Validation Report

AlerTox ELISA Coconut

KIT3056/KT-6285

INDEX

1. Scope

The AlerTox ELISA Coconut is designed for the determination of coconut 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 based on measured ppm of the AlerTox ELISA Coconut

Replicate	**Level 1** [ppm]	**Level 2** [ppm]	**Level 3** [ppm]	
1	4.67	13.0	30.6	
2	4.60	13.1	30.2	
3	5.07	13.4	30.2	
4	4.98	14.0	28.5	
5	5.04	14.4	30.5	
6	4.40	13.7	31.1	
7	4.62	14.1	31.4	
8	5.14	13.7	30.9	
9	4.92	13.5	27.5	
10	4.86	13.4	28.2	
11	4.41	13.3	30.0	
12	4.90	14.9	30.3	
13	4.86	14.3	29.8	
14	4.66	13.9	29.0	
15	4.90	14.8	30.1	
16	4.81	12.9	30.3	
17	4.62	13.8	29.0	
18	4.92	14.5	29.1	
19	4.78	14.1	28.6	
20	4.81	13.8	28.5	
Mean	4.80	13.8	30.0	
SD	0.20	0.57	1.06	**RMS**
CV [%]	**4.2**	**4.1**	**3.5**	**4.0**

The coefficient of variation is ranging from 3.5% to 4.2% depending on the concentration.

RMS = Root Mean Square

Table 2: Intra-assay variation based on measured OD values of the AlerTox ELISA Coconut

Replicate	Level 1 OD_{450nm}	Level 2 OD_{450nm}	Level 3 OD_{450nm}	
1	0.362	0.814	1.662	
2	0.358	0.821	1.646	
3	0.383	0.838	1.647	
4	0.346	0.869	1.570	
5	0.359	0.886	1.660	
6	0.389	0.852	1.684	
7	0.376	0.873	1.698	
8	0.373	0.854	1.675	
9	0.347	0.842	1.524	
10	0.375	0.836	1.554	
11	0.373	0.831	1.637	
12	0.361	0.911	1.651	
13	0.375	0.884	1.627	
14	0.370	0.923	1.592	
15	0.359	0.861	1.639	
16	0.376	0.908	1.651	
17	0.371	0.809	1.590	
18	0.385	0.855	1.595	
19	0.368	0.892	1.573	
20	0.380	0.870	1.567	
Mean	0.369	0.859	1.622	
SD	0.012	0.032	0.048	**RMS**
CV [%]	**3.2**	**3.8**	**3.0**	**3.4**

The coefficient of variation is ranging from 3.0% to 3.8% 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 3: Inter-assay variation based on measured ppm of the AlerTox ELISA Coconut

Assay No.	**Level 1** [ppm]	**Level 2** [ppm]	**Level 3** [ppm]	
1	4.16	13.1	24.9	
2	4.54	13.3	23.7	
3	5.09	15.3	27.0	
4	4.09	12.7	25.3	
Mean	4.47	13.6	25.2	
SD	0.46	1.17	1.39	**RMS**
CV [%]	**10.2**	**8.6**	**5.5**	**8.3**

The coefficient of variation is ranging from 5.5% to 10.2% depending on the concentration.

Table 4: Inter-assay variation based on OD values of the AlerTox ELISA Coconut

Assay No.	**Level 1** OD_{450nm}	**Level 2** OD_{450nm}	**Level 3** OD_{450nm}	
1	0.396	0.987	1.613	
2	0.410	0.958	1.563	
3	0.451	1.115	1.818	
4	0.410	1.006	1.700	
Mean	0.417	1.017	1.674	
SD	0.024	0.069	0.112	**RMS**
CV [%]	**5.7**	**6.7**	**6.7**	**6.4**

The coefficient of variation is ranging from 5.7% to 6.7% depending on the concentration.

3. Recovery

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

Table 5: Recovery of various samples tested with the AlerTox ELISA Coconut

Cookies

Target Value	Actual Concentration	Recovery [%]
5 ppm	4.69	94
15 ppm	13.4	89
30 ppm	27.9	93
	Mean	92

Cornflakes

Target Value	Actual Concentration	Recovery [%]
5 ppm	4.62	92
15 ppm	16.6	111
30 ppm	31.0	103
	Mean	102

Ice-cream

Target Value	Actual Concentration	Recovery [%]
5 ppm	3.44	69
15 ppm	11.2	74
30 ppm	23.8	79
	Mean	74

Chocolate

Target Value	Actual Concentration	Recovery [%]
5 ppm	4.57	91
15 ppm	12.6	84
30 ppm	25.7	86

	Mean	87

Sausage

Target Value	**Actual Concentration**	**Recovery [%]**
5 ppm	3.51	70
15 ppm	12.7	85
30 ppm	25.1	84
	Mean	80

Mean recoveries are ranging from 74% to 102% depending on the sample matrix.

4. Analytical Sensitivity

For determination of the analytical sensitivity sample diluent and coconut-free cookies, cornflakes, ice-cream, chocolate and sausage 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 6: Matrix-dependent and matrix-independent analytical sensitivity of the AlerTox ELISA Coconut

Replicate	Sample diluent [OD]	Cookie matrix [OD]	Cornflakes matrix [OD]	Ice-cream matrix [OD]	Chocolate matrix [OD]	Sausage matrix [OD]
1	0.100	0.051	0.061	0.130	0.159	0.076
2	0.114	0.059	0.067	0.126	0.155	0.067
3	0.092	0.051	0.063	0.132	0.139	0.081
4	0.099	0.057	0.068	0.133	0.131	0.082
5	0.083	0.057	0.067	0.131	0.114	0.057
6	outlier	0.055	0.059	0.124	0.088	0.057
7	0.114	0.047	0.059	0.126	0.085	0.064
8	0.078	0.055	0.058	0.131	0.090	0.067
9	0.103	0.048	0.060	0.119	0.160	0.064
10	0.100	0.051	0.063	0.125	0.140	0.059
11	0.100	0.050	0.058	0.121	0.138	0.060
12	0.091	0.056	0.065	0.118	0.137	0.062
13	0.099	0.052	0.066	0.147	0.123	0.055
14	0.084	0.047	0.057	0.126	0.102	0.067
15	0.081	0.055	0.058	0.129	0.084	0.059
16	0.078	0.051	0.068	0.131	0.093	0.077

17	0.095	0.067	0.072	0.113	0.161	0.085
18	0.095	0.066	0.069	0.121	0.160	0.054
19	0.102	0.057	0.070	0.126	0.131	0.053
20	0.099	0.058	0.069	0.115	0.129	0.059
21	0.088	0.077	0.072	0.117	0.121	0.064
22	0.080	0.060	0.075	0.120	0.098	0.059
23	0.082	0.063	0.068	0.120	0.112	0.061
24	0.096	0.065	0.070	0.122	0.093	0.066
Mean	**0.094**	**0.056**	**0.065**	**0.125**	**0.123**	**0.065**
SD	**0.010**	**0.007**	**0.005**	**0.007**	**0.026**	**0.009**
Limit of Detection	**0.38 ppm**	**0.43 ppm**	**0.17 ppm**	**0.28 ppm**	**0.46 ppm**	**0.44 ppm**

With respect to the sample matrix limits of detection vary from 0.17 to 0.46 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 (LOQ) to assure that all uncontaminated matrices result in concentrations lower than this value.

5. Linearity

Linearity was determined by spiking cookies, cornflakes, ice-cream, chocolate and sausage samples with coconut 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 7: Matrix dependent linearity of the AlerTox ELISA Coconut

Cookies

Target Value	Concentration [ppm]	Recovery [%]
30 ppm	29.2	100
15 ppm	15.1	103
7.5 ppm	8.17	112
3.75 ppm	3.66	100
1.875 ppm	1.82	99
	Mean [%]	104

Cornflakes

Target Value	Concentration [ppm]	Recovery [%]
30 ppm	30.0	100
15 ppm	15.6	104
7.5 ppm	8.27	110
3.75 ppm	4.09	109
1.875 ppm	1.86	99
	Mean [%]	102

Ice-cream

Target Value	Concentration [ppm]	Recovery [%]
30 ppm	28.1	100
15 ppm	15.0	107
7.5 ppm	8.12	116
3.75 ppm	4.24	121
1.875 ppm	2.14	122
	Mean [%]	116

Chocolate

Target Value	Concentration [ppm]	Recovery [%]
30 ppm	21.8	100
15 ppm	13.5	123
7.5 ppm	7.27	133
3.75 ppm	3.48	128
1.875 ppm	1.58	116
	Mean [%]	125

Sausage

Target Value	Concentration [ppm]	Recovery [%]
30 ppm	25.1	100
15 ppm	12.3	98
7.5 ppm	6.77	108
3.75 ppm	3.41	109
1.875 ppm	1.45	93
	Mean [%]	102

For different matrices the mean linearity is ranging from 102% to 125%. 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

No cross-reactivities could be determined.

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

Table 8: Non-cross-reactive food matrices in the AlerTox ELISA Coconut

Raw material	c [ppm]
Almond	0.00
Apricot	0.00
Barley	0.64
Bean. white	0.00
Beef	0.13
Brazil nut	0.21
Buckwheat	0.00
Carob gum	0.00
Carrot	0.00
Cashew	0.00
Celery	0.00
Cherry	0.17
Chervil	0.00
Chestnut	0.17
Chick pea	0.00
Chicken	0.00
Cocoa	0.00
Cod	0.00
Corn	0.13
Cress	0.33
Egg	0.00
Egg white powder	0.00
Ewe's milk	0.00
Gelatin	0.00
Gliadin	0.00
Goat's milk	1.51
Guar flour	0.00
Hazelnut	0.00
Isinglass	0.95

Raw material	c [ppm]
Lentil	0.48
Lupin	0.00
Macadamia nut	0.00
Milk	0.22
Mustard	0.02
Oats	0.20
Pea	0.00
Peach	0.00
Peanut	0.00
Pecan	0.76
Pine seed	0.11
Pistachio	0.04
Plum	0.20
Poppy seed	0.37
Pork	1.24
Potato	1.11
Pumpkin seed	0.39
Rice	0.64
Rye	0.49
Sucrose	0.00
Sesame	1.30
Shrimp. cooked	0.00
Shrimp. raw	0.00
Soy	0.12
Soy lecithin	0.31
Sunflower seed	0.64
Tofu	0.10
Tomato	0.00
Walnut	1.21

Kiwi	0.00	Wheat	0.41

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 cookie sample and a sample spiked with 10 ppm of coconut were analyzed respectively.

A) Variation of extraction temperature

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

Table 9: Variation of extraction temperature in the AlerTox ELISA Coconut

Sample	Result 60 °C	Result 25 °C	Result 40 °C	Result 70 °C
Cookie 0 ppm	0.0 ppm	0.0 ppm	0.0 ppm	0.1ppm
Cookie 10 ppm	7.8 ppm	8.1 ppm	8.0 ppm	7.6 ppm

Under consideration of the intra-assay and inter-assay variations, the results do not differ significantly.

B) Variation of extraction time

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

Table 10: Variation of extraction time in the AlerTox ELISA Coconut

Sample	Result 15 min	Result 10 min	Result 20 min
Cookie 0 ppm	0.0 ppm	0.0 ppm	0.0 ppm
Cookie 10 ppm	10.9 ppm	9.5 ppm	9.1 ppm

Under consideration of the intra-assay and inter-assay variations, the results do not differ significantly.

C) Drift

In contrast to the test procedure as defined in the instruction manual the incubation time of the samples was extended and reduced by 4 minutes compared to the calibrators (20 min).

Table 11: Drift in the AlerTox ELISA Coconut

Sample	Result 20 min	Result 16 min	Result 24min
Cookie 0 ppm	0.0 ppm	0.0 ppm	0.0 ppm
Cookie 10 ppm	8.8 ppm	7.2 ppm	10.9 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.

Date: Apr 2015