Validation Report

AlerTox ELISA Lysozyme
KIT3044/KT-5757

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1. Scope

The AlerTox ELISA Lysozyme is designed for the determination of Lysozyme 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 samples of various concentration levels in 20-fold replicates.

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

Replicate	Level 1	Level 2	Level 3	
1	56.9	108	250	
2	53.6	108	224	
3	52.8	104	229	
4	54.8	110	240	
5	54.4	108	244	
6	51.4	102	216	
7	61.6	103	234	
8	48.0	108	210	
9	54.0	106	251	
10	53.0	106	228	
11	53.7	110	223	
12	55.1	110	238	
13	55.3	105	226	
14	53.5	106	218	
15	52.5	103	231	
16	52.7	105	198	
17	54.8	107	225	
18	53.5	108	224	
19	55.3	105	210	
20	55.2	104	221	
Mean	54.1	106	227	
SD	2.5	2.5	13.6	**Mean**
CV [%]	**4.7**	**2.3**	**6.0**	**4.3**

The coefficient of variation is ranging from 2.3% to 6.0% depending on the concentration.

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 Lysozyme

Assay No.	Level 1	Level 2	Level 3	
1	49.9	98.2	184	
2	50.4	101	181	
3	51.8	103	192	
4	49.7	103	184	
Mean	50.5	101	185	
SD	0.9	2.3	4.9	Mean
CV [%]	1.8	2.3	2.6	2.2

The coefficient of variation is ranging from 1.8% to 2.6% depending on the concentration.

3. Recovery

3.1 Wine

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

Table 3.1a: Recovery of various wine samples tested with the AlerTox ELISA Lysozyme

Sample / Target Value	Actual Concentration [ppb]	Recovery [%]
White wine 1 (50 ppb)	62.1	124
White wine 1 (100 ppb)	117	117
White wine 1 (200 ppb)	228	114
Red wine 1 (50 ppb)	44.2	88
Red wine 1 (100 ppb)	96.2	96
Red wine 1 (200 ppb)	185	92
White wine 2 (100 ppb)	86.0	86
Rosé wine (100 ppb)	90.3	90
Red wine 2 (100 ppb)	86.5	87
Mean		99

Table 3.1b: Concentration of non-spiked wine samples tested with the AlerTox ELISA Lysozyme

Sample	Actual Concentration [ppb]
White wine 1	0.4
Red wine 1	0.0
White wine 2	0.0
Rosé wine	0.0
Red wine 2	0.0

The mean recovery for wine is 99%.

3.2 Cheese

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

Table 3: Recovery of various cheese samples tested with the AlerTox ELISA Lysozyme

Camembert, 35% fat in dry weight

Target Value	Actual Concentration	Recovery [%]
250 ppb	195	78
500 ppb	363	73
1000 ppb	803	80
Mean		77

Maasdamer, 45% fat in dry weight

Target Value	Actual Concentration	Recovery [%]
250 ppb	253	101
500 ppb	451	90
1000 ppb	1065	107
Mean		99

Soft cheese, 50% fat in dry weight

Target Value	Actual Concentration	Recovery [%]
250 ppb	214	86
500 ppb	378	76
1000 ppb	802	80
	Mean	80

Table 3.1b: Concentration of non-spiked cheese samples tested with the AlerTox ELISA Lysozyme

Sample	Actual Concentration [ppb]
Camembert, 35% fat	0.0
Maasdamer, 45% fat	0.0
Soft cheese, 50% fat	0.0

The mean recovery for cheese is 85%.

4. Analytical Sensitivity

4.1 LOD

For determination of the analytical sensitivity sample diluent and lysozyme-free wine and cheese 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 Lysozyme

Replicate	Sample Diluent [OD]	White Wine Matrix [OD]	Red Wine Matrix [OD]	Camembert Matrix [OD]	Maasdamer Matrix [OD]	Soft Cheese Matrix [OD]
1	0.056	0.040	0.048	0.005	0.007	0.006
2	0.040	0.041	0.045	0.003	0.004	0.004
3	0.064	0.046	0.038	0.004	0.004	0.003
4	0.042	0.040	0.045	0.006	0.007	0.004
5	0.038	0.056	0.045	0.007	0.006	0.006
6	0.038	0.047	0.044	0.004	0.005	0.004
7	0.040	0.047	0.047	0.006	0.007	0.005
8	0.042	0.043	0.041	0.009	0.007	0.005
9	0.050	0.043	0.040	0.006	0.005	0.004
10	0.043	0.046	0.049	0.004	0.003	0.003
11	0.043	0.044	0.048	0.003	0.003	0.004
12	0.042	0.048	0.044	0.006	0.004	0.004
13	0.048	0.063	0.048	0.006	0.005	0.004
14	0.040	0.048	0.043	0.006	0.008	0.004
15	0.040	0.053	0.043	0.009	0.003	0.004
16	0.042	0.053	0.047	0.005	0.005	0.007
17	0.052	0.057	0.053	0.004	0.006	0.005
18	0.048	0.051	0.047	Outlier	0.004	0.003
19	0.044	0.046	0.043	0.008	0.003	0.003
20	0.042	0.044	0.040	0.007	0.004	0.005
21	0.056	0.046	0.048	0.005	0.005	0.005
22	0.039	0.043	0.042	0.007	0.004	0.005
23	0.040	0.049	0.046	0.009	0.005	0.004
24	0.050	0.045	0.041	0.009	0.007	0.007
Mean	**0.045**	**0.047**	**0.045**	**0.006**	**0.005**	**0.005**
SD	**0.0067**	**0.00578**	**0.00358**	**0.00191**	**0.00152**	**0.00114**
Limit of Detection	**2.3 ppb**	**2.2 ppb**	**1.2 ppb**	**3.0 ppb**	**2.5 ppb**	**2.0 ppb**

With respect to the sample matrix, limits of detection vary from 1.2 to 3.0 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 not determined in the same test runs.

4.2 LOQ

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

5.1 Wine

Linearity was determined by spiking wine samples with lysozyme 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 percent of this reference after consideration of the dilution factor.

Table 5.1: Matrix dependent linearity of the AlerTox ELISA Lysozyme for wine samples

White Wine

Target Value	Concentration [ppb]	Recovery [%]
250 ppb	249	100
125 ppb	120	96
62.5 ppb	53.5	86
31.25 ppb	23.7	76
15.625 ppb	17.9	115
Mean		95

Red Wine

Target Value	Concentration [ppb]	Recovery [%]
250 ppb	239	100
125 ppb	111	93
62.5 ppb	50.5	84
31.25 ppb	23.5	79
15.625 ppb	11.8	79
Mean		87

For different wine matrices the mean linearity is ranging from 87% to 95%. The linearity may be affected by the intra-assay and inter-assay variation as stated in chapter 2.

5.2 Cheese

Linearity was determined by spiking cheese samples with lysozyme 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 percent of this reference after consideration of the dilution factor.

Table 5.2: Matrix dependent linearity of the AlerTox ELISA Lysozyme for cheese samples

Camembert, 35% fat in dry weight

Target Value	Concentration [ppb]	Recovery [%]
1000 ppb	803	100
500 ppb	389	97
250 ppb	196	98
125 ppb	100	99
62.5 ppb	48	96
Mean		97

Maasdamer, 45% fat in dry weight

Target Value	Concentration [ppb]	Recovery [%]
1000 ppb	1065	100
500 ppb	533	100
250 ppb	270	101
125 ppb	143	107
62.5 ppb	55	83
Mean		98

Soft Cheese, 50% fat in dry weight

Target Value	Concentration [ppb]	Recovery [%]
1000 ppb	802	
500 ppb	384	96
250 ppb	189	94
125 ppb	93	93
62.5 ppb	42	84
Mean		92

For different cheese matrices the mean linearity is ranging from 92% to 98%. The linearity may be affected by the intra-assay and inter-assay variation as stated in chapter 2.

6. Cross-Reactivity

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

Table 6.1: Non-cross-reactive food matrices in the the AlerTox ELISA Lysozyme

Raw material	c [ppb]	Raw material	c [ppb]
Adzuki	0.0	Linseed	0.0
Almond	0.4	Lupin	0.0
Apricot	0.0	Macadamia	0.0
Barley	0.0	Mustard	5.2
Bean, white	1.8	Nutmeg	0.0
Beef	0.0	Oats	0.5
Bovine gelatin	2.1	Onion	9.9
Brazil nut	1.0	Paprika	1.0
Buckwheat	3.0	Pea	0.6
Caraway	0.0	Peach	0.2
Carob gum	0.0	Peanut	0.7
Carrot	0.0	Pecan	0.0
Cayenne	0.8	Pepper	1.4
Celery	1.8	Pine seed	0.3
Cherry	0.0	Pistachio	0.0
Chestnut	1.4	Poppy seed	0.7
Chia	19.6	Pork	5.1
Chickpea	0.0	Potato	0.0
Chili	1.7	Prawn, cooked	0.0
Cocoa	2.0	Prawn, raw	0.0
Coconut	6.6	Pumpkin seed	1.4
Cod	0.0	Rice	0.0
Corn	0.1	Rye	0.0
Cow's milk	17.8	Saccharose	0.0
Cumin	1.9	Sesame	1.2
Duck	2.8	Shrimps	1.4
Fenugreek	2.4	Soy	0.5
Gliadin	6.4	Soy lecithin	0.0
Goat's milk	0.0	Split peas	0.0
Guar gum	2.6	Sunflower seeds	0.0
Hazelnut	0.0	Tomato	0.5
Kidney bean	0.0	Turkey	10.8
Kiwi	2.0	Walnut	0.6
Lamb	2.7	Wheat	0.0
Lentil	0.0		

The following cross-reactivities could be determined:

Table 6.2: Cross-reactive food matrices in the AlerTox ELISA Lysozyme

Raw material	Cross-reactivity [%]
Ovalbumin	< 0.0001
Ovomucoid	< 0.0001
Conalbumin	< 0.0001
Cashew	<0.000005%
Chicken	<0.000006%

Since it was not clear that the tested egg proteins were absolutely pure, it cannot be excluded that the minor cross-reactivities of ovalbumin, ovomucoid and conalbumin are a result of a lysozyme 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 unspiked red wine sample and a sample spiked with 100 ppb of lysozyme were analyzed respectively.

7.1 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 Lysozyme

Sample	Result 20 min	Result 25 min	Result 15 min
Red Wine 0 ppb	0 ppb	0 ppb	0 ppb
Red Wine 100 ppb	90.9 ppb	99.8 ppb	75.4 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.