APPLICATION NOTE

Rapid Enzymatic Dipstick Assay

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Validation of the HistaStrip™ Test Kit for the Detection of Histamine in Seafood and Other Food Matrices

General Information

Histamine poisoning is the second leading cause of food-borne illness in the United States, accounting for nearly one in fifty of the total cases of food-borne illness in the US. The expensive, time-consuming, and labor-intensive nature of the available detection methods for histamine dramatically limit the capacity to test large numbers of samples. This restricts the ability of analysts to test the overall food supply and quarantine contaminated samples. PerkinElmer has developed a novel reader-based quantitative colorimetric strip test for the determination of histamine in seafood. The HistaStrip™ Test Kit offers a convenient solution for quick, quantitative, field-based screening of seafood samples. Fresh/frozen tuna, canned tuna, frozen mahi mahi, milk, and fish sauce samples were used for the single lab validation study. The linearity of the assay ranged from 0-75 ppm. Overall recoveries for all tested matrices were within the acceptable range (80-120%). A 1-year claimed shelf-life of the kit at 4°C was verified by accelerated stability study data collected at 1 day, 15 days, and 1-month time points at 25°C. A previous study conducted for AOAC PTM certification produced data which show that the enzyme used does not have cross-reactivity or interference with other biological amines. This study validated the performance of PerkinElmer's HistaStrip™ Test Kit as a rapid and accurate screening tool.

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Materials and Methods

Naturally contaminated samples of known concentration were not available, therefore artificially spiked samples were used. Seafood and other food matrices were sourced from local food markets. The spike solution was prepared from USP histamine dihydrochloride (certified reference PHR1357-500mg; Sigma Aldrich). To make 1000 ppm spike solution, 82.5 ± 0.2mg of histamine dihydrochloride was dissolved in 50mL of distilled water in a 50mL volumetric flask.

Sample Preparation

Seafood (Fresh/Frozen Tuna & Canned Tuna)

1. Thaw sample and homogenize about 40g of sample.
2. Transfer 4.0 g of sample to a new 50mL tube. Add 16.0mL of prepared Seafood/Milk Buffer.
3. Vortex sample at maximum speed, or shake vigorously, for 30 seconds.
4. Incubate sample for 1 minute at room temperature.
5. Vortex sample, or shake vigorously, for an additional 30 seconds.
6. Incubate sample for 5 minutes at room temperature to allow solid debris to settle to the bottom of the tube.
7. Transfer the top, clear layer for testing.

Note: Dilution factor = 5

Milk

1. Transfer 800 µL of milk to a 2mL tube containing 800µL of prepared Seafood/Milk Buffer.
2. Vortex sample for 1 minute at maximum speed.
3. Transfer 500µL of sample to a new 2mL tube containing 100µL of Balance Buffer.
4. Vortex sample for 1 minute at maximum speed.

Note: Dilution factor = 2.4

Fish Sauce

1. Transfer 500µL of sample to a tube containing 12mL of deionized water.
2. Vortex sample manually for 1 minute at maximum speed.
3. Transfer 500µL of the diluted sample to a new 2mL tube containing 100µL of Balance Buffer.
4. Vortex sample for 1 minute at maximum speed.

Note: Dilution factor = 25

Linearity of the Assay

Linearity of the assay was demonstrated by measuring replicates of histamine dissolved in water at six concentrations (0, 5, 7.5, 10, 12.5, 15 ppm; dilution factor 5). The assay was carried out and the histamine concentration was quantitatively measured using the QuickSTAR™ Strip Reader. The results were plotted with spiked concentration on the x-axis and determined concentration on the y-axis. The linearity of the assay extends beyond the claimed limit of 50 ppm (Figure 1).

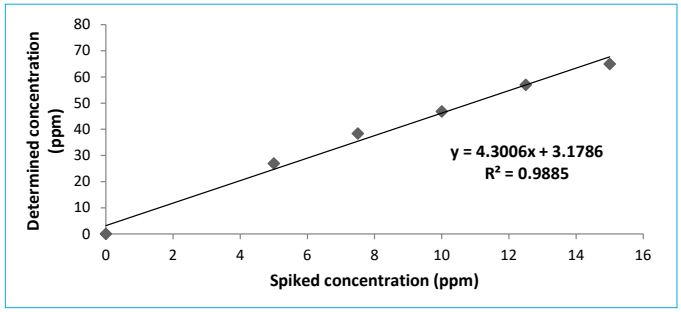

Figure 1. Graph representing linearity of the assay

Cross Reactivity/Selectivity

The enzymatic determination is specific to histamine. Cross-reactivity for the method with biological amines and related compounds was assessed in the absence of histamine. Interference was tested in the presence of histamine. In the previous AOAC PTM validation for the enzymatic method, these studies were carried out using the same enzyme (Gone, Kosa, & Krebs, 2017). The results revealed minimal cross reactivity (Table 1) and no interference (Table 2) for the enzyme.

Compound	A450	Normalized	% Activity
Histamine	0.55	0.4080	100.00
Tyramine	0.15	0.0007	0.17
Anserine	0.16	0.0010	0.24
L-Histidine	0.16	0.0016	0.40
Spermine	0.16	0.0010	0.24
L-Tyrosine	0.15	0.0005	0.12
L-Tryptophan	0.15	0.0005	0.12
Agmatine	0.32	0.0167	4.10
Cadaverine	0.15	0.0005	0.12
Tryptamine	0.15	0.0005	0.12
Carnosine	0.15	0.0001	0.02
3-Methyl Histamine	0.15	0.0001	0.02
Putrescine	0.19	0.0038	0.93
Spermidine	0.17	0.0020	0.50
L-Phenylalanine	0.16	0.0014	0.34
Water Control	0.15	0.0000	0.00

Note: A450 = Absorbance at 450 nm

Table 1. Summary of cross-reactivity testing with related biological amines from MaxSignal® Histmaine Enzymatic Assay Validation Report. No significant cross-reactivity was observed.

Compound	Recovery %
Tyramine	98.0
L-Histidine	99.1
L-Tyrosine	99.4
L-Tryptophan	98.1
Tryptamine	93.0
3-Methyl Histamine	98.4
Spermidine	99.1
L-Phenylalanine	99.4
Anserine	96.0
Spermine	98.0
Agmatine	129.0
Cadaverine	100.0
Carnosine	96.2
Putrescine	105.0
Water + Histamine	95.0

Table 2. Summary of interference testing with related biological amines from MaxSignal® Histmaine Enzymatic Assay Validation Report. No interference was observed with other biological amines.

Food Matrix Study

Frozen tuna, canned tuna, mahi mahi, milk, and fish sauce were spiked with five concentrations of histamine to cover the analytical range (0, 25, 40, 50, and 60 ppm). Five replicates were spiked per concentration. The mean concentration, standard deviation, repeatability precision, and bias were calculated (Table 3). The spiked samples indicated an overall recovery of 94% from frozen tuna, 96% from canned tuna, 92% from mahi mahi, 94% from fish sauce, and 96% from milk (Table 3). The overall recovery is within the 80-120% range.

Matrix	Spike (ppm)	Mean (n=5)	Recovery %	SD	RSD	Bias (ppm)
Frozen Tuna	0	4.2		0.59	14.14	-4.2
	25	26.9	107.6	0.74	2.75	-1.9
	40	38.1	95.25	0.82	2.15	1.9
	50	44.2	88.4	0.75	1.71	5.8
	60	50.6	84.3	0.41	0.82	9.4
Mean			94			
Canned Tuna	0	3.5		1.41	40.2	-3.5
	25	26.8	107.2	1.03	3.84	-1.8
	40	38.3	95.7	1.44	3.75	1.7
	50	46.5	93	1.62	3.48	3.5
	60	52.2	87	1.3	2.49	7.8
Mean			96			
Mahi Mahi	0	6.3		1.25	19.9	6.3
	25	26.4	105.6	2.48	9.4	-1.4
	40	35.9	89.75	1.41	3.9	4.1
	50	44.2	88.4	0.98	2.05	5.8
	60	50.7	84.5	1.15	2.27	9.3
Mean			92			
Fish Sauce	0	1.8		0.447	24.8	-1.8
	25	26.3	105.2	0.57	2.16	-1.3
	40	36.3	91.2	0.836	2.3	3.7
	50	44	88	0.79	1.79	6
	60	53.4	91	0.961	1.79	6.6
Mean			94			
Milk	0	0.3		0.447	149	-0.3
	25	26.2	104.8	1.823	6.95	-1.2
	40	37.9	94.75	1.917	5.05	2.1
	50	47.2	94.4	0.758	1.6	2.8
	60	53.7	89.5	1.095	2.03	6.3
Mean			96			

Note: SD = standard deviation; RSD = repeatability precision

Table 3. Summary of the food matrix studies.

Stability

To validate the shelf life of the kit at 4°C, an accelerated stability study was performed based on the Arrhenius model, where the activation energy, Ea, is 20 kcal, and 1 year at 5°C represents 32 days at 25°C. Unspiked and 25 ppm histamine

spiked samples were tested with kits that were staggered at 25°C. Testing was carried out on day 1, 15, and 32 (Table 4). The kit performed with the same accuracy after 1 month at room temperature.

ACCELERATED HISTASTRIP™ STABILITY AT RT		
	Unspiked	**25 ppm**
Day 1	3	24
	4	23
	4	24
Mean	3.7	24
Day 15	5	28
	5	27
	3	26
Mean	4.3	27
Day 32	4	27
	6	26
	4	26
Mean	4.6	26.3

Table 4. Accelerated stability of HistaStrip™ Test Kit at room temperature.

Conclusions

PerkinElmer's HistaStrip™ Test Kit demonstrated high precision for the determination of histamine in various food matrices (frozen/fresh tuna, canned tuna, mahi mahi, milk, and fish sauce). Repeatability standard deviation of histamine at defect action level of 50 ppm imposed by USFDA is less than 4%. The linearity of the assay ranged from 0-75 ppm. Overall recoveries for all tested matrices were within the acceptable range (80-120%). A 1-year claimed shelf-life of the kit at 4°C was verified by accelerated stability study data collected at 1 day, 15 days, and 1 month time points at 25°C.

References

1. Gone, S., Kosa, N., Krebs, J. (2017). Validation Study of MaxSignal Histamine Enzymatic Assay for the Detection of Histamine in Fish/Seafood. *Journal of AOAC International, 101*. Retrieved from https://doi.org/10.5740/jaoacint.17-0289

Reagent Kit Used

Kit	Part Number
HistaStrip™	FOOD-1100-001

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