Validation Report

AlerTox ELISA Ovalbumin

KIT3045/KT-5759

INDEX

1. Scope

The AlerTox ELISA Ovalbumin is designed for the determination of ovalbumin residues in wine and other food matrices. The present report describes the validation process for wine and its results.

2. Precision

A) Intra-Assay Variation

The intra-assay variation was determined by testing three samples of various concentration levels in 20fold replicates.

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

Replicate	Level 1	Level 2	Level 3	
1	51.4	188	356	
2	52.1	176	350	
3	49.5	175	347	
4	50.0	176	360	
5	51.0	176	357	
6	48.7	168	349	
7	50.2	177	357	
8	45.6	160	322	
9	51.9	177	343	
10	49.8	175	347	
11	49.8	171	344	
12	50.9	171	355	
13	50.0	176	362	
14	50.7	167	349	
15	49.1	174	353	
16	47.9	158	327	
17	53.0	172	354	
18	52.6	171	346	
19	51.0	171	339	
20	52.6	174	349	
Mean	50.4	173	348	
SD	1.77	6.44	10.1	**RMS**
CV [%]	**3.5**	**3.7**	**2.9**	**3.4**

The coefficient of variation is ranging from 2.9% to 3.7% depending on the concentration.

RMS = Root Mean Square

B) Inter-Assay Variation

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

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

Assay No.	Level 1	Level 2	Level 3	
1	51.7	170	317	
2	49.8	164	321	
3	50.7	168	327	
4	47.8	170	305	
Mean	50.0	168	318	
SD	1.63	2.71	9.69	**RMS**
CV [%]	**3.3**	**1.6**	**3.1**	**2.8**

The coefficient of variation is ranging from 1.6% to 3.3% depending on the concentration.

3. Recovery

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

Table 3a: Recovery of various wine samples tested with the AlerTox ELISA Ovalbumin

Sample / Target Value	Actual Concentration [ppb]	Recovery [%]
White wine 1 (50 ppb)	54.3	109
White wine 1 (150 ppb)	172	115
White wine I (300 ppb)	318	106
Red wine 1 (50 ppb)	54.5	109
Red wine 1 (150) ppb	163	109
Red wine 1 (300 ppb)	291	97
White wine 2 (100 ppb)	103	103
Rosé wine (100 ppb)	104	104
Red wine 2 (100 ppb)	95.2	95
	Mean	105

Table 3b: Concentration of non-spiked wine samples tested with the AlerTox ELISA Ovalbumin

Sample	Actual Concentration [ppb]
White wine 1	0.0
Red wine 1	1.2
White wine 2	2.2
Rosé wine	2.5
Red wine 2	2.1

The mean recovery for wine is 105%.

4. Analytical Sensitivity

For determination of the analytical sensitivity sample diluent and ovalbumin free wine, 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 Ovalbumin

Replicate	Sample Diluent [OD]	White Wine Matrix [OD]	Red Wine Matrix [OD]
1	0.069	0.017	0.024
2	0.061	0.019	0.023
3	0.063	0.025	0.023
4	0.068	0.024	0.033
5	0.071	0.029	0.033
6	0.064	0.030	0.040
7	0.066	0.030	0.028
8	0.061	0.041	0.018
9	0.067	0.024	0.024
10	0.061	0.024	0.025
11	0.069	0.024	0.024
12	0.064	0.023	0.026
13	0.066	0.026	0.016
14	0.066	0.040	0.028
15	0.062	0.027	0.036
16	0.058	0.026	0.019
17	0.055	0.017	0.024
18	0.054	0.025	0.029
19	0.064	0.029	0.038
20	0.075	0.030	0.034
21	0.053	0.042	0.029
22	0.069	0.029	0.028

Replicate	Sample Diluent [OD]	White Wine Matrix [OD]	Red Wine Matrix [OD]
23	0.064	0.027	0.037
24	0.075	0.033	0.017
Mean	**0.064**	**0.028**	**0.027**
SD	**0.00578**	**0.00654**	**0.00672**
LOD	**4.4 ppb**	**< 0 Std.**	**< 0 Std.**

With respect to the sample matrix, limits of detection vary from 0 to 4.4 ppb. 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 determined in the same test runs.

The lowest positive standard (25 ppb) was defined as limit of quantification to assure that all uncontaminated matrices result in concentrations lower than this value.

5. Linearity

Linearity was determined by spiking wine samples with ovalbumin 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 Ovalbumin

White Wine

Target Value	Concentration [ppb]	Recovery [%]
400 ppb	386	100
200 ppb	185	96
100 ppb	90.8	94
50 ppb	44.9	93
25 ppb	20.7	86
	Mean	94

Red Wine

Target Value	Concentration [ppb]	Recovery [%]
400 ppb	400	100
200 ppb	209	104
100 ppb	99.2	99
50 ppb	46.6	93
25 ppb	20.4	82
	Mean	96

For different wine matrices the mean linearity is ranging from 94% to 96%. The linearity may be affected by the intra-assay and inter-assay variation.

6. Cross-Reactivity

Since ovalbumin is an egg white protein, some potentially cross-reactive egg white proteins as well as other fining reagents were tested for cross-reactivity. The following cross-reactivities could be determined:

Table 6: Cross-reactivity of proteins and fining reagents in the AlerTox ELISA Ovalbumin

Protein	Cross-reactivity [%]
Lysozyme	< 0.02
Ovomucoid	< 0.02
Conalbumin	< 0.2
Whole Egg Powder	15
Non Fat Dry Milk	0
Fish	0
Bovine Gelatin	0

Since it was not clear that the tested egg proteins were absolutely pure, it cannot be excluded that the minor cross-reactivities of lysozyme, ovomucoid and conalbumin are a result of an ovalbumin contamination in the tested material.

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 red wine sample and a sample spiked with 100 ppb of ovalbumin were analyzed respectively.

Drift

In contrast to the test procedure as 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 7: Drift in the AlerTox ELISA Ovalbumin

Sample	Result 20 min	Result 25 min	Result 15 min
Red Wine 0 ppb	0 ppb	0 ppb	0 ppb
Red Wine 100 ppb	92.1 ppb	101 ppb	66.6 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.

Date: Dec 2014