coefficient of variation is ranging from 5.4% to 12.8% depending on the concentration.

B) Inter-Assay Variation

The inter-assay variation 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 Sesame

Assay No.	Level 1	Level 2	Level 3	
1	6.34	20.4	31.8	
2	5.52	17.0	33.1	
3	5.53	17.6	32.6	
4	6.12	16.4	34.6	
Mean	5.88	17.8	33.1	
SD	0.42	1.77	1.18	Mean
VK [%]	7.1	9.9	3.6	6.9

The coefficient of variation is ranging from 3.6% to 9.9% depending on the concentration.

3. Recovery

For recovery experiments different sample matrices were spiked with sesame to obtain various final concentrations after performing all sample pre-treatment steps. Tested samples and results were as follows.

Table 3: Recovery of various samples tested with the AlerTox ELISA Sesame

Soup

Target Value	Actual Concentration	Recovery [%]
5 ppm	5.41	108
15 ppm	19.5	130
40 ppm	36.7	92
	Mean	110

Ice-cream

Target Value	Actual Concentration	Recovery [%]
5 ppm	4.75	95
15 ppm	13.4	89
40 ppm	28.1	70
	Mean	85

Sausage

Target Value	Actual Concentration	Recovery [%]
5 ppm	4.12	82
15 ppm	15.0	100
40 ppm	37.2	93
	Mean	92

Salad Sauce

Target Value	Actual Concentration	Recovery [%]
5 ppm	4.99	100
15 ppm	16.4	109
40 ppm	28.2	71
	Mean	93

Cracker

Target Value	Actual Concentration	Recovery [%]
5 ppm	6.22	124
15 ppm	14.2	94
40 ppm	43.9	110
	Mean	109

Mean recoveries are ranging from 85% to 110% depending on the sample matrix.

4. Analytical Sensitivity

For determination of the analytical sensitivity sample diluent and sesame free soup, ice-cream, sausage, salad sauce and cracker samples respectively were assayed in 24fold replicates. After identification of possible outliers the OD mean and standard deviation were calculated. The corresponding concentration of the OD mean + 3x standard deviation was defined as limit of detection. This results in limits of detection according to the following table:

Table 4: Matrix-dependent and matrix-independent analytical sensitivity of the AlerTox ELISA Sesame

Replicate	Sample diluent [OD]	Biscuit matrix [OD]	Ice-cream matrix [OD]	Sausage matrix [OD]	Salad Sauce matrix [OD]	Cracker matrix [OD]
1	0.132	0.110	0.122	0.139	0.105	0.073
2	0.140	0.096	0.119	0.100	0.090	0.071
3	0.114	0.099	0.109	0.116	0.090	0.065
4	0.120	0.110	0.109	0.120	0.126	0.072
5	0.134	0.113	outlier	0.124	0.115	0.079
6	0.135	0.111	0.107	0.122	0.129	0.073
7	0.158	0.113	0.104	0.133	0.099	0.073
8	0.133	0.119	0.110	0.118	0.094	0.075
9	0.128	0.106	0.122	0.123	0.106	0.068
10	0.128	0.106	0.118	0.118	0.087	0.062
11	0.168	0.100	0.101	0.118	0.084	0.059
12	0.122	0.110	0.113	0.118	0.096	0.064
13	0.137	0.107	0.110	0.118	0.093	0.066
14	0.146	0.107	0.113	0.126	outlier	0.064
15	0.126	0.111	0.107	0.116	0.083	0.070
16	0.163	0.103	0.106	0.116	0.087	0.071
17	0.127	0.114	0.127	0.127	0.095	0.074
18	0.139	0.104	0.120	0.104	0.112	0.065
19	0.150	0.101	0.118	0.108	0.093	0.063
20	0.165	outlier	0.111	0.115	0.097	0.070
21	0.157	0.118	0.108	0.113	0.093	0.068
22	0.183	0.122	0.108	0.120	0.091	0.063
23	0.133	0.121	0.117	0.115	0.092	0.067
24	0.133	0.110	0.107	0.108	0.093	0.071
Mean	0.140	0.109	0.112	0.118	0.098	0.069
SD	0.017	0.007	0.007	0.009	0.012	0.005
Limit of Detection	0.17 ppm	0.12 ppm	0.01 ppm	0.06 ppm	0.04 ppm	0.01 ppm

With respect to the sample matrix limits of detection vary from 0.01 to 0.17 ppm. Note that the derived limits of detection are strictly dependent on the coefficient of variation and may thus vary in every individual test. The data for sample diluent and matrices respectively were not determined in the same test runs.

The lowest positive standard (2 ppm) was defined as limit of quantification to assure that all important matrices like milk, egg, wheat, rye, oats and barley result in concentrations lower than this value.

5. Linearity

Linearity was determined by spiking soup, ice-cream, sausage, salad sauce and cracker samples with sesame and testing subsequent dilutions of the resulting extracts. For calculation of the linearity the highest concentration was defined as reference value (100%) and further dilutions were expressed in percent of this reference after consideration of the dilution factor.

Table 5: Matrix dependent linearity of the AlerTox ELISA Sesame

Soup

Target Value	Concentration [ppm]	Recovery [%]
60 ppm	59.4	100
30 ppm	21.7	73
15 ppm	12.4	83
7.5 ppm	6.12	82
3.75 ppm	3.25	88
	Mean [%]	82

Ice-cream

Target Value	Concentration [ppm]	Recovery [%] 1)
60 ppm	64.0	100
30 ppm	29.4	92
15 ppm	17.6	110
7.5 ppm	9.95	124
3.75 ppm	5.17	129
	Mean [%]	114

Sausage

Target Value	Concentration [ppm]	Recovery [%] 1)
60 ppm	60.5	100
30 ppm	24.9	82
15 ppm	14.2	94
7.5 ppm	7.39	98
3.75 ppm	4.03	107
	Mean [%]	95

Salad Sauce

Target Value	Concentration [ppm]	Recovery [%] 1)
60 ppm	49.9	100
30 ppm	27.6	111
15 ppm	15.2	122
7.5 ppm	7.78	125
3.75 ppm	3.85	123
	Mean [%]	120

Cracker

Target Value	Concentration [ppm]	Recovery [%] 1)
60 ppm	30.12	100
30 ppm	11.14	74
15 ppm	6.89	92
7.5 ppm	4.09	109
3.75 ppm	2.32	123
	Mean [%]	99

For different matrices the mean linearity is ranging from 82% to 120%. The linearity is independent of the specific concentration and may only be affected by the intra-assay and inter-assay variation as stated in chapter 2.

6. Cross-Reactivity

For the following foods no cross-reactivity (results < LOQ) could be detected:

Table 6: Non-cross-reactive food matrices in the AlerTox ELISA Sesame

Almond	Chickpea	Hazelnut	Pistachio
Barley	Chili	Isinglass	Plum
Bean, white	Cocoa	Kiwi	Poppy seed
Beef	Coconut	Lamb	Pork
Bovine gelatin	Cod	Lentil	Potato
Brazil nut	Corn	Lupin	Pumpkin seed
Buckwheat	Cow's milk	Macadamia	Rice
Caraway	Crab, cooked	Mustard	Rye
Carob gum	Crab, raw	Nutmeg	Saccharose
Carrot	Cress	Oats	Shrimp, cooked
Cashew	Cumin	Paprika	Shrimp, raw
Celery	Egg	Pea	Soy flour
Cherry	Fish gelatin	Peanut	Soy lecithin
Chervil	Gliadin	Pecan	Tomato
Chestnut	Goat's milk	Pepper	Walnut
Chicken	Guar gum	Pine seed	Wheat

The following cross-reactivities could be determined:

Table 7: Cross-reactive food matrices in the AlerTox ELISA Sesame

Food	Cross-reactivity [%]
Chia	0.36
Cayenne	0.0006
Onion	0.0007
Sunflower seeds	0.0003

7. Robustness

Robustness was determined by variation of different handling parameters as defined in the instruction manual. The results were compared with the results of samples analyzed according to the intended method. An unspiked soup sample and a sample spiked with 15 ppm of sesame were analyzed respectively.

Variation of extraction temperature

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

Table 8: Variation of extraction temperature in the AlerTox ELISA Sesame

Sample	Result 60 °C	Result 25 °C	Result 40 °C	Result 70 °C
Soup 0 ppm	0.82 ppm	0.94 ppm	0.83 ppm	0.81 ppm
Soup 15 ppm	13.3 ppm	10.5 ppm	12.4 ppm	14.3 ppm

Under consideration of the intra-assay and inter-assay variations as stated in chapter 2 the results do not differ significantly.

Variation of extraction time

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

Table 9: Variation of extraction time in the AlerTox ELISA Sesame

Sample	Result 15 min	Result 10 min	Result 20 min
Soup 0 ppm	0.82 ppm	1.00 ppm	0.91 ppm
Soup 15 ppm	13.3 ppm	12.6 ppm	11.5 ppm

Under consideration of the intra-assay and inter-assay variation as stated in chapter 2, the results do not differ significantly.

Drift

In contrast to the test procedure as 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 Sesame

Sample	Result 20 min	Result 15 min	Result 25 min
Soup 0 ppm	0.82 ppm	0.25 ppm	1.00 ppm
Soup 15 ppm	13.3 ppm	8.23 ppm	16.4 ppm

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