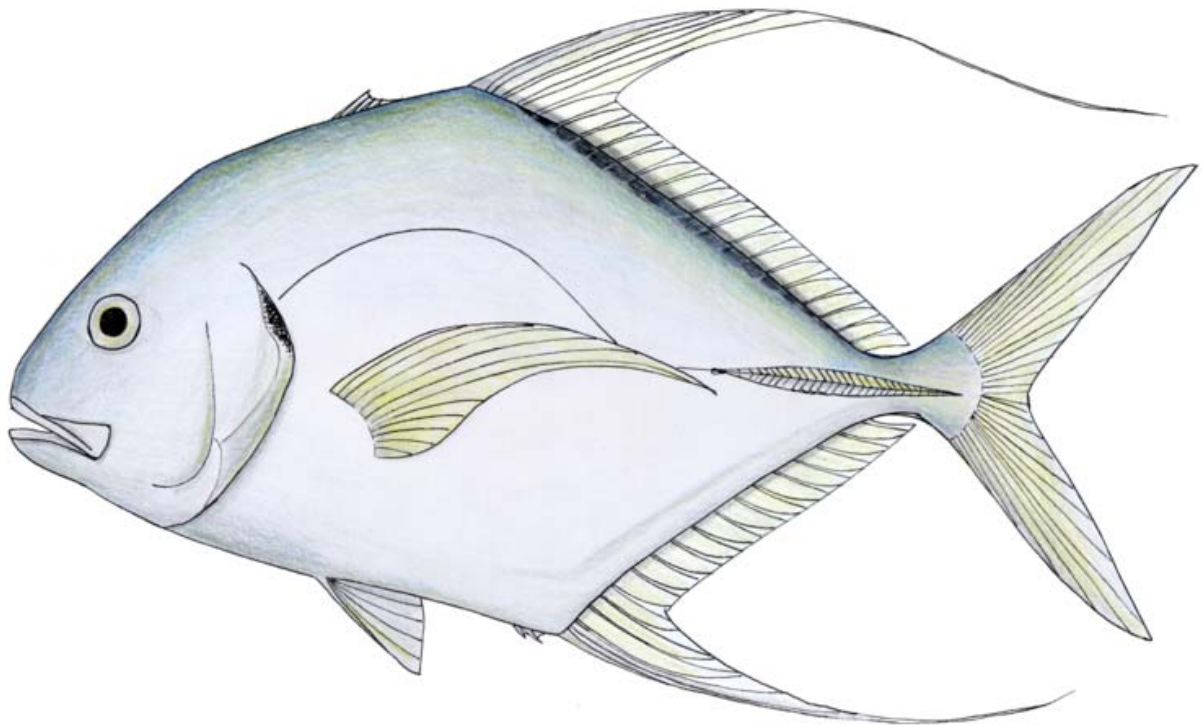




CD/K/581:2010
ICS 67.120.30

EAST AFRICAN STANDARD

Fresh and frozen threadfin — Specification



EAST AFRICAN COMMUNITY

Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in East Africa. It is envisaged that through harmonized standardization, trade barriers which are encountered when goods and services are exchanged within the Community will be removed.

In order to meet the above objectives, the EAC Partner States have enacted an East African Standardization, Quality Assurance, Metrology and Test Act, 2006 (EAC SQMT Act, 2006) to make provisions for ensuring standardization, quality assurance, metrology and testing of products produced or originating in a third country and traded in the Community in order to facilitate industrial development and trade as well as helping to protect the health and safety of society and the environment in the Community.

East African Standards are formulated in accordance with the procedures established by the East African Standards Committee. The East African Standards Committee is established under the provisions of Article 4 of the EAC SQMT Act, 2006. The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

Article 15(1) of the EAC SQMT Act, 2006 provides that "Within six months of the declaration of an East African Standard, the Partner States shall adopt, without deviation from the approved text of the standard, the East African Standard as a national standard and withdraw any existing national standard with similar scope and purpose".

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

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Introduction

In the preparation of this East African Standard, the following sources were consulted extensively:

IS 14892:2000, *Threadfin — Fresh and Frozen — Specification*

CAC/RCP 52:2003(Rev. 4:2008), *Code of practice for fish and fishery products*

IS 4303-1:1975, *Code of hygienic conditions for fish industry — Part 1: Pre-processing stage*

IS 4303-2:1975, *Code of hygienic conditions for fish industry — Part 2: Canning stage*

Codex Alimentarius website: http://www.codexalimentarius.net/mrls/vetdrugs/jsp/vetd_q-e.jsp

USDA Foreign Agricultural Service website: <http://www.mrlatabase.com>

USDA Agricultural Marketing Service website: <http://www.ams.usda.gov/AMSv1.0/Standards>

European Union: http://ec.europa.eu/enterprise/sectors/pharmaceuticals/veterinary-use/maximum-residue-limits/index_en.htm

Assistance derived from these sources is hereby acknowledged.

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Fresh and frozen threadfin — Specification

1 Scope

This standard prescribes the requirements and the method of sampling and test for fresh threadfin and frozen eviscerated threadfin.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

CAC/GL 21, *Principles for the establishment and application of microbiological criteria for foods*

CAC/RCP 1, *Recommended international code of practice — General principles of food hygiene*

CAC/GL 30, *Principles and guidelines for the conduct of microbiological risk assessment*

CAC/GL 31, *Guidelines for the sensory evaluation of fish and shellfish in laboratories*

CD/K/572:2010, *Fish and fisheries products — Methods of sampling*

CAC/RCP 52[CD/K/521:2010], *Code of practice for fish and fishery products*

EAS 35, *Edible salt — Specification*

EAS 12, *Drinking (potable water) — Specification*

EAS 38, *Labelling of prepackaged foods — Specification*

EAS 41, *Fruits, vegetables and derived products — Sampling and methods of test*

EAS 103, *Schedule for permitted food additives*

EAS 123, *Distilled water — Specification*

ISO 4831, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique*

ISO 4832, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique*

ISO 4833, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of microorganisms — Colony-count technique at 30 degrees C*

ISO 6579, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Salmonella spp.*

ISO 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 6887-2, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products*

ISO 6887-3, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products*

ISO 6888-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 1: Technique using Baird-Parker agar medium*

ISO 6888-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 2: Technique using rabbit plasma fibrinogen agar medium*

ISO 6888-3, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 3: Detection and MPN technique for low numbers*

ISO 7251, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique*

ISO 7937, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of Clostridium perfringens — Colony-count technique*

ISO 13720, *Meat and meat products — Enumeration of Pseudomonas spp.*

ISO 16050, *Foodstuffs — Determination of aflatoxin B₁ and the total content of aflatoxin B₁, B₂, G₁ and G₂ in cereals, nuts and derived products — High performance liquid chromatographic method*

ISO 16654, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Escherichia coli O157*

ISO 21567, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Shigella spp.*

ISO/TS 21872-1:2007, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of potentially enteropathogenic Vibrio spp. — Part 1: Detection of Vibrio parahaemolyticus and Vibrio cholerae*

ISO/TS 21872-2:2007, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of potentially enteropathogenic Vibrio spp. — Part 2: Detection of species other than Vibrio parahaemolyticus and Vibrio cholerae*

ISO 11290-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Listeria monocytogenes — Part 1: Detection method*

ISO 11290-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Listeria monocytogenes — Part 2: Enumeration method*

3 Definitions

The term threadfin shall apply to the following species:

Eleutheronema tetradactylum

Polydactylus indicus

Polynemus heptadactylus

4 Grades

4.1 Each threadfin, fresh or frozen shall be either of the following three grades based on the mass of a single threadfin. For frozen threadfin, the mass shall be taken after thawing:

Grade designation	Mass, kg
Extra large	Above 10
Large	5 to 10
Small	Below 5

NOTE The mass of frozen threadfin shall be taken after thawing.

5 Description

The fish (fresh or frozen), its skin and flesh shall have characteristic colour. The gills should be reddish and free from discoloured mucous. The meat and stomach portion should be firm.

6 Requirements

6.1 The material shall be prepared and processed as given in Annex A, under hygienic conditions as prescribed in CAC/RCP 52.

6.2 The material shall be clean, wholesome and free from defects.

6.3 The fish used for freezing shall be whole or gutted. In case the fish is gutted, the entrails, air bladder and the membrane of the gut cavity shall be removed and the eviscerated fish shall be washed thoroughly with clean potable water to remove blood.

6.4 The frozen threadfin, on thawing, shall be in sound, intact and undamaged conditions.

6.5 The product shall be free from foreign matter.

6.6 The material shall conform to the requirements prescribed in Table 1.

6.7 No sample of fresh and frozen threadfin shall contain histamine content exceeding 20 mg/kg when tested by the method given in Annex B.

7 Packing and marking

7.1 Packing

The material shall be packed in suitable container as agreed between the purchaser and the processor. In the absence of any such agreement the material shall be packed in containers which may withstand the stress and strain of transportation and can prevent deterioration during transportation and frozen storage. A layer of polyethylene shall be used between the material and the container when individually frozen threadfins are packed.

7.2 Marking

7.2.1 Each container of fresh material or each wrapped frozen material shall be marked or labelled with the following particulars:

- a) Name and type of the material (fresh or frozen);
- b) Name and address of the processor;
- c) Batch or code number;
- d) Grade;
- e) Net mass;
- f) Date of packing;
- g) The words 'Best before (month and year to be indicated)'; and

h) Any other requirement as given OIML R87, *Quantity of product in prepackages*.

7.2.2 Certification marking

The product may also be marked with the relevant Standard Mark.

Table 1 — Requirements for fresh and frozen threadfin

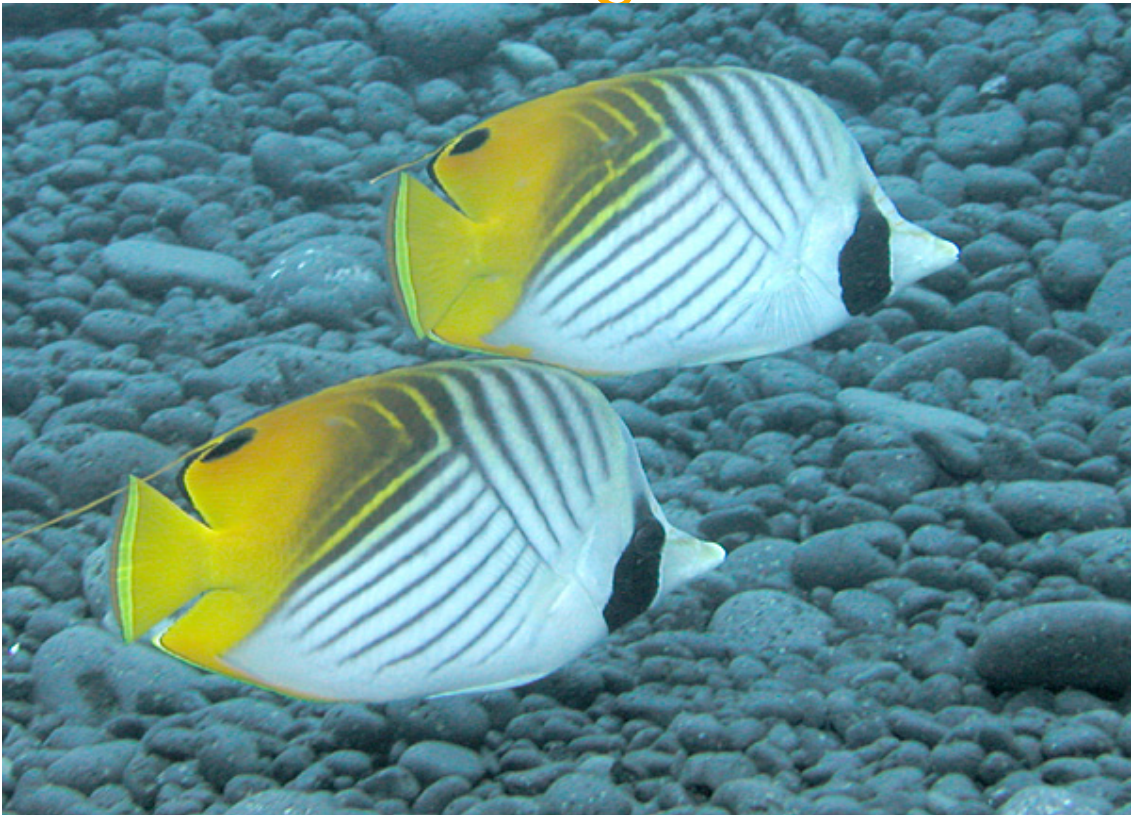
Characteristic	Requirement		Method of test
	Fresh	Frozen	
(1)	(2)	(3)	(4)
i) Total bacterial count/g, in the finished product, Max	100 000	100 000	ISO 4833
ii) <i>Escherichia coli</i> count/g, Max	20	20	ISO 7251
iii) Faecal <i>Streptococci</i> count/g, Max	100	100	Annex C
iv) Coagulase positive <i>Staphylococci</i> /g, Max	100	100	ISO 6888
v) <i>Salmonella</i> , per 25 g	Absent	Absent	ISO 6579
vi) <i>Shigella</i> , per 25 g	Absent	Absent	ISO 21567
vii) <i>Vibrio cholerae</i> , per 25 g	Absent	Absent	ISO/TS 21872
viii) <i>Listeria monocytogenes</i> , per 25 g	Absent	Absent	ISO 11290
ix) Formaldehyde mg/kg, Max	10.0	10.0	Annex D
x) Indole, mg/kg, Max	2.5	2.5	Annex E
xi) Heavy metals:			
a) Mercury, mg/kg, Max	0.5	0.5	EAS 41
b) Copper, mg/kg, Max	20.0	20.0	EAS 41
c) Zinc, mg/kg, Max	50.0	50.0	EAS 41
f) Arsenic, mg/kg, Max	0.1	0.1	EAS 41
e) Lead, mg/kg, Max	0.3	0.3	EAS 41
f) Tin, mg/kg, Max			
(i) For product packed in tin plate	50.0	50.0	EAS 41
(ii) For product packed in other packing containers	250.0	250.0	EAS 41
g) Cadmium	0.3	0.3	EAS 41
h) Methylmercury	0.5	0.5	EAS 41

8 Sampling

The method of drawing representative samples of the material, both fresh and frozen for test and the criteria for conformity shall be according to the method prescribed in CD/K/572:2010.



Frozen golden threadfin



Threadfin butterfly

Draft



Fresh threadfin



Threadfin Shad



Threadfin Geophagus



Frozen threadfin bream

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Annex A
(normative)

Fresh and frozen threadfin

A.1 Fresh threadfin

A.1.1 Preparation

A.1.1.1 The material shall be washed in clean potable water containing 5mg/kg chlorine to remove all adhering impurities and shall be iced immediately in suitable containers.

A.1.1.2 The material shall be grouped according to the grade of the fish (see 4.1).

A.1.1.3 The temperature of the fish in the container shall not exceed 5 °C at any time.

A.2 Frozen threadfin

A.2.1 Processing

A.2.1.1 Clean, wholesome and fresh threadfin which do not show any signs of spoilage shall be used (see 6.1 to 6.6). In case the fish is gutted, the entrails, air bladder and the membrane of the gut cavity shall be removed and the eviscerated fish shall be washed thoroughly with clean potable water to remove the blood.

A.2.1.2 The fish shall be properly iced and maintained at a temperature not exceeding 5 °C till transported to the factory for freezing.

A.2.1.3 The material shall be quick frozen at a temperature not exceeding -40 °C in the minimum possible time and the quick frozen material shall be stored in the cold storage at a temperature not below -23 °C.

A.2.1.4 The material may be grouped according to grades (see 4.1).

Annex B (normative)

Determination of histamine

B.1 Principle

Sample is extracted with 75% (v/v) methanol. Extract is passed through ion exchange column. *o*-Phthaldialdehyde solution is added to eluate to form fluorescent histamine derivatives. Fluorescent intensity of derivatives is measured using fluorometer and histamine is quantified using external standards.

B.2 Apparatus

Rinse all plastic and glass containers with HCl (1 + 3) and H₂O before use.

- (a) **Chromatographic tube** — 200 ×7 id mm polypropylene tube fitted with small plastic stopcocks and ca 45 cm Teflon tubing. Control flow rate at >3 ml/min by adjusting height of column relative to tubing outlet. Alternatively, use 2-way valve in place of tubing.
- (b) **Photofluorometer** — Equipped with medium pressure Hg lamp with excitation at 350 nm and measuring emission at 444 nm.
- (c) **Repipets** — 1 and 5 ml.

B.3 Reagents

- (a) **Ion-exchange resin** — Bio-Rad AG 1-X8, 50–100 mesh or Dowex 1-X8, 50–100 mesh. Convert to -OH form by adding ca 15 ml 2M NaOH/g resin to beaker. Swirl mixture and let stand <30min. Decant liquid and repeat with additional base. Thoroughly wash resin with H₂O, slurry into fluted paper and wash again with H₂O. Prepare resin fresh weekly and store under H₂O. Place glass wool plug in base of tube, B.2(a), and slurry in enough resin to form 8 cm bed. Maintain H₂O level above top of resin bed at all times. Do not regenerate resin in packed column; rather, use batch regeneration in beaker when necessary. Wash column with ca 10 ml H₂O before applying each extract.
- (b) **Phosphoric acid** — 3.57N. Dilute 121.8 ml 85% H₃PO₄ to 1 L. For other concentration H₃PO₄, volume required for 1 L 1.19M acid = 17493/(density H₃PO₄ × percent H₃PO₄). Standardize 5.00 ml by titration with 1.00M NaOH to phenolphthalein end point, and adjust concentration if necessary.
- (c) ***o*-Phthaldialdehyde (OPT) solution** — 0.1% (w/v). Dissolve 100 mg OPT in 100 ml distilled-in-glass methanol. Store in amber bottle in refrigerator. Prepare fresh weekly.
- (d) **Histamine standard solutions** — Store in refrigerator.
 - (1) **Stock solution** — 1 mg/ml as free base. Accurately weigh ca 169.1 mg histamine 2HCl (98%) into 100 ml volumetric flask, and dissolve and dilute to volume with 0.1M HCl. Prepare fresh weekly.
 - (2) **Intermediate solution** — 10 µg/ml. Pipet 1 ml stock solution into 100 ml volumetric flask, and dilute to volume with 0.1M HCl. Prepare fresh weekly.
 - (3) **Working solutions** — 0.5, 1.0, and 1.5 µg/5 ml. Pipet 1, 2, and 3 ml intermediate solution into separate 100 ml volumetric flasks, and dilute each to volume with 0.1M HCl. Prepare fresh daily.

- (e) **Methanol** — 75% (v/v). Place 75 ml MeOH (distilled in glass) into 100 ml volumetric flask or stoppered graduated cylinder. Dilute to volume with H₂O. Swirl flask while adding H₂O.

B.4 Preparation of standard curve

Pipet duplicate 5 ml aliquots of each working standard solution into separate 50 ml glass or polypropylene Erlenmeyers. Pipet in 10 mL 0.1M HCl to each flask and mix. Pipet in 3 ml 1M NaOH and mix. Within 5 min, pipet in 1 ml OPT solution and mix immediately. After exactly 4 min, pipet in 3 ml 3.57NH₃PO₄ and mix immediately. It is important to mix thoroughly after each addition and at least once during OPT reaction. (Run 6– 10 OPT reactions simultaneously by adding reagents to Erlenmeyers in set order.) Prepare blank by substituting 5 ml 0.1M HCl for histamine solution. Within 1.5 h, record fluorescence intensity (*I*) of working standard solutions with H₂O in reference cell, using excitation wavelength of 350 nm and emission wavelength of 444 nm. Plot *I* (corrected for blank) against µg histamine/5 ml aliquot.

B.5 Determination

Extract prepared sample with 75% (v/v) methanol. Pass 4–5 ml H₂O through column, C.2(a), and discard eluate. Pipet 1 ml extract onto column and add 4–5 ml H₂O. Immediately initiate column flow into 50 ml volumetric flask containing 5.00 ml 1.00M HCl. When liquid level is ca 2 mm above resin, add ca 5 ml H₂O and let elute. Follow with H₂O in larger portions until ca 35 ml has eluted. Stop column flow, dilute to volume with H₂O, stopper, and mix. Refrigerate eluate.

Pipet 5 ml eluate into 50 ml Erlenmeyer, and pipet in 10 ml 0.1M HCl. Proceed as in B.4, beginning "Pipet in 3 ml 1M NaOH . . .".

If test sample contains >15 mg histamine/100 g fish, pipet 1 ml sample–OPT mixture into 10 ml beaker containing exactly 2 ml blank–OPT mixture, and mix thoroughly. Read fluorescence of new solution. Dilute and mix aliquots with blank–OPT mixture as needed to obtain measurable reading. This approximation indicates proper dilution of eluate required prior to second OPT reaction needed for reliable quantitation of test sample. Alternatively, use sensitivity range control of fluorometer (if instrument has one) to estimate dilution. Use these approximations to prepare appropriate dilution of aliquot of eluate with 0.1NHCl, and proceed as in B.4, beginning "Pipet in 3 ml 1M NaOH . . .".

B.6 Calculations

Plot of *I* (measured by meter deflection or recorder response and corrected for blank) against µg histamine/5 ml test solution should be straight line passing through origin with slope = $m = [(I_a / 1.5) + I_b + 2I_c] / 3$.

$$\text{mg Histamine/100 g fish} = (10)(F)(1/m)(I_s)$$

$$\mu\text{g Histamine/g fish} = 10 \times (\text{mg histamine/100 g fish})$$

where *I_s*, *I_a*, *I_b*, and *I_c* = fluorescence from test sample, 1.5, 1.0, and 0.5 µg histamine standards, respectively; and *F* = dilution factor = (ml eluate + ml 0.1M HCl)/ml eluate. *F* = 1 for undiluted eluate.

If calibration plot is not linear, use standard curve directly for quantitation. Each subdivision on abscissa should be ≤0.1 µg histamine/5 ml test solution. Read all values from curve to nearest 0.05 µg histamine/5 ml test solution.

$$\text{mg Histamine/100 g fish} = (10)(F)(W)$$

$$\mu\text{g Histamine/g fish} = 10 \times (\text{mg histamine/100 g fish})$$

where *W* = µg histamine/5 ml test solution as determined from standard curve.

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