Validation Report

AlerTox ELISA Pistachio

KIT3054/KT-5917

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1. Scope

The AlerTox ELISA Pistachio is designed for the determination of pistachio 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 Pistachio

Replicate	Level 1	Level 2	Level 3	
1	4.8	8.8	37.3	
2	3.7	8.6	34.7	
3	4.1	8.9	32.5	
4	3.6	8.2	35.6	
5	3.9	7.3	38.7	
6	4.0	7.9	34.1	
7	4.0	9.3	35.0	
8	4.2	8.3	37.2	
9	3.6	8.9	35.4	
10	4.1	9.3	35.2	
11	3.8	9.2	35.7	
12	3.5	8.6	37.4	
13	4.0	8.2	41.3	
14	4.1	9.1	35.9	
15	3.9	7.5	40.5	
16	4.0	8.5	39.6	
17	3.5	7.5	37.5	
18	4.6	8.1	35.0	
19	4.2	8.0	36.3	
20	4.0	7.1	36.9	
Mean	4.0	8.4	36.6	
SD	0.3	0.7	2.2	**RMS**
CV [%]	**8.1**	**8.0**	**6.0**	**7.4**

The coefficient of variation is ranging from 6.0% to 8.1% depending on the concentration.

RMS = Root Mean Square

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 Pistachio

Assay No.	Level 1	Level 2	Level 3	
1	4.5	9.0	32.7	
2	4.3	10.0	38.7	
3	4.5	11.0	41.8	
4	4.0	8.4	36.1	
Mean	4.3	9.6	37.3	
SD	0.2	1.1	3.8	**RMS**
CV [%]	**5.2**	**11.9**	**10.3**	**9.6**

The coefficient of variation is ranging from 5.2% to 11.9% depending on the concentration.

3. Recovery

For recovery experiments different sample matrices were spiked with pistachio 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 Pistachio

Cookies

Target Value	Actual Concentration	Recovery [%]
4 ppm	3.4	85
10 ppm	10.2	102
40 ppm	37.8	94
	Mean	**94**

Cornflakes

Target Value	Actual Concentration	Recovery [%]
4 ppm	3.6	89
10 ppm	10.0	100
40 ppm	38.9	97
	Mean	**96**

Ice-cream

Target Value	Actual Concentration	Recovery [%]
4 ppm	4.0	100
10 ppm	8.2	82
40 ppm	34.1	85
	Mean	**89**

Chocolate

Target Value	Actual Concentration	Recovery [%]
4 ppm	3.1	78
10 ppm	8.4	84
40 ppm	32.5	81
	Mean	**81**

Sausage

Target Value	Actual Concentration	Recovery [%]
4 ppm	3.6	89
10 ppm	9.3	93
40 ppm	35.1	88
	Mean	**90**

Mean recoveries are ranging from 81% to 96% depending on the sample matrix.

4. Analytical Sensitivity

For determination of the analytical sensitivity sample diluent and pistachio 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 4: Matrix-dependent and matrix-independent analytical sensitivity of the AlerTox ELISA Pistachio

Replicate	Sample diluent [OD]	Cookie matrix [OD]	Cornflakes matrix [OD]	Ice-cream matrix [OD]	Chocolate matrix [OD]	Sausage matrix [OD]
1	0.041	0.034	0.039	0.052	0.033	0.021
2	0.048	0.035	0.034	0.047	0.028	0.021
3	0.051	0.030	0.029	0.043	0.030	0.019
4	0.045	0.045	0.040	0.068	0.028	0.020
5	0.047	0.035	0.037	0.047	0.033	0.023
6	0.037	0.035	0.034	0.042	0.028	0.021
7	0.039	0.035	0.037	0.041	0.029	0.022
8	0.064	0.037	0.031	0.043	0.032	0.024
9	0.040	0.039	0.038	0.049	0.034	0.032
10	0.039	0.040	0.036	0.044	0.035	0.020
11	0.040	0.034	0.028	0.037	0.028	0.018
12	0.049	0.071	0.039	0.059	0.054	0.021
13	0.038	0.041	0.038	0.045	0.033	0.026
14	0.038	0.045	0.035	0.040	0.029	0.022
15	0.040	0.038	0.038	0.043	0.032	0.023
16	0.039	0.034	0.030	0.041	0.028	0.026
17	0.037	0.039	0.036	0.048	0.033	0.021
18	0.046	0.034	0.034	0.039	0.031	0.018
19	0.049	0.032	0.030	0.033	0.030	0.018
20	0.047	0.055	0.039	0.057	0.048	0.020
21	0.037	0.035	0.040	0.045	0.035	0.024
22	0.037	0.037	0.031	0.044	0.030	0.023
23	0.045	0.033	0.033	0.046	0.032	0.026
24	0.050	0.034	0.037	0.036	0.035	0.027
Mean	**0.043**	**0.039**	**0.035**	**0.045**	**0.033**	**0.022**
SD	**0.006**	**0.009**	**0.004**	**0.008**	**0.006**	**0.003**
Limit of Detection	**0.13 ppm**	**0.18 ppm**	**0.01 ppm**	**0.20 ppm**	**0.09 ppm**	**0.17 ppm**

The limit of detection (LOD) is 0.13 ppm of pistachio. With respect to the sample matrix limits of detection vary from 0.01 to 0.20 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 (1 ppm) was defined as limit of quantification (LOQ).

5. Linearity

Linearity was determined by spiking cookie, cornflakes, ice-cream, chocolate and sausage samples with pistachio 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 Pistachio

Cookies

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	37.7	100
20 ppm	20.2	107
10 ppm	8.2	87
5 ppm	4.8	101
2.5 ppm	2.4	100
	Mean [%]	**99**

Cornflakes

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	37.9	100
20 ppm	19.9	105
10 ppm	7.8	83
5 ppm	3.9	81
2.5 ppm	2.1	90
	Mean [%]	**90**

Ice-cream

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	37.6	100
20 ppm	23.5	125
10 ppm	10.1	107
5 ppm	4.4	93
2.5 ppm	2.6	111
	Mean [%]	**109**

Chocolate

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	37.0	100
20 ppm	23.4	126
10 ppm	9.4	102
5 ppm	4.6	100
2.5 ppm	2.5	109
	Mean [%]	**109**

Sausage

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	35.1	100
20 ppm	21.7	123
10 ppm	9.8	111
5 ppm	5.0	113
2.5 ppm	2.5	115
	Mean [%]	**116**

For different matrices the mean linearity is ranging from 90% to 116%. The linearity is independent of the specific concentration and may only be affected by the intra-assay and inter-assay variation.

6. Cross-Reactivity

The following cross-reactivities could be determined:

Table 6: Cross-reactive food matrices in the AlerTox ELISA Pistachio

Raw material	Cross-reactivity
Cashew	12%
Hazelnut	0.17%
Walnut	0.0008%
Pecan	0.0005%
Sunflower seed	0.0002%

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

Table 7: Non-cross-reactive food matrices in the AlerTox ELISA Pistachio

Almond	Chicken	Macadamia	Rye
Apricot	Chickpea	Mustard	Sucrose
Barley	Cocoa	Oats	Sesame
Bean	Coconut	Pea	Shrimps, boiled
Beef	Cod	Peanut	Shrimps, un-boiled
Bovine gelatine	Corn	Pine seed	Soy
Brazil nut	Cow's milk	Plum	Soy lecithin
Carob gum	Egg	Poppy seed	Tomato
Carrot	Guar gum	Pork meat	Wheat
Celery	Kiwi	Potato	
Cherry	Lentil	Pumpkin seed	
Chestnut	Lupine	Rice	

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 cookie sample and a sample spiked with 10 ppm of pistachio 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 Pistachio

Sample	Result 60 °C	Result 25 °C	Result 40 °C	Result 70 °C
Cookie 0 ppm	0.06 ppm	0.03 ppm	0.08 ppm	0.05 ppm
Cookie 10 ppm	10.5 ppm	12,1 ppm	8,0 ppm	11.9 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 9: Variation of extraction time in the AlerTox ELISA Pistachio

Sample	Result 15 min	Result 10 min	Result 20 min

Cookies 0 ppm	0.06 ppm	0.05 ppm	0.03 ppm
Cookies 10 ppm	10.5 ppm	11.3 ppm	8.9 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 10: Drift in the AlerTox ELISA Pistachio

Sample	Result 20 min	Result 16 min	Result 24min
Cookies 0 ppm	0.06 ppm	0.00 ppm	0.00 ppm
Cookies 10 ppm	10.5 ppm	6.8 ppm	13.2 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.