CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

021402

The AOAC Research Institute hereby certifies that the performance of the test kit known as:

HistaSure™ ELISA Fast Track

manufactured by

LDN Labor Diagnostika Nord GmbH & Co
KG Am Eichenhain 1
48531 Nordhorn
Germany

This method has been evaluated in the AOAC® *Performance Tested Methods*SM Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC® Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested* SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above-mentioned method for a period of one calendar year from the date of this certificate (September 14, 2021 – December 31, 2022). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

September 14, 2021
Date

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KIT NAME(S)
HistaSure^{TM} ELISA $^{Fast\ Track}$

CATALOG NUMBERS
FC E-3600, FC E-3900

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APPLICABILITY OF METHOD
Target analyte– histamine

Matrixes – (10 g samples) - fresh/frozen yellowfin tuna, canned tuna-chunk light in water, fresh/frozen mahi mahi, canned sardines in oil, and fish meal

Performance claims
Selectivity: Cross reactivity with components of similar behavior < 2%
Accuracy: %RSD < 15% (above LOQ)
Precision: recovery within 80 – 120 %
Detection limit: LOQ < 3 ppm (Corresponding to Control 2 of the ELISA)
Stability: 2-year shelf life at 2 – 8°C. Acceptable OD range of control 0: 1.250 – 2.750.
Method comparison LDN HistaSure ELISAFast Track vs AOAC 977.13. Correlation Coefficient r>0.9. Ratio AOAC 977.13 / HistaSure ELISA$^{Fast\ Track}$ 0.8 – 1.2

REFERENCE METHOD
W. Horwitz, G.W. Latimer, 2005. AOAC Official Method 977.13. Official Methods of analysis of AOAC International 18th Edition 35.1.32 (13)

ORIGINAL CERTIFICATION DATE
February 03, 2014

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2022

METHOD MODIFICATION RECORD
1. **November 2017 Level 1**
2. **November 2019 Level 1**
3. **September 2020 Level 1**

SUMMARY OF MODIFICATION
1. **Editorial changes**
2. **Editorial changes**
3. **Editorial changes to labels**

Under this AOAC® *Performance Tested*^{SM} License Number, 021402 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*^{SM} License Number, 021402 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
Histamine is extracted from the fish using a simple water extraction. After filtration or centrifugation, the clear supernatant is used for the subsequent step, the derivatization of histamine.
By use of the acylation reagent, histamine from the calibrators and histamine from the food extracts is quantitatively derivatized into N-acylhistamine. The competitive Histamine ELISA kit uses the microtiter plate format. Histamine is bound to the solid phase of the microtiter plate. Acylated histamine and solid phase bound histamine compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum-peroxidase complexes are removed by washing. The substrate TMB/peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase histamine is inversely proportional to the histamine concentration of the sample.

DISCUSSION OF THE VALIDATION STUDY (1)
In the AOAC PTM validation study the performance of the LDN HistaSure^{TM} ELISA$^{Fast\ Track}$ was at least equivalent to the AOAC **977.13** reference method. The LDN HistaSure ELISA$^{Fast\ Track}$ showed good recovery rates for all tested matrixes (fresh/frozen tuna, canned tuna, fresh/frozen mahi mahi, canned sardines and fish meal) and repeatability precision was excellent at the 50 ppm defect action level. No solvents are required for extraction. The method is robust, fast, efficient and suitable for high throughput screening. Based on the results of this study it could be recommended that the LDN HistaSure ELISA$^{Fast\ Track}$ receives PTM certification.

Table 9: Method comparison results overview AOAC 977.13 vs. LDN HistaSure™ ELISA Fast Track (1)

	Fortification level (ppm)	AOAC 977.13 Mean concentration (n=5) (ppm)	AOAC 977.13 SD	AOAC 977.13 %RSD	AOAC 977.13 Average % recovery (n=5)	LDN HistaSure™ ELISA Fast Track Mean concentration (n=7) (ppm)	LDN SD	LDN %RSD	LDN Average % recovery (n=7)
Fresh/frozen Tuna	0	0.51	0.63	123.5	/	0.27 (< LOQ)	0.11	38.7	/
	5	4.92	0.81	16.4	88.1	5.25	0.40	7.52	99.6
	20	17.6	2.81	16.0	85.4	19.2	2.08	10.8	94.6
	50	43.9	7.37	16.8	86.8	42.9	1.77	4.13	85.2
	100	87.9	15.1	17.2	87.4	92.6	6.99	7.55	92.4
	250	261.8	27.2	10.4	104.5	218.6	17.3	7.90	87.3
	Mean recovery				**90.4**				**91.8**
Canned tuna	0	0.22	0.22	99.7	/	0.50 (< LOQ)	0.17	33.7	/
	5	4.84	0.16	3.21	92.4	5.49	0.76	13.9	99.8
	20	18.3	2.35	12.8	90.6	20.2	2.01	9.92	98.6
	50	52.3	2.89	5.52	104.2	47.8	4.10	8.57	94.7
	100	99.5	7.12	7.15	99.3	99.8	8.10	8.12	98.7
	250	238.9	33.8	14.2	95.5	267.3	8.46	3.17	106.5
	Mean recovery				**96.4**				**99.8**
Frozen mahi mahi	0	1.52	0.96	63.3	/	1.22 (< LOQ)	0.21	17.3	/
	5	5.44	0.77	14.3	78.4	6.38	0.61	9.56	103.1
	20	17.9	1.69	9.45	82.0	18.9	1.28	6.78	88.2
	50	45.8	7.68	16.8	88.5	43.5	2.29	5.26	84.6
	100	93.9	6.30	6.71	92.4	82.7	2.46	2.97	81.5
	250	242.6	19.9	8.22	96.4	199.0	12.6	6.35	79.1
	Mean recovery				**87.5**				**87.3**
Canned sardines	0	0.21	0.32	155.1	/	0.67 (< LOQ)	0.19	28.9	/
	5	4.24	0.28	6.57	80.6	5.27	0.28	5.36	92.1
	20	20.7	1.10	5.32	102.3	18.2	1.24	6.78	87.8
	50	41.6	4.89	11.8	82.9	41.5	0.75	1.80	81.6
	100	86.1	10.8	12.5	85.9	77.5	3.94	5.08	76.8
	250	230.3	32.2	14.0	92.0	249.9	23.0	9.22	99.7
	Mean recovery				**89.0**				**87.6**
Fish meal	0	6.31	0.73	11.6	/	9.4	0.54	5.71	/
	5	5.30	0.30	5.73	-20.1	14.0	1.11	7.92	92.3
	20	15.5	1.42	9.12	46.2	25.5	1.02	3.98	80.5
	50	38.5	4.11	10.7	64.5	48.9	1.02	2.09	79.0
	100	95.1	2.78	2.92	88.8	93.8	3.73	3.97	84.4
	250	212.9	19.8	9.29	82.6	244.2	15.8	6.47	93.9
	Mean recovery				**52.4**				**86.0**
Naturally contaminated	Sample 1	61	3.06	5.04	/	58	5.2	8.95	/
	Sample 2	203	13.7	6.74	/	133	10.7	7.99	/
	Sample 3	7	1.00	14.3	/	8.6	0.92	10.7	/
	Sample 4	54	3.21	5.99	/	48	2.8	5.83	/
	Sample 5	57	1.53	2.66	/	69	5.3	7.75	/

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