AlerTox® ELISA Lupine Kit

Claim Support Summary

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1. Scope

The AlerTox® ELISA Lupine Kit is designed to determine lupine content in food. This report describes the validation process and its results.

Definitions

CV = Coefficient of variation

IQR = Interquartile range

LOD = Limit of detection

LOQ = Limit of quantification

OD = Optical density

ppm = parts per million = mg of whole lupine seed per kg or L of sample (mg/kg or mg/L)

PT = Proficiency testing

SD = Standard deviation

2. Precision

2.1 Intra-Assay Variation

The intra-assay variation (repeatability) was determined by testing a control at 3 concentrations (5, 15 and 30 ppm) in 20-fold replicates (Table 1).

Table 1. AlerTox ELISA Lupine Kit Intra-Assay Variation.

Replicate	5 ppm	15 ppm	30 ppm	
1	5.7	20	34	
2	6.1	18	36	
3	6.2	22	38	
4	6.2	19	37	
5	6.3	19	34	
6	6.3	20	35	
7	7.0	20	36	
8	5.7	16	31	
9	5.4	20	35	
10	5.7	20	39	
11	6.0	21	34	
12	5.6	21	35	
13	6.0	17	34	
14	6.2	19	32	
15	6.2	20	28	
16	6.1	18	29	
17	5.3	18	29	
18	5.8	20	30	
19	6.0	22	33	
20	6.3	21	31	
Mean	6.0	20	33	
SD	0.4	1.5	3.0	Mean
CV [%]	**6.1**	**7.8**	**9.1**	**7.7**

The coefficient of variation (CV) ranged from 6.1 to 9.1%, depending on the concentration tested.

2.2 Inter-Assay Variation

The inter-assay variation (intermediate precision) was determined by testing a control at 3 concentrations (5, 15 and 30 ppm) in 4 different test runs of the same kit lot (Table 2).

Table 2. AlerTox ELISA Lupine Kit Inter-Assay Variation.

Assay Number	5 ppm	15 ppm	30 ppm	
1	4.6	13	27	
2	5.1	13	22	
3	4.6	14	23	
4	4.6	14	25	
Mean	4.7	13	24	
SD	0.2	0.5	2.2	Mean
CV [%]	**5.1**	**4.1**	**9.3**	**6.6**

The CV ranged from 4.1 to 9.3%, depending on the concentration tested.

3. Analytical Sensitivity

To determine the analytical sensitivity of the AlerTox ELISA Lupine Kit, sample diluent (matrix independent) and various representative, lupine-free sample matrices (matrix dependent) were assayed in 24-fold replicates. The data from sample diluent and matrices were assessed in separate test runs.

After identifying possible outliers, the mean and standard deviation of the optical density (OD) were calculated. The corresponding concentration of the mean OD + 3X standard deviation (SD) was defined as the limit of detection (LOD) (Table 3).

Table 3. Matrix-Independent and Matrix-Dependent Analytical Sensitivity of the AlerTox ELISA Lupine Kit.

Replicate	Sample Diluent [OD]	Biscuit Matrix [OD]	Orange Juice Matrix [OD]	Sausage Matrix [OD]	Ketchup Matrix [OD]	Croquette Matrix [OD]
1	0.070	0.121	0.356	0.089	0.094	0.099
2	0.063	0.125	0.321	0.090	0.066	0.091
3	0.056	0.114	0.336	0.083	0.081	0.102
4	0.054	0.120	0.342	0.078	0.070	0.098
5	0.086	0.106	0.330	0.068	0.077	0.081
6	0.066	0.105	0.330	0.073	0.080	0.083
7	0.081	0.112	0.341	0.073	0.098	0.106
8	0.071	0.098	0.291	0.063	0.079	0.087
9	0.074	0.106	0.334	0.085	0.083	0.113
10	0.079	0.102	0.303	0.084	0.070	0.102
11	0.060	0.113	0.320	0.076	0.082	0.096
12	0.069	0.113	0.367	0.079	0.074	0.105
13	0.074	0.127	0.333	0.084	0.085	0.084
14	0.068	0.105	0.321	0.067	0.078	0.091
15	0.072	0.096	0.332	0.077	0.074	0.093
16	0.071	0.116	0.298	0.066	0.062	0.080
17	0.084	0.095	0.344	0.093	0.080	0.104
18	0.068	0.131	0.317	0.083	0.079	0.104
19	0.072	0.115	0.320	0.074	0.074	0.091
20	0.075	0.114	0.305	0.074	0.072	0.089
21	0.070	0.125	0.301	0.073	0.068	0.082
22	0.072	0.115	0.321	0.069	0.073	0.104
23	0.068	0.113	0.325	0.081	0.078	0.092
24	0.070	0.125	0.309	0.062	0.071	0.093
Mean	0.071	0.113	0.325	0.077	0.077	0.095
SD	0.008	0.010	0.018	0.009	0.008	0.009
LOD [ppm]	**0.21 ppm**	**0.25 ppm**	**0.69 ppm**	**0.16 ppm**	**0.06 ppm**	**0.23 ppm**
Mean LOD	—	**0.278 ppm**				

LODs vary from 0.21 ppm for sample diluent to an average of 0.278 ppm for matrices. Therefore, the LOD was set to 0.2 ppm. Note that the derived LODs are strictly dependent on the CV and thus may vary in individual tests.

The lowest positive standard (2 ppm) was defined as the limit of quantification (LOQ) to ensure that measurements of all uncontaminated matrices result in concentrations lower than this value.

4. Recovery

For recovery experiments, different sample matrices were spiked with lupine at 3 final concentrations (5, 15 and 30 ppm) after performing all sample pre-treatment steps. The tested samples and calculated results are shown in Table 4.

Table 4. Recovery of Various Samples Tested with the AlerTox ELISA Lupine Kit.

Biscuit

Spiked Value	Measured Concentration [ppm]	Recovery [%]
5 ppm	5.9	119
15 ppm	16.7	111
30 ppm	32.7	109
	Mean	113

Orange Juice

Spiked Value	Measured Concentration [ppm]	Recovery [%]
5 ppm	6.1	122
15 ppm	15.1	100
30 ppm	26.4	88
	Mean	104

Sausage

Spiked Value	Measured Concentration [ppm]	Recovery [%]
5 ppm	5.3	105
15 ppm	14.7	98
30 ppm	28.5	95
	Mean	99

Ketchup

Spiked Value	Measured Concentration [ppm]	Recovery [%]
5 ppm	5.4	109
15 ppm	14.0	93
30 ppm	27.4	91
	Mean	98

Croquette

Spiked Value	Measured Concentration [ppm]	Recovery [%]
5 ppm	6.4	127
15 ppm	16.3	109
30 ppm	28.9	96
	Mean	111

Mean recoveries ranged from 98 to 113%, depending on the sample matrix.

5. Linearity

Linearity was determined by spiking various matrices with lupine and testing serial dilutions (30 to 1.875 ppm) of the resulting matrices (Table 5). For calculating linearity, the highest concentration was defined as the reference value (100%), and further dilutions were expressed as a percentage of this reference after accounting for the dilution factor.

Table 5. Matrix-Dependent Linearity of the AlerTox ELISA Lupine Kit.

Biscuit

Spiked Value	Measured Concentration [ppm]	Recovery [%]
30 ppm	32.7	Reference* (100)
15 ppm	14.7	90
7.5 ppm	8.6	105
3.75 ppm	4.0	98
1.875 ppm	1.6	78
	Mean	93

Orange Juice

Spiked Value	Measured Concentration [ppm]	Recovery [%]
30 ppm	27.1	Reference* (100)
15 ppm	17.1	126
7.5 ppm	8.3	123
3.75 ppm	3.7	108
1.875 ppm	1.2	70
	Mean	107

Sausage

Spiked Value	Measured Concentration [ppm]	Recovery [%]
30 ppm	28.4	Reference* (100)
15 ppm	14.6	103
7.5 ppm	7.3	103
3.75 ppm	3.8	107
1.875 ppm	1.6	91
	Mean	101

* The highest concentration was defined as the reference value (100%). Further dilutions were expressed as a percentage of this reference after accounting for the dilution factor.

Ketchup

Spiked Value	Measured Concentration [ppm]	Recovery [%]
30 ppm	27.4	Reference* (100)
15 ppm	14.3	104
7.5 ppm	7.8	114
3.75 ppm	4.0	117
1.875 ppm	1.5	90
	Mean	106

Croquette

Spiked Value	Measured Concentration [ppm]	Recovery [%]
30 ppm	28.9	Reference* (100)
15 ppm	16.1	112
7.5 ppm	7.5	103
3.75 ppm	3.8	106
1.875 ppm	1.7	95
	Mean	104

* The highest concentration was defined as the reference value (100%). Further dilutions were expressed as a percentage of this reference after accounting for the dilution factor.

For different matrices, the mean linearity ranged from 93 to 107%. The linearity was independent of the specific concentration and may have been affected by the precision, as stated in Section 2.

6. Cross-Reactivity

No cross-reactivity is defined as a result that is less than the LOQ. For the AlerTox ELISA Lupine Kit, the LOQ is 2 ppm whole lupine seed. During testing, no cross-reactivity was detected in the foods included in Table 6.

Table 6. Non-Cross-Reactive Food Matrices with the AlerTox ELISA Lupine Kit.

Non-Cross-Reactive Matrices				
Apricot	Almond	Barley	Bean, white*	Beef (cooked)
Beef (raw)	Brazil nut	Buckwheat	Cabbage, white	Caraway
Cardamom	Carob gum	Carrot	Cashew	Cayenne*
Celery	Cherry*	Chestnut	Chia	Chicken
Chili	Coconut	Cod	Corn	Cumin
Dill	Duck	Egg	Fennel	Flaxseed
Garden cress	Garlic (fresh)	Garlic (granulated)	Gelatin, cow	Ginger (fresh)
Ginger (ground)*	Gliadin	Guar gum*	Gum arabic	Hazelnut*
Horseradish	Kiwi	Lamb	Leek	Macadamia
Milk, cow	Milk, goat	Mustard	Oats	Onion
Orange	Paprika	Pea	Peach	Peanut
Pepper, black	Pine seed	Pistachio	Poppy	Pork
Potato	Prawn (cooked)	Prawn (raw)	Pumpkin seed	Radish
Rapeseed	Rice	Rye	Saccharose	Sesame
Shrimps	Split pea*	Strawberry	Sunflower seed	Thyme*
Tofu		Tomato		Turkey
Turmeric		Walnut		Wheat

* Bean (white), cayenne, cherry, ginger (ground), guar gum, hazelnut, split pea and thyme showed results between 0.5 LOQ and 1 LOQ and may provide values above the LOQ.

The following cross-reactivities were determined (Table 7):

Table 7. Cross-Reactive Food Matrices with the AlerTox ELISA Lupine Kit.

Cross-Reactive Matrix	Percent Cross-Reactivity (%)
Soy flour	0.0065
Clove	0.0009
Fenugreek	0.0006
Cinnamon	0.0005
Adzuki bean	0.0003
Chickpea	0.0003
Cocoa	0.0003

Cross-Reactive Matrix	Percent Cross-Reactivity (%)
Kidney bean	0.0003
Lentil	0.0003
Nutmeg	0.0003
Pecan	0.0003
Plum	0.0003
Soy lecithin	0.0003

7. Robustness

Robustness was determined by the variation of different 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 biscuit sample and a sample spiked with 5 ppm lupine were analyzed.

7.1 Variation of Extraction Temperature

The extraction temperature, defined as 60 °C, was changed to 25, 40 and 70 °C (Table 8).

Table 8. Variation of Extraction Temperature with the AlerTox ELISA Lupine Kit.

Sample	Result, 60 °C	Result, 40 °C	Result, 70 °C
Biscuit, 0 ppm lupine	**0.15 ppm**	0.13 ppm	0.22 ppm
Biscuit, 5 ppm lupine	**4.47 ppm**	4.73 ppm	4.18 ppm

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

7.2 Variation of Extraction Time

The extraction time, defined as 15 min, was changed to 10 and 20 min (Table 9).

Table 9. Variation of Extraction Time with the AlerTox ELISA Lupine Kit.

Sample	Result, 15 min	Result, 10 min	Result, 20 min
Biscuit, 0 ppm lupine	**0.15 ppm**	0.26 ppm	0.15 ppm
Biscuit, 5 ppm lupine	**4.47 ppm**	5.13 ppm	5.41 ppm

Considering the precision data (Section 2), the results did *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 5 minutes compared to the calibrators (20 min) (Table 10).

Table 10. Drift in the AlerTox ELISA Lupine Kit.

Sample	Result, 20 min	Result, 15 min	Result, 25 min
Biscuit, 0 ppm lupine	**0.15 ppm**	0.02 ppm	0.05 ppm
Biscuit, 5 ppm lupine	**4.47 ppm**	2.85 ppm	6.55 ppm

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

8. Summary

- The AlerTox ELISA Lupine Kit is sensitive with a matrix-independent LOD of 0.2 ppm (mg of whole lupine seed per kg or L of sample) and a matrix-dependent LOD range of 0.06 to 0.69 ppm (mg/kg or mg/L). The LOQ is 2 ppm whole lupine seed (mg/kg or mg/L).
- The AlerTox ELISA Lupine Kit provides consistent, precise results, as indicated by results of the intra- and inter-assay studies (CV < 10%, Section 2).
- Robust recovery (Section 4) and linearity (Section 5) mean that samples and their dilutions are quantitated accurately.
- Extensive cross-reactivity studies showed high specificity (Section 6) with slight cross-reactivity with 13 matrices. Soy flour has the highest percent cross reactivity at 0.0065%, while those for the other matrices ranged from 0.0003 to 0.0009.
- **Important**: Drift in large test runs can be avoided by pipetting calibrators once before and once after the samples and using the calibrator mean values for the calculations.