

# Validation report



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## Tetracycline ELISA

Validation report for the enzyme immunoassay for the quantitative determination of Tetracyclines in food.

**REF**

**DETCYE01**



**96 wells**

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## 1. Scope

The **Tetracycline ELISA** is designed for the fast determination of Tetracyclines in different food matrices. The present report describes the efforts made for validation purposes and summarizes its results.

## 2. Precision

### 2.1. Intra-Assay Variation

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

**Table 1: Intra-assay variation of the Tetracycline ELISA**

Replicate	Level 1		Level 2		Level 3	
	OD	c [pg/mL]	OD	c [pg/mL]	OD	c [pg/mL]
1	1.066	88	0.702	350	0.422	1154
2	1.104	74	0.643	437	0.456	971
3	1.133	65	0.666	400	0.452	991
4	1.153	59	0.702	450	0.475	1344
5	1.115	71	0.678	383	0.424	1142
6	1.037	99	0.678	383	0.433	1090
7	1.088	80	0.636	449	0.433	1090
8	1.074	85	0.669	396	0.418	1179
9	1.093	78	0.664	403	0.423	1148
10	1.165	56	0.665	401	0.424	1142
11	1.191	49	0.732	313	0.450	1000
12	1.157	58	0.667	398	0.442	1041
13	1.201	46	0.698	354	0.404	1272
14	1.105	74	0.624	470	0.403	1279
15	1.101	75	0.645	433	0.429	1112
16	1.122	68	0.677	383	0.414	1205
17	1.149	61	0.653	420	0.432	1095
18	1.176	52	0.690	365	0.399	1307
19	1.083	82	0.696	357	0.412	1217
20	-	-	0.650	425	0.401	1293
<b>Mean</b>	1.122	69	0.672	399	0.427	1154
<b>SD</b>	0.045	14.3	0.026	39.3	0.020	109.0
<b>CV</b>	<b>4.0</b>	<b>20.5</b>	<b>3.9</b>	<b>9.9</b>	<b>4.7</b>	<b>9.5</b>

The coefficient of variation is ranging from 9.5% to 20.5% referring to the concentration. The magnitude of the CV is dependent on the slope of the calibration curve associated with a peculiar concentration. Consequently the range of CVs for the corresponding absorptions, which are independent of the shape of the calibration curve, is more constant and lower in magnitude (3.9% to 4.7%).

## 2.2. Inter-Assay Variation

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

**Table 2: Inter-assay variation of the Tetracycline ELISA**

Assay No	Level 1	Level 2	Level 3
	c [pg/mL]	c [pg/mL]	c [pg/mL]
1	138	439	1191
2	113	346	859
3	127	477	964
4	126	358	994
<b>Mean</b>	126	405	1002
<b>SD</b>	10.2	63.3	138.7
<b>CV(%)</b>	<b>8.1</b>	<b>15.6</b>	<b>13.8</b>

The coefficient of variation is ranging from 8.1% to 15.6% referring to the concentration.

## 3. Recovery

For recovery experiments different sample matrices were spiked with Tetracycline to yield 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 Tetracycline ELISA**

Sample	Target Value	Actual Concentration	Recovery
Meat	2 ppb	1.5 ppb	87%
	8 ppb	6.8 ppb	
	20 ppb	19.9 ppb	
Milk	2 ppb	2.2 ppb	94%
	8 ppb	6.3 ppb	
	20 ppb	18.7 ppb	
Nonfat Dry Milk	10 ppb	10.4 ppb	90%
	40 ppb	27.8 ppb	
	100 ppb	96.2 ppb	
Cheese	5 ppb	3.6 ppb	82%
	20 ppb	17.0 ppb	
	50 ppb	44.7 ppb	
Shrimps	2 ppb	2.0 ppb	89%
	8 ppb	6.9 ppb	
	20 ppb	16.7 ppb	
Honey	10 ppb	7.3 ppb	103%
	40 ppb	47.1 ppb	
	100 ppb	118 ppb	

Mean recoveries are ranging from 82% to 103% depending on the sample matrix. Under consideration of the intra-assay and inter-assay variation as stated in chapter 2. this may not differ significantly from 100%.

#### 4. Analytical Sensitivity

##### 4.1. LOD / LOQ

For determination of the Limit of Detection (LOD) and the Limit of Quantification (LOQ) sample diluent and different matrices were assayed in 20fold replicates. After identification of possible outliers the OD mean was calculated as well as its standard deviation.

The corresponding concentration of OD mean – 3 x standard deviation was defined as limit of detection, the corresponding concentration of OD mean – 9 x standard deviation was defined as limit of quantification.

**Table 4: Matrix independent analytical sensitivity of the Tetracycline ELISA**

Replicate	Sample Diluent [OD]	Meat [OD] Dil.F. = 20	Milk [OD] Dil.F. = 20	Nonfat Dry Milk [OD] Dil.F. = 100	Cheese [OD] Dil.F. = 50	Shrimps [OD] Dil.F. =20	Honey [OD] Dil.F. = 50
1	2.026	2.304	1.727	1.737	2.407	2.029	1.437
2	2.015	2.398	1.773	1.806	2.275	2.045	1.368
3	2.048	2.348	1.786	1.776	2.426	2.040	1.416
4	2.157	2.438	1.816	1.891	2.524	2.079	1.254
5	2.150	2.423	1.886	1.852	2.194	2.063	1.352
6	2.051	2.375	1.783	1.774	2.225	2.071	1.391
7	2.058	2.435	1.809	1.619	2.290	2.063	1.386
8	2.081	2.297	1.798	1.736	2.318	2.026	1.434
9	2.176	2.311	1.660	1.806	2.377	2.103	1.446
10	2.139	2.366	1.785	1.837	2.334	2.004	1.417
11	2.214	2.363	1.783	1.854	2.342	2.077	1.418
12	2.313	2.421	1.863	1.807	2.259	2.099	1.321
13	2.145	2.740	1.917	1.643	2.135	2.105	1.386
14	2.101	2.565	1.789	1.750	2.228	2.079	1.291
15	2.115	2.410	1.847	1.657	2.266	1.987	1.292
16	2.143	2.255	1.777	1.861	2.248	1.943	1.345
17	2.245	2.197	1.776	1.824	2.370	1.989	1.340
18	2.296	2.284	1.723	1.688	2.302	1.976	1.299
19	2.293	2.393	1.819	1.767	2.345	2.034	1.441
20	2.206	2.384	1.904	1.591	2.129	2.028	1.381
<b>Mean</b>	2.149	2.385	1.801	1.764	2.300	2.042	1.371
<b>SD</b>	0.091	0.115	0.062	0.086	0.097	0.045	0.057
<b>LOD</b>	<b>24 pg/mL</b>	<b>0.5 ppb</b>	<b>1.1 ppb</b>	<b>6.7 ppb</b>	<b>1.4 ppb</b>	<b>0.5 ppb</b>	<b>2.2 ppb</b>
<b>LOQ</b>	<b>72 pg/mL</b>	<b>1.4 ppb</b>	<b>2.0 ppb</b>	<b>14.0 ppb</b>	<b>3.2 ppb</b>	<b>1.1 ppb</b>	<b>3.3 ppb</b>

With respect to the sample matrix and dilution factors, limits of detection vary from 0.5 to 6.7 ppb and the limits of quantification vary from 1.1 ppb to 14.0 ppb. 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 Tetracycline 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 Tetracycline ELISA**

***Meat (Dilution Factor = 20)***

Target Value	Concentration [ppb]	Recovery [%]
32 ppb	27.9	-
16 ppb	15.2	109
8 ppb	6.2	89
4 ppb	2.8	81
2 ppb	1.7	96
	<b>Mean [%]</b>	<b>94</b>

***Milk (Dilution Factor = 20)***

Target Value	Concentration [ppb]	Recovery [%]
32 ppb	45.7	-
16 ppb	25.1	110
8 ppb	12.0	105
4 ppb	5.8	102
2 ppb	2.7	94
	<b>Mean [%]</b>	<b>103</b>

***Nonfat Dry Milk (Dilution Factor = 100)***

Target Value	Concentration [ppb]	Recovery [%]
160 ppb	218.8	-
80 ppb	134.4	123
40 ppb	68.5	125
20 ppb	27.4	100
10 ppb	9.4	69
	<b>Mean [%]</b>	<b>104</b>

***Cheese (Dilution Factor = 50)***

Target Value	Concentration [ppb]	Recovery [%]
80 ppb	60.6	-
40 ppb	31.9	105
20 ppb	17.6	116
10 ppb	5.8	76
5 ppb	2.3	61
	<b>Mean [%]</b>	<b>90</b>

**Shrimps (Dilution Factor = 20)**

Target Value	Concentration [ppb]	Recovery [%]
32 ppb	41.0	-
16 ppb	16.3	80
8 ppb	8.7	84
4 ppb	4.1	81
2 ppb	2.3	89
	<b>Mean [%]</b>	<b>83</b>

**Honey (Dilution Factor = 50)**

Target Value	Concentration [ppb]	Recovery [%]
80 ppb	83.2	-
40 ppb	48.4	116
20 ppb	24.7	119
10 ppb	10.7	103
5 ppb	5.4	103
	<b>Mean [%]</b>	<b>110</b>

For different matrices the mean linearity is ranging from 83% to 110%. 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 chapter 2.

**6. Cross-Reactivity**

Different derivatives of Tetracycline were tested in different concentrations in the Tetracycline ELISA test kit in order to cover the total range of the calibration curve. The mean concentrations as indicated by the ELISA were expressed in per cent of the actual concentration.

**Table 6: Cross-Reactivity of various Tetracycline derivatives in the Tetracycline ELISA**

Substance	Cross-Reactivity (Mean)
Tetracycline	100%
4-Epitetracycline	111%
Rolitetracycline	82%
Chlortetracycline	42%
Doxycycline	41%
Demeclocycline	37%
Oxytetracycline	34%
4-Epioxytetracline	34%
4-Epichlortetracycline	11%
Methacycline	9%
Minocycline	1%

Cross-reactivity is affected by the special chemical characteristics of a compound. 4-Epitetracycline shows the greatest structural similarities with Tetracycline in a region essential for antibody induction and thus the greatest cross-reactivity.