AlerTox® ELISA Peanut
KIT3048

Index

1. Scope

The AlerTox® ELISA Peanut Kit is designed for the determination of peanut in food, based on a polyclonal antibody. 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 Peanut Kit in Sample Diluent.

Replicate	Level 1	Level 2	Level 3	
1	2.3	12.5	41.5	
2	2.4	12.3	46.3	
3	2.7	11.9	43.7	
4	2.7	13.0	44.4	
5	2.9	12.8	49.3	
6	2.7	12.6	42.1	
7	3.0	12.3	43.6	
8	3.0	12.7	39.4	
9	2.6	11.8	41.9	
10	2.6	12.3	42.0	
11	3.0	11.8	43.0	
12	3.1	12.7	46.2	
13	3.1	10.6	45.5	
14	2.7	10.7	38.6	
15	3.2	11.0	38.6	
16	3.1	10.0	37.6	
17	2.5	11.8	37.2	
18	3.2	12.2	38.8	
19	3.0	12.0	38.7	
20	3.2	12.9	39.3	
Mean	2.9	12.0	41.9	
SD*	0.29	0.84	3.37	Mean
CV† [%]	10.1	7.0	8.0	8.4

* SD = standard deviation † CV = coefficient of variation

The coefficient of variation (CV) ranges from 7.0 to 10.1%, depending on the concentration.

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 Peanut Kit in Sample Diluent.

Assay Number	Level 1	Level 2	Level 3	
1	2.4	12.3	46.7	
2	2.3	10.6	52.8	
3	2.4	10.0	44.0	
4	2.3	10.6	40.9	
Mean	2.4	10.9	46.1	
SD*	0.05	1.03	5.05	Mean
CV† [%]	2.2	9.5	11.0	7.5

* SD = standard deviation † CV = coefficient of variation

The coefficient of variation (CV) ranges from 2.2 to 11.0%, depending on the concentration.

3. Analytical Sensitivity

For determination of the analytical sensitivity (limit of detection, LOD), sample diluent and various representative peanut-free sample matrices were assayed in 24-fold 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 the limit of detection. This results in the limits of detection shown in Table 3.

Table 3. Matrix-Dependent and Matrix-Independent Analytical Sensitivity of the AlerTox ELISA Peanut Kit (complete data).

Replicate	Sample Diluent [OD]	Cookie Matrix [OD]	Ice Cream Matrix [OD]	Chocolate Matrix [OD]	Muesli Matrix [OD]	Hazelnut Matrix [OD]
1	0.039	0.022	0.072	0.033	0.047	0.041
2	0.038	0.027	0.081	0.034	0.041	0.026
3	0.034	0.028	0.074	0.036	0.052	0.028
4	0.033	0.028	0.047	0.031	0.044	0.032
5	0.033	0.030	0.041	0.034	0.039	0.035
6	0.028	0.026	0.052	0.031	0.039	0.034
7	0.033	0.023	0.044	0.032	0.044	0.040
8	0.048*	0.032	0.039	0.040	0.055	0.048*
9	0.033	0.024	0.039	0.030	0.040	0.029
10	0.028	0.023	0.044	0.040	0.040	0.031
11	0.031	0.025	0.050	0.033	0.050	0.028
12	0.041	0.025	0.040	0.031	0.043	0.032
13	0.029	0.027	0.040	0.033	0.052	0.035
14	0.029	0.019	0.050	0.029	0.040	0.038
15	0.033	0.024	0.043	0.032	0.042	0.035
16	0.034	0.018	0.052	0.043	0.043	0.033
17	0.039	0.022	0.040	0.032	0.044	0.035
18	0.033	0.020	0.042	0.035	0.039	0.031
19	0.036	0.035	0.043	0.032	0.040	0.030
20	0.031	0.022	0.044	0.038	0.041	0.033
21	0.028	0.025	0.039	0.037	0.049	0.031
22	0.025	0.017	0.041	0.031	0.046	0.032
23	0.027	0.019	0.040	0.029	0.049	0.037
24	0.035	0.019	0.055	0.036	0.042	0.036
Mean	0.033	0.024	0.046	0.034	0.044	0.034
SD	0.005	0.005	0.049	0.004	0.005	0.004
LOD [ppm]	0.08	0.06	0.25	0.24	0.25	0.14
0 ppm	0.027	0.027	0.024	0.022	0.024	0.021
1 ppm	0.261	0.223	0.163	0.119	0.163	0.194
4 ppm	0.762	0.684	0.547	0.340	0.547	0.591
10 ppm	1.440	1.298	1.118	0.738	1.118	1.170
40 ppm	2.664	2.538	2.268	1.712	2.268	2.268

* identified as an outlier by 1.5 IQR testing; not considered in the calculation.

With respect to the sample matrix, limits of detection vary from 0.06 to 0.25 ppm. Note that the derived limits of detection are 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 (1 ppm) was defined as the limit of quantification 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 peanut to obtain various 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 Peanut Kit.

Cookies

Target Value	Actual Concentration [ppm]	Recovery [%]
1 ppm	0.89	89
5 ppm	4.3	85
15 ppm	17.4	116
	Mean	97

Cornflakes

Target Value	Actual Concentration [ppm]	Recovery [%]
1 ppm	1.05	105
5 ppm	4.6	92
15 ppm	16.2	108
	Mean	102

Chocolate

Target Value	Actual Concentration [ppm]	Recovery [%]
1 ppm	0.67	67
5 ppm	4.7	94
15 ppm	18.9	126
	Mean	96

Ice Cream

Target Value	Actual Concentration [ppm]	Recovery [%]
1 ppm	0.99	99
5 ppm	4.2	84
15 ppm	14.3	95
	Mean	93

Hazelnut

Target Value	Actual Concentration [ppm]	Recovery [%]
1 ppm	0.85	85
5 ppm	4.9	98
15 ppm	14.2	95
	Mean	96

Mean recoveries range from 93 to 102%, depending on the sample matrix.

5. Linearity

Linearity was determined by spiking cookies, chocolate, cornflakes and ice cream samples with peanut and testing subsequent dilutions of the resulting extracts. For calculation of the linearity, the highest concentration was defined as the 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 Peanut Kit.

Cookies

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	39.25	100
20 ppm	18.89	96
10 ppm	10.39	106
5 ppm	4.61	94
2.5 ppm	2.39	97
	Mean	98

Chocolate

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	36.55	100
20 ppm	15.72	86
10 ppm	9.39	103
5 ppm	3.89	85
2.5 ppm	2.44	107
	Mean	95

Cornflakes

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	33.22	100
20 ppm	14.18	85
10 ppm	7.46	90
5 ppm	3.58	86
2.5 ppm	1.97	95
	Mean	89

Ice cream

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	32.84	100
20 ppm	18.12	110
10 ppm	7.62	93
5 ppm	4.10	100
2.5 ppm	2.36	115
	Mean	105

For different matrices, the mean linearity ranges from 85 to 115%. The linearity is independent of the specific concentration and may only be affected by the intra-assay and inter-assay variation as stated in Section 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 Peanut Kit.

Raw material	Concentration [ppm]	Raw material	Concentration [ppm]	Raw material	Concentration [ppm]
Adzuki bean	0.08	Egg	0.00	Peach	0.07
Almond	0.60	Fennel	0.08	Pecan	0.59
Apple	0.12	Fenugreek	0.52	Pepper, black	0.09
Apricot	0.00	Flaxseed	0.00	Pine seed	0.00
Barley	0.09	Garden cress	0.45	Pistachio	0.00
Bean, white	0.15	Garlic (fresh)	0.00	Poppy	0.22
Beef	0.00	Garlic (granulated)	0.00	Pork	0.02
Beef (cooked)	0.00	Gelatin, cow	0.12	Potato	0.00
Brazil nut	0.01	Ginger (ground)	0.13	Prawn (cooked)	0.07
Buckwheat	0.07	Ginger (fresh)	0.20	Prawn (raw)	0.13
Cabbage, white	0.00	Gliadin	0.05	Pumpkin seed	0.25
Caraway	0.11	Guar gum	0.07	Radish	0.00
Cardamom	0.14	Hazelnut	0.00	Rapeseed	0.07
Carob gum	0.90	Horseradish	0.06	Rice	0.01
Carrot	0.47	Kidney bean	0.44	Rye	0.08
Cashew	0.29	Kiwi	0.06	Saccharose	0.00
Celery	0.00	Lamb	0.02	Sesame	0.24
Cherry	0.23	Leek	0.31	Shrimps	0.00
Chestnut	0.03	Lentil	0.65	Soy flour	0.90
Chia	0.10	Lupin	0.19	Soy lecithin	0.00
Chicken	0.04	Macadamia	0.24	Split pea	0.25
Chickpea	0.18	Milk (powder)	0.15	Sunflower seed	0.04
Chili	0.00	Milk, cow	0.00	Thyme	0.24
Cinnamon	0.30	Milk, goat	0.00	Tofu	0.00
Coconut	0.00	Mustard	0.00	Tomato	0.01
Cod	0.04	Nutmeg	0.45	Turkey	0.00
Corn	0.32	Oats	0.20	Turmeric	0.08
Cumin	0.03	Onion	0.07	Walnut	0.04
Dill	0.09	Paprika	0.29	Wheat	0.00
Duck	0.15	Pea	0.05		

The following cross-reactivities could be determined:

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

Raw material	Concentration [ppm]	Cross-reactivity [%]
Cayenne	12.3	0.0012
Clove	1.3	0.0001
Cocoa	1.6	0.0002
Gum arabic	5.9	0.0006

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 cookie sample and a sample spiked with 10 ppm peanut extract were analyzed.

7.1 Variation of Extraction Temperature

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

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

Sample	Result 60 °C	Result 40 °C	Result 70 °C
Cookie 0 ppm	0 ppm	0 ppm	0 ppm
Cookie 10 ppm	11.3 ppm	12.5 ppm	9.1 ppm

Considering the precision data (Section 2), the results do not differ significantly.

7.2 Variation of Extraction Time

The extraction time, defined as 15 min, was changed to 5 min, 10 min and 20 min.

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

Sample	Result 15 min	Result 5 min	Result 10 min	Result 20 min
Cookies 0 ppm	0 ppm	0 ppm	0 ppm	0 ppm
Cookies 10 ppm	11.3 ppm	14.5 ppm	13.0 ppm	11.1 ppm

Considering the precision data (Section 2), the results do 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 4 minutes compared to the calibrators (20 min).

Table 10. Drift in the AlerTox ELISA Peanut Kit.

Sample	Result 20 min	Result 16 min	Result 24 min
Cookie 0 ppm	0 ppm	0 ppm	0 ppm
Cookie 10 ppm	9.7 ppm	7.9 ppm	10.8 ppm

Considering the intra-assay and inter-assay variations (Section 2), the results do not differ significantly. Drift in extensive test runs can be avoided by pipetting calibrators once before and once after the samples and using the mean value for the calculation.