AlerTox® ELISA Histamine
KIT3065

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1. Scope

The AlerTox® ELISA Histamine kit is designed for the determination of histamine in fish and wine. 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 20-fold replicates.

Table 1: Intra-assay variation of the AlerTox® ELISA Histamine

Replicate	Level 1		Level 2		Level 3	
	OD	[ppm]	OD	[ppm]	OD	[ppm]
1	1.670	3.2	1.255	4.3	0.611	21.4
2	1.648	3.3	1.128	4.9	0.592	22.2
3	1.615	3.5	1.188	4.6	0.631	20.6
4	1.564	3.8	1.138	4.9	0.615	21.3
5	1.159	3.7	1.157	4.8	0.615	21.3
6	1.690	3.0	1.049	5.3	0.593	22.2
7	1.612	3.5	1.028	5.4	0.630	20.7
8	1.521	4.1	1.163	4.8	0.593	22.2
9	1.592	3.7	1.142	4.9	0.668	19.1
10	1.619	3.5	1.087	5.1	0.593	22.2
11	1.603	3.6	1.170	4.7	0.637	20.4
12	1.647	3.3	1.090	5.1	0.615	21.3
13	1.590	3.7	1.037	5.4	0.656	19.6
14	1.513	4.2	1.139	4.9	0.574	22.9
15	1.601	3.6	1.123	5.0	0.551	23.9
16	1.620	3.5	1.183	4.7	0.569	23.1
17	1.533	4.0	1.134	4.9	0.657	19.6
18	1.517	4.1	1.179	4.7	0.629	20.7
19	1.579	3.7	1.118	5.0	0.607	21.6
20	1.639	3.4	1.239	4.4	0.577	22.8
Mean	1.577	3.6	1.137	4.9	0.611	21.5
SD	0.110	0.3	0.060	0.3	0.031	1.3
CV [%]	**7.0**	**8.7**	**5.3**	**5.9**	**5.1**	**5.9**

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 Histamine

	Level 1	Level 2	Level 3
Assay No.	**[ppm]**	**[ppm]**	**[ppm]**
1	3.2	6.7	36.2
2	3.1	7.5	34.7
3	3.1	8.5	39.0
4	3.1	7.8	30.6
Mean	3.1	7.6	35.1
SD	0.1	0.7	3.5
CV [%]	**1.6**	**9.8**	**10.0**

The coefficient of variation ranges from 1.6% to 10% depending on the concentration.

3. Recovery

For recovery experiments, different sample matrices were spiked with histamine 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 Histamine

Sample	Target Value	Actual Concentration	Recovery
Trout	3 ppm	2.8 ppm	**96%**
	10 ppm	9.7 ppm	
	40 ppm	39.6 ppm	
Codfish	3 ppm	2.6 ppm	**102%**
	10 ppm	11.5 ppm	
	40 ppm	41.5 ppm	
Tuna	3 ppm	2.7 ppm	**99%**
	10 ppm	10.1 ppm	
	40 ppm	42.2 ppm	
Salmon	3 ppm	2.6 ppm	**84%**
	10 ppm	7.5 ppm	
	40 ppm	35.8 ppm	
Plaice	3 ppm	2.8 ppm	**95%**
	10 ppm	7.8 ppm	
	40 ppm	45.8 ppm	
Red Wine	1 ppm	0.9 ppm	**97%**
	3 ppm	3.3 ppm	
	9 ppm	8.4 ppm	

Mean recoveries range from 84% to 102% depending on the sample matrix. In combination with the results of the intra-assay and inter-assay experiments (Section 2), this may not differ significantly from 100%.

4. Analytical Sensitivity

For determination of the Limit of Detection (LOD) and the Limit of Quantification (LOQ), sample diluent and different matrices were assayed in 20-fold replicates. After identification of possible outliers, the OD mean was calculated as well as its standard deviation. According to AOAC guidelines the corresponding concentration of OD mean – 3 x standard deviation was defined as limit of detection, the corresponding concentration of OD mean – 10 x standard deviation was defined as limit of quantification.

Table 4: Matrix-dependent analytical sensitivity of the AlerTox® ELISA Histamine

Replicate	Sample diluent	Trout	Salmon	Codfish	Plaice	Tuna	Red Wine
	[OD]	[OD]	[OD]	[OD]	[OD]	[OD]	[OD]
1	1.618	1.598	1.544	1.568	1.454	1.487	1.792
2	1.568	1.669	1.530	1.563	1.445	1.432	1.796
3	1.576	1.670	1.531	1.546	1.454	1.464	1.771
4	1.583	1.717	1.526	1.565	1.460	1.475	1.844
5	1.619	1.655	1.482	1.542	1.533	1.448	1.766
6	1.624	1.593	1.556	1.528	1.528	1.492	1.749
7	1.647	1.723	1.584	1.566	1.561	1.489	1.764
8	1.680	1.745	1.605	1.577	1.534	1.510	1.768
9	1.601	1.576	1.471	1.510	1.513	1.479	1.708
10	1.563	1.608	1.456	1.590	1.539	1.433	1.746
11	1.606	1.609	1.462	1.544	1.528	1.466	1.763
12	1.553	1.627	1.507	1.549	1.543	1.455	1.803
13	1.651	1.640	1.502	1.507	1.539	1.457	1.795
14	1.645	1.584	1.470	1.522	1.498	1.462	1.789
15	1.653	1.770	1.511	1.562	1.566	1.462	1.755
16	1.689	1.552	1.499	1.581	1.508	1.482	1.791
17	1.568	1.640	1.463	1.486	1.518	1.435	1.714
18	1.544	1.595	1.470	1.415	1.519	1.395	1.802
19	1.546	1.635	1.503	1.454	1.578	1.439	1.783
20	1.559	1.687	1.507	1.485	1.550	1.413	1.788
Mean	**1.605**	**1.645**	**1.509**	**1.533**	**1.518**	**1.459**	**1.774**
SD	**0.045**	**0.060**	**0.041**	**0.045**	**0.039**	**0.028**	**0.031**
LOD	**7 ppb**	**0.7 ppm**	**1.1 ppm**	**0.4 ppm**	**0.4 ppm**	**0.5 ppm**	**0.3 ppm**
LOQ	**24 ppb**	**2.6 ppm**	**2.5 ppm**	**2.0 ppm**	**1.7 ppm**	**1.5 ppm**	**0.4 ppm**

With respect to the sample matrix, limits of detection vary from 0.3 to 0.7 ppm and the limits of quantification vary from 0.4 ppm to 2.6 ppm. Note that the derived LODs and LOQs 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.

5. Linearity

Linearity was determined by spiking different sample matrices with histamine 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: Matrix dependent linearity of the AlerTox® ELISA Histamine

Trout

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	38.5	100
20 ppm	18.8	98
10 ppm	11.5	119
5 ppm	4.8	99
	Mean [%]	**105**

Salmon

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	40.7	100
20 ppm	20.5	101
10 ppm	11.2	110
5 ppm	4.9	96
	Mean [%]	**102**

Tuna

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	35.2	100
20 ppm	16.2	92
10 ppm	8.6	97
5 ppm	3.8	86
	Mean [%]	**92**

Cod

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	38.0	100
20 ppm	18.6	98
10 ppm	11.9	125
5 ppm	4.8	101
	Mean [%]	**108**

Plaice

Target Value	Concentration [ppm]	Recovery [%]
40 ppm	36.3	100
20 ppm	17.2	95
10 ppm	9.8	108
5 ppm	4.3	95
	Mean [%]	**99**

Red Wine

Target Value	Concentration [ppm]	Recovery [%]
10 ppm	9.3	100
5 ppm	5.0	106
2.5 ppm	2.4	103
1.25ppm	1.4	118
	Mean [%]	**109**

For different matrices, the mean linearity ranges from 92% to 109%. The linearity seems to be relatively independent of the specific concentration and may moreover be affected by the intra-assay and inter-assay variation as stated in Section 2.

6. Cross-Reactivity

Various histamine derivates and possible cross-reacting substances were tested in different concentrations in the AlerTox® ELISA Histamine kit. The mean concentrations as indicated by the ELISA were expressed in percent of the actual concentration.

Table 6: Non-cross-reactive food matrices in the AlerTox® ELISA Histamine

Substance	Target Value [µg/mL]	Measured Concentration [µg/mL]	Cross Reactivity [%]	Mean
Serotonin	1,000 µg/mL	< 0.05	-	**0%**
	100 µg/mL	< 0.05	-	
	10 µg/mL	< 0.05	-	
	1 µg/mL	< 0.05	-	
	0.1 µg/mL	< 0.05	-	
1-Methyl-Histamine	1,000 µg/mL	> 5	-	**5%**
	100 µg/mL	2.0	2	
	10 µg/mL	0.4	4	
	1 µg/mL	0.1	10	
	0.1 µg/mL	< 0.05	-	
Histidine	5,000 µg/mL	< 0.05	-	**0%**
	500 µg/mL	< 0.05	-	
	50 µg/mL	< 0.05	-	
	5 µg/mL	< 0.05	-	
	0.5 µg/mL	< 0.05	-	
N-acetyl histamine	5,000 µg/mL	0.75	0.015	**0.019%**
	500 µg/mL	0.11	0.022	
	50 µg/mL	< 0.05	-	
	5 µg/mL	< 0.05	-	
	0.5 µg/mL	< 0.05	-	

Cross reactivity is affected by the special chemical characteristics of a compound. 1-methyl-histamine shows the greatest structural similarities with histamine in a region essential for antibody induction and thus the greatest cross-reactivity.