

# Development and validation of BioSystems Y15 Histamine method for the quantification of Histamine in fish and fishery products (AOAC PTM<sup>SM</sup> #072001)

BioSystems

human - centred biotech

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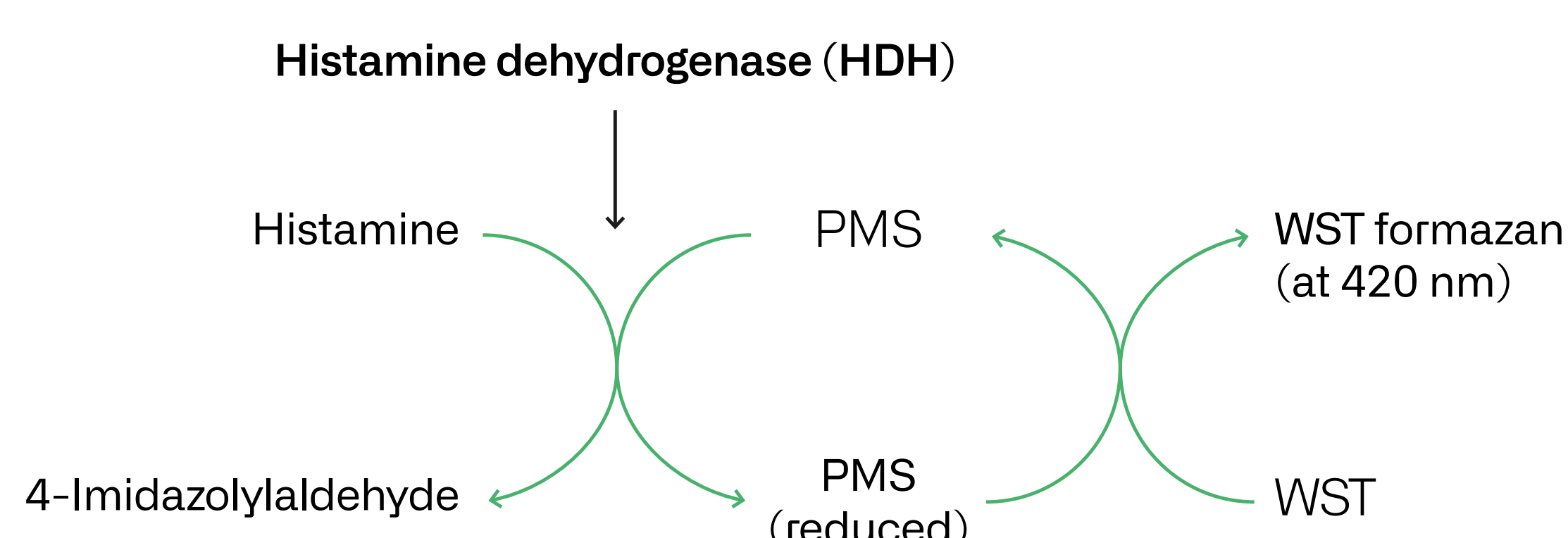
## Introduction

The presence of histamine in fish is becoming increasingly important to consumers and producers alike due to its potential toxicity and subsequent health implications. Additionally, histamine can indicate unsanitary conditions during fish handling. Current methods for histamine analysis, such as HPLC, ELISA, and fluorimetry, often require expensive and sophisticated instrumentation, as well as skilled technicians. BioSystems introduces a new, simple, and rapid enzymatic method for determining histamine in fish that can be automated. This method is based on the specific reaction of histamine with recombinant histamine dehydrogenase (from *E. coli*), which reduces a soluble tetrazolium salt to form formazan salt that absorbs at 420 nm. This absorption can be measured by visible spectrophotometry and correlated with known histamine concentrations through calibration.

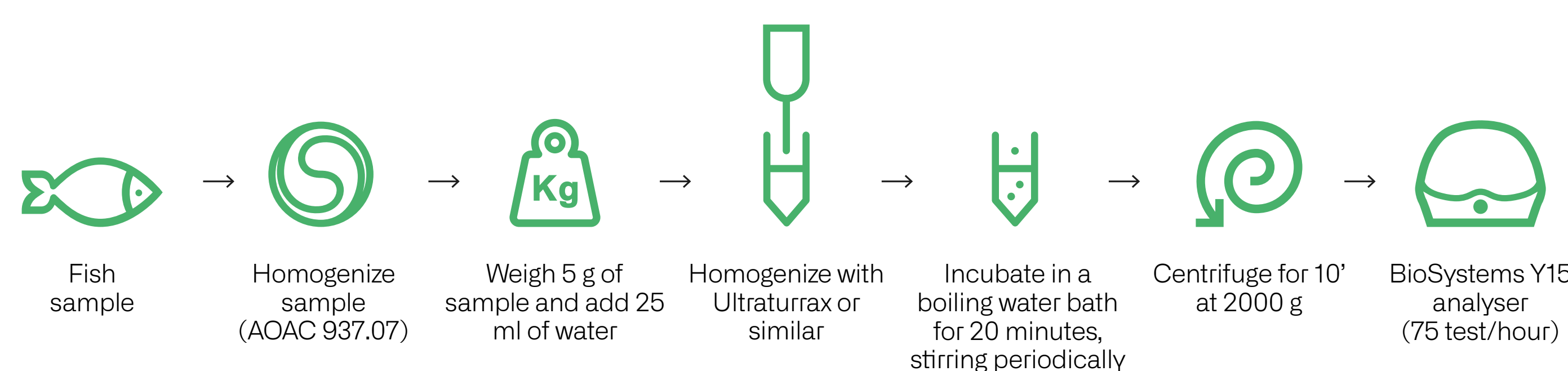
The new reagent is liquid, ready-to-use, and highly stable, minimizing end-user influence. Linearity, limit of quantification (LoQ), repeatability, within-laboratory reproducibility, trueness, and recovery were characterized using a BioSystems Y15 automated analyser and validated against the HPLC method according to AOAC RI Guidelines to obtain certification as a Performance Tested Method (PTM).

## Measurement procedure

Histamine in the sample forms a colored complex through the coupled reactions described below, which can be measured by spectrophotometry:



### Protocol:



## Conclusions

The validation data for the BioSystems Y15 Histamine method shows a linear range 0 to 200 mg/kg ( $r^2 > 0.99$ ). The LOQ is 10 mg/kg in all the matrixes. Recoveries range from 75 to 107% at concentrations from 5 to 200 mg/kg, with repeatability precision values between 0.8 and 5.5%. Comparison with the HPLC reference method shows a good correlation. Cross-reactivity of the assay is negligible for all biogenic amines tested except for agmatine (6.3%). Product consistency was verified by validating lot-to-lot variations and variations within the same lot. Shelf life was verified by real-time stability testing during 40 months at 2–8°C. No differences in histamine detection were observed in robustness testing.

The BioSystems Y15 Histamine method has been validated for quantification of histamine in fish and fishery products and proves to be a valuable tool for automated, rapid, accurate, and straightforward histamine determination. Automating the measurement process in the BioSystems Y15 analyser enhances precision and eliminates potential user errors, allowing for the analysis of a wide range of sample sizes, from a few to a high throughput of 75 samples per hour.

Additionally, the BioSystems Y15 automation capability provides the opportunity to combine histamine analysis with other control parameters like sulfite levels or organic acids. This advantage improves efficiency and speed in decision-making during the fish control.

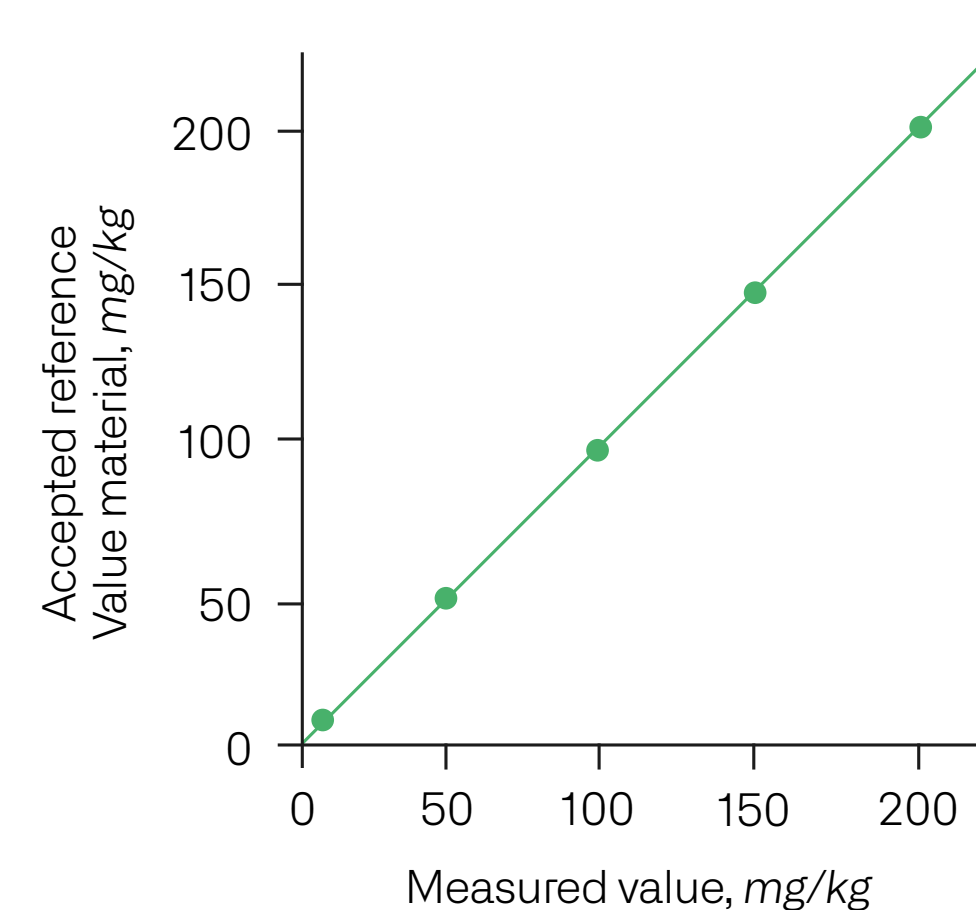
## Product performance

The method validation has been conducted according to the criteria established by the AOAC (Appendix F: Guidelines for Standard Method Performance Requirements).

### Measurement range - Linearity

The measurement range of the kit is from 10 (LoQ) to 200 mg/kg of histamine.

Reference value (mg/kg)	Calculated values				
	Mean value (n=5)	Sr (mg/kg)	RSDr (%)	Bias (mg/kg)	
0	-0.1	0.12	-	-0.1	
10	9.9	0.11	1.08	-0.1	
50	50.7	0.11	0.22	0.7	
100	99.5	0.20	0.20	-0.5	
150	150.5	0.35	0.24	0.5	
200	199.1	0.64	0.32	-0.9	

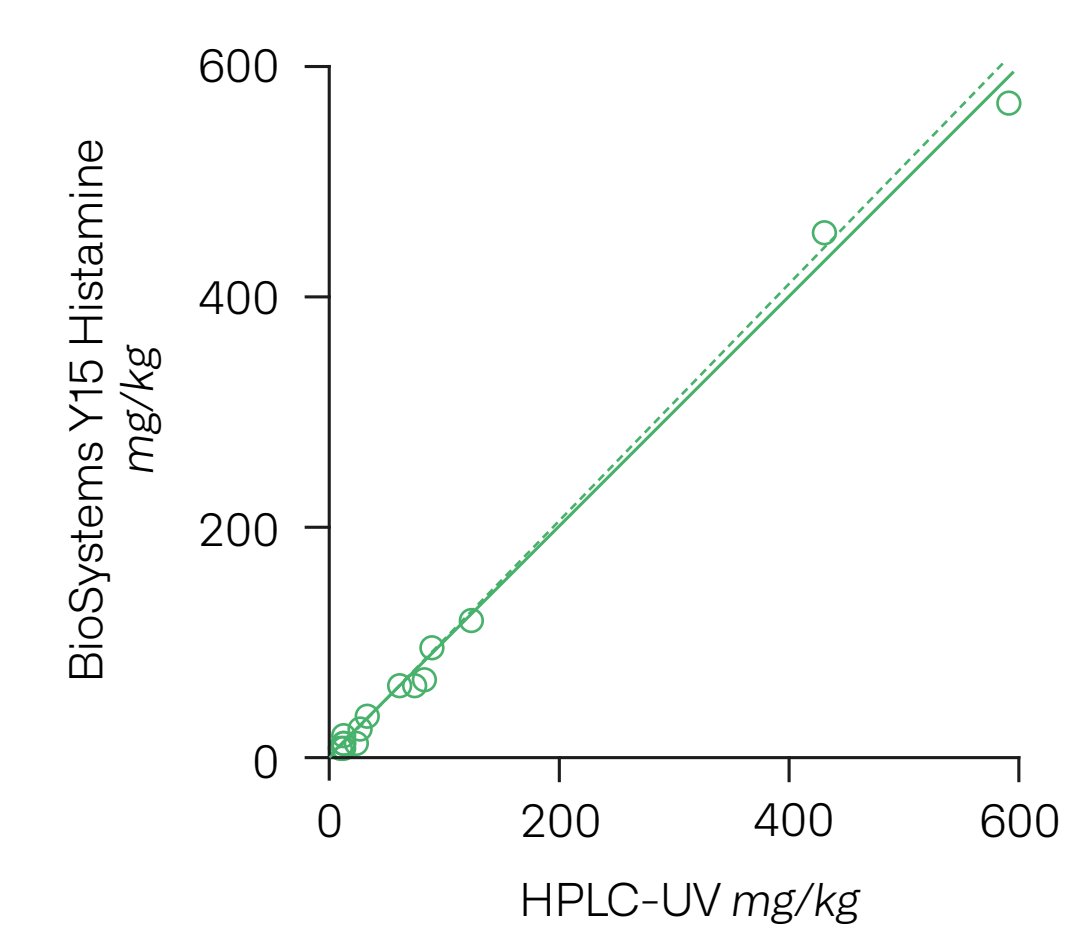


### Precision and recovery

Matrix	BioSystems Y15 Histamine results							
	Endogenous histamine, mg/kg	Spiking, mg/kg	Total histamine, mg/kg	Mean (n=5), mg/kg	Sr, mg/kg	RSDr, %	Recovery, %	Bias mg/kg
Raw tuna	1.2	0	1.2	1.2				
		10	11.2	10.3	0.09	7.81	92%	-0.8
		50	51.2	48.9	0.33	3.16	96%	-2.3
Oil-packed tuna	4.2	0	4.2	4.2				
		10	14.2	14.2	0.04	0.90	100%	0.0
		50	54.2	55.6	0.18	1.26	103%	1.4
Raw sardines	5.1	0	5.1	5.1				
		10	15.1	15.2	0.25	4.95	101%	0.1
		50	55.1	55.3	0.27	1.76	100%	0.2
Semi-preserved anchovy fillets	3.3	0	3.3	3.3				
		10	13.3	13.1	0.14	4.26	98%	-0.2
		50	53.3	54.7	0.45	3.44	103%	1.4

### Methods comparison

	ISO 19343:2017 HPLC mg/kg	BioSystems Y15 Histamine mg/kg
Raw mackerel	34	39
Raw mackerel (spiked)	61	64
Raw sardine	10	12
Raw sardine (spiked)	89	97
Pickled anchovy	437	450
Pickled anchovy (spiked)	592	567
Anchovy pate	<10 (4)	4
Raw tuna sirloin	<10 (0)	0
Raw tuna sirloin (spiked)	74	65
Oil-packed tuna	<10 (3)	2
Oil-packed tuna (spiked)	<10 (8)	8
Oil-packed tuna (spiked)	122	121
Oil-packed sardines	<10 (7)	7
Oil-packed sardines (spiked)	27	28
Oil-packed mackerel	<10 (0)	1
Oil-packed mackerel (spiked)	81	69
Raw tuna	<10 (1.1)	1.1
Water-packed tuna	<10 (5.5)	5.5
Oil-packed tuna	<10 (6.6)	6.5
Semi-preserved anchovy fillets	10.7	10.7



Samples Equation  
R square  
 $Y = 0.9813 \cdot X + 0.7169$   
0.9978

A representative sample of the data and validated matrices are presented. For complete validation information, please refer to the paper published in the Journal of AOAC INTERNATIONAL, "Validation Study of the BioSystems Y15 Histamine Dehydrogenase Kit for the Detection of Histamine in Fish and Fishery Products: AOAC Performance Tested Method<sup>SM</sup> 072001," accessible via the QR code.

AOAC International paper:



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