Validation Report

AlerTox ELISA BLG

KIT3042/KT-5919

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1. Scope

The AlerTox ELISA BLG is designed for the determination of ß-lactoglobulin 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 two controls of various concentration levels in 20fold replicates.

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

Replicate	Level 1	Level 2	
1	11.1	89.0	
2	12.0	96.5	
3	10.7	86.4	
4	11.9	79.7	
5	12.2	90.9	
6	10.2	86.4	
7	11.0	85.9	
8	10.9	86.4	
9	10.2	83.8	
10	10.5	83.4	
11	10.3	77.0	
12	10.7	98.6	
13	10.3	96.1	
14	9.1	83.6	
15	10.0	95.5	
16	9.5	89.1	
17	11.5	92.3	
18	11.4	92.7	
19	11.0	98.3	
20	11.3	99.0	
Mean	10.8	89.5	
SD	0.80	6.48	**RMS**
CV [%]	**7.5**	**7.2**	**7.4**

The coefficient of variation is ranging from 7.2% to 7.5% depending on the concentration.

RMS = Root Mean Square

B) Inter-Assay Variation

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

Table 2: Inter-assay variation of the AlerTox ELISA BLG

Assay No.	Level 1	Level 2	
1	11.8	84.8	
2	11.3	93.6	
3	9.6	72.4	
4	10.7	76.5	
Mean	10.8	81.8	
SD	0.96	9.39	RMS
CV [%]	**8.8**	**11.5**	**10.2**

The coefficient of variation is ranging from 8.8% to 11.5% depending on the concentration.

3. Recovery

For recovery experiments different sample matrices were spiked with ß-lactoglobulin 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 BLG

Cornflakes

Target Value	Actual Concentration	Recovery [%]
10 ppb	10.0	100
100 ppb	87.4	87
	Mean	**94**

Cookies

Target Value	Actual Concentration	Recovery [%]
10 ppb	9.4	94
100 ppb	81.6	82
	Mean	**88**

Sausage

Target Value	Actual Concentration	Recovery [%]
10 ppb	12.8	128

100 ppb	84.8	85
Mean		**107**

Chocolate

Target Value	Actual Concentration	Recovery [%]
10 ppb	9.1	91
100 ppb	80.9	81
Mean		**86**

White wine

Target Value	Actual Concentration	Recovery [%]
10 ppb	9.4	94
100 ppb	70.0	70
Mean		**82**

Soy milk

Target Value	Actual Concentration	Recovery [%]
10 ppb	7.2	72
100 ppb	69.0	69
Mean		**70**

Orange juice

Target Value	Actual Concentration	Recovery [%]
10 ppb	10.4	104
100 ppb	92.4	92
Mean		**98**

Mean recoveries are ranging from 70% to 107% depending on the sample matrix.

4. Analytical Sensitivity

For determination of the analytical sensitivity sample diluent was assayed in 24fold replicates. After identification of possible outliers the OD mean and standard deviation was 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-independent analytical sensitivity of the AlerTox ELISA BLG

Replicate	Sample diluent [OD]
1	0.106
2	0.062
3	0.066
4	0.064
5	0.077
6	0.064
7	0.069
8	0.059
9	0.080
10	0.070
11	0.084
12	0.069
13	0.071
14	0.063
15	0.064
16	0.068
17	0.070
18	0.069
19	0.081
20	0.069
21	0.086
22	0.063
23	0.061
24	0.065
Mean	0.071
SD	0.010
Limit of Detection	**1.5 ppb**

The limit of detection is 1.5 ppb of ß-lactoglobulin. The lowest positive standard (10 ppb) was defined as limit of quantification.

5. Linearity

Linearity was determined by spiking orange juice, white wine, soy milk, cornflakes, cookies, sausage and chocolate samples with ß-lactoglobulin 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 per cent of this reference after consideration of the dilution factor.

Table 5: Matrix dependent linearity of the AlerTox ELISA BLG

Orange juice

Target Value	Concentration [ppb]	Recovery [%]
400 ppb	389	100
200 ppb	266	137
100 ppb	96.1	99
50 ppb	68.4	141
25 ppb	23.6	97
	Mean [%]	**118**

White wine

Target Value	Concentration [ppb]	Recovery [%]
400 ppb	395	100
200 ppb	236	119
100 ppb	94.4	96
50 ppb	52.7	107
25 ppb	24.7	100
	Mean [%]	**106**

Soy milk

Target Value	Concentration [ppb]	Recovery [%]
400 ppb	409	100
200 ppb	249	122
100 ppb	130	127
50 ppb	45.3	89
25 ppb	24.7	97
	Mean [%]	**109**

Cornflakes

Target Value	Concentration [ppb]	Recovery [%]
400 ppb	337	100
200 ppb	200	119
100 ppb	101	120
50 ppb	40.0	95
25 ppb	20.8	99
	Mean [%]	**108**

Cookies

Target Value	Concentration [ppb]	Recovery [%]
400 ppb	410	100

200 ppb	228	111
100 ppb	94.9	93
50 ppb	51.3	100
25 ppb	27.8	109
	Mean [%]	**103**

Sausage

Target Value	Concentration [ppb]	Recovery [%]
400 ppb	383	100
200 ppb	234	122
100 ppb	98.5	103
50 ppb	54.3	114
25 ppb	27.2	114
	Mean [%]	**113**

Chocolate

Target Value	Concentration [ppb]	Recovery [%]
400 ppb	326	100
200 ppb	208	127
100 ppb	88.3	108
50 ppb	54.2	133
25 ppb	28.1	138
	Mean [%]	**127**

For different matrices the mean linearity is ranging from 103% to 127%. The linearity is independent of the specific concentration and may only be affected by the intra-assay and inter-assay variation.

6. Cross-Reactivity

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

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

Egg	Corn	Chick pea	Chestnut
Wheat	Buckwheat	Walnut	Soy lecithin
Rye	Poppy seed	Bovine serum albumin	Peach
Barley	Sesame	Pork	Apricot
Oats	Sunflower seed	Pecan nut	Cherry
Cocoa	Pumpkin seed	Brazil nut	Plum
Orange	Pine seed	Coconut	Sucrose
Soy	Cashew	Almond	Pea
Wine	Peanut	Pistachio	Bean
Rice	Hazelnut	Macadamia nut	Beef

Chicken			

The following cross-reactivities were determined:

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

Food	Cross-reactivity [%]
Ewe's milk	< 0.2
Goat's milk	< 0.002
Casein	< 0.02

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 un-spiked cornflakes sample and a sample spiked with 100 ppb of ß-lactoglobulin 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 8: Variation of extraction temperature in the AlerTox ELISA BLG

Sample	Result 60 °C	Result 25 °C	Result 40 °C	Result 70 °C
Cornflakes 0 ppb	0 ppb	0 ppb	0 ppb	0 ppb
Cornflakes 100 ppb	100 ppb	107 ppb	95 ppb	87 ppb

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

B) 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 BLG

Sample	Result 15 min	Result 10 min	Result 20 min
Cornflakes 0 ppb	0 ppb	0 ppb	0 ppb
Cornflakes 100 ppb	102 ppb	103 ppb	79 ppb

The results differ significantly. The incubation time should not exceed 15 min.

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 10: Drift in the AlerTox ELISA BLG

Sample	Result 20 min	Result 16 min	Result 24min
Cornflakes 0 ppb	0 ppb	0 ppb	0 ppb
Cornflakes 100 ppb	100 ppb	73 ppb	102 ppb

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.

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