# CERTIFICATION

## AOAC Research Institute

## *Performance Tested Methods*[SM]

Certificate No.

**070703**

**The AOAC Research Institute hereby certifies the method known as:**

## Veratox® Quantitative Histamine Test

manufactured by

**Neogen Corporation**
**620 Lesher Place**
**Lansing, Michigan 48912**
**USA**

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*[SM] Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*[SM] certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

| | |
|---|---|
| Issue Date | December 27, 2023 |
| Expiration Date | December 31, 2024 |

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**SUBMITTING COMPANY**
**Neogen Corporation**
**620 Lesher Place**
**Lansing, Michigan 48912**

**METHOD NAME**
Veratox® Quantitative Histamine Test

**CATALOG NUMBERS**
9505, 9506

**INDEPENDENT LABORATORY**
Rhode Island Department of Health
50 Orms St.
Providence, RI USA
SAMPLE PREPERATION
Aquatic Food Products Laboratory, University of Florida
P.O. Box 110375
Gainesville, FL USA

**APPLICABILITY OF METHOD**
**Target analytes – The primary amine compound histamine**

**Matrixes – (10 g) – canned tuna, fresh tuna**

**Performance claims – Precision - Less than 10% CV at the 2.5 ppm level, less than 5% CV at the 20 0ppm level, less than 5% CV at the 50 ppm level. Accuracy - 83% agreement with mean values of Canadian Check Sample Program. No observed cross reactivity with closely related compounds. LOD - Defined as the mean value of 10 negative samples + 3 SDs and found to be 0.53 ppm for canned tuna and 0.29 ppm for frozen tuna steak. Range of quantitation - Range at which the results obtained can be accurate. The assay has a range of quantitation between 2.5 and 40 ppm (without additional dilution).**

**REFERENCE METHOD**

**AOAC Official Method 977.13 Histamine in Seafood (35.1.32)**

**ORIGINAL CERTIFICATION DATE**
**June 2007**

**CERTIFICATION RENEWAL RECORD**
**Renewed Annually through December 2024.**

**METHOD MODIFICATION RECORD**
1. **August 2022 Level 2**

**SUMMARY OF MODIFICATION**
1. **Change in substrate composition for regulation and labelling requirements.**

**Under this AOAC *Performance Tested Methods*$^{SM}$ License Number, 070703 this method is distributed by:**
**NONE**

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**NONE**

**PRINCIPLE OF THE METHOD** (1)

Veratox for Histamine is a competitive direct enzyme-linked immunosorbent assay (CD-ELISA) that allows the user to obtain exact concentrations of histamine in parts per million (ppm). Free histamine in the samples and controls is allowed to compete with enzyme-labeled histamine (conjugate) for the antibody binding sites. After a wash step, substrate is added, which reacts with the bound conjugate to produce blue color. More blue color means less histamine. The test is read in a microwell reader to yield optical densities. The optical densities of the controls form the standard curve, and the sample optical densities are plotted against the curve to calculate the exact concentration of histamine.

**DISCUSSION OF THE VALIDATION STUDY (1)**

The independent laboratory validation study demonstrates no significant difference between results generated with the Veratox method and AOAC Method **977.13**. The Veratox method shows a mean 95.4% overall histamine recovery when directly compared to AOAC Method **977.13**. The Veratox method also demonstrated a mean RSD across all samples of 11.68, with a minimum RSD of 5.46 and a maximum RSD of 17.7 compared to AOAC **977.13**, which showed a mean RSD of 11.93 and a minimum RSD of 3.8 and a maximum RSD of 26.8. All other criteria for robustness, cross-reactivity, precision, and accuracy were also met. Based on the material presented in the study, method equivalence has been adequately demonstrated. It is recommended that the Veratox for histamine assay be considered for PTM status.

**Table 4. Comparison of Veratox and AOAC Method 977.13[a]**

| Sample ID | Sample type | Mean result | | |
|---|---|---|---|---|
| | | Immunoassay | Fluorometry | |
| H5 | Tissue | 69.75 | 38.70 | 63.51 |
| H6 | Tissue | 10.50 | 7.91 | 12.50 |
| H7 | Sauce | 10.98 | 6.27 | 9.12 |
| H8 | Sauce | 0.11 | 0.45 | 0.41 |
| H9 | Tissue | 4.13 | 4.48 | 5.11 |
| H10 | Tissue | 11.66 | 14.11 | 14.47 |
| H11 | Sauce | 9.00 | 10.53 | 12.36 |
| H12 | Sauce | 15.38 | 15.15 | 17.87 |
| H13 | Tissue | 1.78 | 1.66 | 2.10 |
| H14 | Tissue | 14.75 | 12.98 | 12.78 |
| H15 | Sauce | 8.85 | 7.39 | 9.93 |
| H16 | Sauce | 12.05 | 7.81 | 10.50 |
| H17 | Tissue | 3.83 | 4.08 | 4.24 |
| H18 | Tissue | 21.81 | 18.21 | 19.08 |
| H19 | Sauce | 30.54 | 30.35 | 34.77 |
| H20 | Sauce | 7.29 | 7.15 | 10.97 |
| H21 | Tissue | 7.12 | 7.03 | 7.04 |
| H22 | Tissue | 11.99 | 13.89 | 12.89 |
| H23 | Sauce | 33.12 | 36.59 | 36.31 |
| H24 | Sauce | 9.37 | 9.21 | 9.28 |
| H25 | Tissue | 14.68 | 21.00 | 20.29 |
| H26 | Tissue | 13.67 | 12.61 | 13.08 |
| H27 | Sauce | 2.60 | 3.86 | 2.79 |
| H28 | Sauce | 17.26 | 22.40 | 23.95 |
| H29 | Tissue | 6.39 | 12.23 | 12.35 |
| H30 | Tissue | 3.18 | 4.47 | 4.34 |
| H31 | Sauce | 24.93 | 22.38 | 22.73 |
| H32 | Sauce | 4.80 | 8.59 | 8.99 |
| H33 | Tissue | 19.80 | 15.06 | 13.90 |
| H34 | Tissue | 15.45 | 6.20 | 6.27 |
| H35 | Sauce | 17.63 | 13.04 | 12.26 |
| H36 | Sauce | 23.66 | 19.27 | 19.35 |
| H37 | Tissue | 10.13 | 8.46 | 8.58 |
| H38 | Tissue | 9.88 | 8.14 | 8.49 |
| H39 | Sauce | 19.15 | 14.28 | 16.30 |
| H40 | Sauce | 24.30 | 20.24 | 21.39 |
| H41 | Tissue | 7.83 | 6.43 | 7.22 |
| H42 | Tissue | 13.28 | 11.24 | 12.66 |
| H43 | Sauce | 24.58 | 20.75 | 22.60 |
| H44 | Sauce | 20.10 | 14.25 | 14.85 |
| H45 | Tissue | 15.10 | 6.51 | 6.90 |
| H46 | Tissue | 15.70 | 11.09 | 11.83 |
| H47 | Sauce | 8.88 | 11.59 | 12.68 |
| H48 | Sauce | 3.75 | 5.88 | 5.77 |

**Table 4. Continued**

| Sample ID | Sample type | Immunoassay | Fluorometry | Mean result |
|---|---|---|---|---|
| H49 | Tissue | 16.20 | 13.69 | 12.06 |
| H50 | Tissue | 4.53 | 4.39 | 4.36 |
| H51 | Sauce | 23.33 | 21.24 | 20.50 |
| H52 | Sauce | 5.03 | 6.92 | 6.15 |
| H53 | Tissue | 20.75 | 22.12 | 21.11 |
| H54 | Tissue | 13.65 | 12.84 | 11.54 |
| H55 | Sauce | 8.45 | 14.30 | 15.15 |
| H56 | Sauce | 23.23 | 35.34 | 34.02 |
| H57 | Tissue | 2.13 | 1.74 | 2.36 |
| H58 | Tissue | 27.53 | 22.68 | 21.07 |
| H59 | Sauce | 13.93 | 15.77 | 16.62 |
| H60 | Sauce | 14.35 | 16.22 | 16.91 |
| H61 | Tissue | 9.68 | 6.26 | 6.51 |
| H62 | Tissue | 23.93 | 15.59 | 15.24 |
| H63 | Sauce | 9.75 | 5.02 | 6.44 |
| H64 | Sauce | 26.13 | 15.50 | 17.18 |
| H65 | Tissue | 27.05 | 22.24 | 20.96 |
| H66 | Tissue | 9.10 | 6.20 | 6.32 |
| H67 | Sauce | 25.40 | 14.61 | 15.08 |
| H68 | Sauce | 23.88 | 11.72 | 13.03 |
| H69 | Tissue | 6.59 | 7.18 | 7.01 |
| H70 | Tissue | 4.04 | 3.36 | 3.65 |
| H71 | Sauce | 3.82 | 4.96 | 5.84 |
| H72 | Sauce | 12.34 | 11.25 | 12.68 |
| H73 | Tissue | 26.53 | 21.39 | 21.91 |
| H74 | Tissue | 7.35 | 6.92 | 7.68 |
| H75 | Sauce | 19.60 | 15.84 | 16.74 |
| H76 | Sauce | 5.08 | 5.28 | 6.21 |
| H77 | Tissue | 10.15 | 11.58 | 11.46 |
| H78 | Tissue | 2.35 | 3.58 | 3.79 |
| H79 | Sauce | 4.62 | 13.63 | 14.76 |
| H80 | Sauce | 2.91 | 5.07 | 5.90 |
| H81 | Tissue | 11.83 | 12.00 | 11.80 |
| H82 | Tissue | 6.70 | 7.27 | 7.30 |
| H83 | Sauce | 5.03 | 5.88 | 6.60 |
| H84 | Sauce | 26.45 | 22.43 | 20.52 |
| H97 | Tissue | 7.05 | 7.25 | 6.65 |
| H98 | Tissue | 9.10 | 11.27 | 10.28 |
| H99 | Sauce | 19.30 | 20.86 | 19.73 |
| H100 | Sauce | 4.34 | 5.32 | 6.59 |

[a] All results expressed in % mg histamine.

**REFERENCES CITED**

1. Lupo, A. and Mozola, M., Validation of a Rapid ELISA for Detection of Histamine in Tuna, AOAC *Performance Tested Methods*℠ certification number 070703
2. AOAC Official Method 977.13 Histamine in Seafood (35.1.32).

3. Fowley, M., Gray, R.L., Roman, B., and Donofrio, R., Level 2 Modification Study to Validate Multiple Veratox® ELISA Methods, AOAC *Performance Tested Methods*℠ certification number 050901. Approved August 3, 2022.