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EAST AFRICAN STANDARD

Fresh, frozen and canned mackerel — Specification

EAST AFRICAN COMMUNITY

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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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East African Community

P O Box 1096

Arusha

Tanzania

Tel: 255 27 2504253/8

Fax: 255-27-2504481/2504255

E-Mail: eac@eachq.org

Web: www.each.int

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Introduction

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

IS 14891:2001, *Mackerel — Fresh, Frozen and Canned — 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.mrldatabase.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, frozen and canned mackerel — Specification

1 Scope

1.1 This standard prescribes the requirements and methods of sampling and test for fresh, frozen and canned mackerel.

1.2 The term mackerel shall apply to the *Rastrelliger kanagurta* species.

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 66-3:2000, *Tomato products — Specification — Part 3: Tomato concentrates (puree and paste)*

EAS 66-4:2000, *Tomato products — Specification — Part 4: Highly seasoned tomato products (sauce and ketchup)*

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.*

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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 6887-3, *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 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 Grades

Fresh mackerel and frozen mackerel shall be of the following two grades:

Grade Designation	*Mass*
Large Above	85 g
Small	85 g and below

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

4 General requirements

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

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5 Requirements for fresh mackerel

5.1 Description

The fish, its skin and flesh shall have characteristic colour, free from any discolouration. The gills should be bright red in colour and free from discoloured mucous. The meat and stomach portion should be firm and shall not leave a mark when lightly pressed with finger.

5.2 The material shall also conform with the requirements given in Table 1.

6 Requirements for frozen mackerel

6.1 Fresh mackerel for freezing shall conform with the requirements given in Clause 5.

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

6.3 The fish may be whole or gutted. The entrails, gills, air-bladder and the membrane of the gut cavity shall be removed. Eviscerated fish shall be washed thoroughly with clean water to remove the blood.

6.4 The frozen mackerel, on thawing, shall be in sound intact and undamaged conditions and free from defects.

6.5 The product shall be free from any foreign matter.

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

Table 1 — Requirements for fresh and frozen mackerel

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) *Escherichia coli* count/g, Max	20	10	ISO 7251
iii) Faecal *Streptococci* count/g, Max	100	100	Annex H
iv) Coagulase positive *Staphylococci*/g, Max	100	100	ISO 6888
V) *Salmonella*, per 25 g	Absent	Absent	ISO 6579
vi) *Shigella,* per 25 g	Absent	Absent	ISO 21567
vii) *Vibrio cholerae,* per 25 g	Absent	Absent	ISO/TS 21872
viii) *Listeria monocytogenes,* per 25 g	Absent	Absent	ISO 11290
ix) Formaldehyde mg/kg, Max	10.0	10.0	Annex F
x) Indole, mg/kg, Max	2.5	2.5	Annex G
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

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6.7 No sample of fresh and frozen mackerel shall contain histamine content exceeding 20 mg/kg when tested by the method given in Annex B of IS 4793.

7 Requirements for canned mackerel

7.1 Raw material

The material to be canned may either be fresh or frozen and shall conform to the requirements given in 5 or 6 respectively.

7.1.1 The material to be canned shall be free from heads gills, tail tips and fins. Entrails shall be completely removed.

7.2 Other ingredients

One or more of the following ingredients which are of food grade quality shall be used depending on type of presentation: edible oil, common salt (EAS 35), tomato ketchup (EAS 66-4), tomato puree (EAS 66-3), spices and condiments.

7.3 The material shall be free from artificial colouring matter and firming agents except common salt. Food additives for fish and fisheries products complying with EAS 103 may be used.

7.4 Presentation

The product shall be presented in one or more of the following packing media: own juice, brine or water, edible oil, tomato sauce or curry.

7.5 The can shall not show any visible external defects like denting, panelling, swelling or rusting.

7.5.1 The contents of the can on opening shall not display any appreciable disintegration. Pieces from which portions have separated out would be treated as disintegrated units.

The percentage of detached portion of fish calculated on the basis of the drained mass shall not exceed 5 percent by mass based on the average of 5 cans.

7.6 The product shall have the odour flavour and colour characteristic of the species.

7.7 The product shall be free from foreign materials, filth and from grittiness.

7.8 The can shall give a negative pressure when punctured. If round cans are used, the vacuum shall be not less than 100 mm of Hg. when measured at 27 ± 2°C with a vacuum gauge of the piercing type or an electric vacuum recorder.

7.9 The drained mass of the contents in each can shall be not less than 65 percent of the net water capacity of the can as tested by the method given in Annex B. A tolerance of ±5 percent is permitted provided average content of fish on the basis of 5 cans lot shall not be less than 60 percent of the net mass.

7.10 The percentage of sodium chloride in the final product shall be 3.5 percent in the case of brine treated cans when tested by the method given in Annex C. The acidity of brine as citric acid anhydrous shall be between 0.06 to 0.20 percent (m/v) when tested by the method given in Annex D.

7.11 No sample of canned mackerel shall contain histamine content exceeding 20 mg/100 g when tested by the method given in Annex B of IS 4793.

7.12 The canned mackerel shall also conform to the requirements prescribed in Table 2.

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Table 2 — Requirements for mackerel, canned

	Type of contaminant	Maximum limit (mg/kg)	Method of test
(i)	Microbiological requirements	Shall be commercially sterile	See 8.6
(i)	Arsenic	0.1	EAS 41
(ii)	Copper	0.4	EAS 41
(iii)	Iron	5.0	EAS 41
(iv)	Tin		
	(a) For product packed in tin plate	50.00	EAS 41
	(b) For product packed in other packing containers	250.00	EAS 41
(v)	Mercury	0.5	EAS 41
(vi)	Lead	0.3	EAS 41
(vii)	Cadmium	0.3	EAS 41
(viii)	Methylmercury	0.5	EAS 41
(ix)	Zinc	50.0	EAS 41

8 Packing

8.1 Mackerel (fresh and frozen) 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 prevent deterioration during transportation and frozen storage. A layer of food grade polyethylene shall be used between the material and the container when individually frozen mackerels are packed.

8.2 Canned mackerel shall be packed in suitable containers, free from rust and hermetically sealed.

Cans shall be lacquered, the lacquer used shall be non-toxic and shall be of such quality that it does not impart any foreign taste and smell to the contents of the cans and does not peel off during processing and storage of the product. The lacquer shall not be soluble in oil or brine

9 Marking

9.1 Each package/container of fresh, frozen or canned mackerel shall be marked or labelled with the following particulars:

a) Name and type of the material with indication of fresh, frozen or canned;

b) Name and address of the processor;

c) Batch or code number;

d) Grade in case of fresh or frozen mackerel;

e) Net mass;

f) name of packing medium for canned mackerel;

g) If the fish has been smoked or smoke flavoured, this information shall appear in close proximity to the name;

h) List of ingredients in descending order;

i) Date of packing;

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j) The words 'Best before ……………. (month and year to be indicated)'; and

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

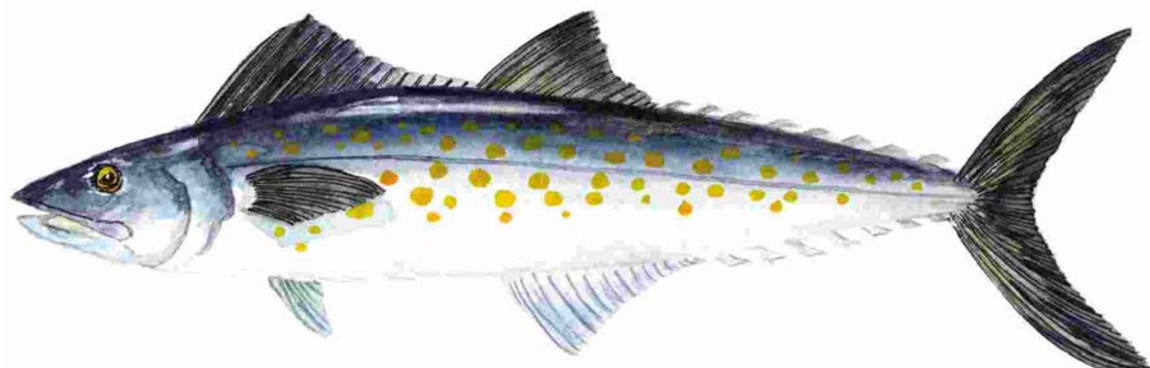
9.2 Certification marking

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

10 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 Indian ocean mackerel



Spanish mackerel

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Fresh frozen mackerel

Mackerel fillets

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Horse mackerel

Indian ocean mackerel

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Horse mackerel

Mackerel canned in own juice

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Mackerel canned in tomato sauce

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Frozen mackerel

Frozen mackerel

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Frozen Spanish mackerel

Frozen mackerel

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Annex A
(normative)

Preparation and processing of mackerel, fresh, frozen and canned

A.1 Fresh mackerel

A.1.1 Preparation

A.1.1.1 The material shall be washed in clean potable water containing 5 mg/kg chlorine to remove all adhering impurities and shall be iced immediately in suitable containers. The top, bottom and sides shal1 be covered with a layer of crushed ice.

A.1.1.2 The material shal1 be grouped according to the grade of the fish (*see* 3.1).

A.1.1.3 The temperature of the fish in the container shal1 not exceed 5 °C at any time.

A.2 Frozen mackerel

A.2.1 Processing

A.2.1.1 Clean, wholesome and fresh mackerel (see A.1.1.1) which do not show any signs of spoilage shall be used.

A.2.1.2 To prevent belly bursting, the fish may be given a dip treatment in 15 percent brine for 30 min before freezing.

A.2.1.3 The material shall be properly arranged and quick frozen quickly 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 of -23 °C or below throughout.

A.2.1.4 The material shall be packed according to grade.

A.3 Canned mackerel

A.3.1 Processing

A.3.1.1 Processing shall be at such a temperature and for such length of time as will ensure thorough cooking and commercial sterility. The water used for cooling cans shall be maintained in clean condition and chlorinated to maintain a minimum free residual chlorine concentration of one ppm.

A.3.1.2 Fish curry, if used shall be prepared as agreed to between the purchaser and the producer, care being taken that during preparation all the fish juices or other ingredients are retained in full.

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Annex B
(normative)

Determination of drained mass and washed drained mass

B.1 Apparatus

B.1.1 Sieve — 2.00 mm for drained mass; 2.8 mm for washed drained mass.

B-2 Procedure

B.2.1 Drained mass for packs in own juice, brine, water or oil

B.2.1.1 Weigh the unopened containers that have been kept at a temperature of not less than 20°C or more than 24°C for a minimum of 12 h prior to examination.

B.2.1.2 After opening, tilt the containers so as to distribute the contents over the meshes of the tared (pre-weighed) circular sieve. Incline the sieve at an angle of approximately 17° - 20° and allow the product to drain 2 min, measured from the time the product is poured into the sieve.

NOTE Collect the drained liquid fur determination of sodium chloride in brine (see C.2).

B.2.1.3 Remove adhering liquids from bottom of the sieve by use of a paper towel. Weigh the sieve containing the drained contents.

B.2.2 Washed drained mass for packs with sauces and curries, also with optional ingredients

B.2.2.1 Weigh the unopened containers that have been kept at a temperature of not less than 20°C or more than 24°C for a minimum of 12 h prior to examination.

B.1.2.1 After opening, tilt the container and wash first the covering sauce and then the full contents with hot tap water (approx 40°C) using a wash bottle on the tared circular sieve.

B.2.2.3 Wash the contents of the sieve with hot water until free of adhering sauce; where necessary separate optional ingredients (spices, vegetables, fruits) with pincers. Incline the sieve at an angle of approximately 17°-20° and allow the contents to drain 2 min, measured from the time the washing procedure has finished.

B.2.2.4 Remove adhering water from the bottom of the sieve by use of a paper towel. Weigh the sieve containing the washed drained contents.

B.2.3 Determination or water capacity of container

B.2.3.1 Select a container which is undamaged in all respects.

B.1.3.1 Wash, dry and weigh the empty container after cutting out the lid without removing or altering the height of the double seam.

B.2.3.3 Fill the container with distilled water at 20° to 5 mm vertical distance below the top level of the container, and weigh the container thus filled.

B.2.3.4 Subtract the mass found in B.2.3.2 from the mass found in B.2.3.3. The difference shall be considered to be the weight of water required to fill the container.

B.3 Calculations

B.3.1 The percentage *m*/*m* drained or washed drained mackerel or jack mackerel is given by the

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following equation:

$$\frac{m_2 - m_1}{m_w} \times 100$$

where

m_1 = mass of the sieve,

m_2 = mass of the sieve plus drained or washed drained product, and

m_w = water capacity of the container.

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Annex C
(normative)

Determination of sodium chloride

C.1 Reagents

C.1.1 Standard Silver Solution — 0.1 N, standardized against 0.1 N sodium chloride solution.

C.1.2 Dilute Nitric Acid — 1:4.

C.1.3 Ferric ammonium indicator solution — A saturated solution of ferric alum $Fe (NH_4) (SO_4)_2.12H_2O$.

C.1.4 Standard Potassium Thiocyanate Solution — 0.1N

C.2 Procedure

C.2.1 Wash the emptied can thoroughly with water and wash the residue on the sieve at least thrice with cold water. Collect the drained liquid and all the washings together in a 1 000 ml graduated flask and make up the volume. Centrifuge the made-up liquid for at least 5 min at 1 000 rev/min.

C.2.2 Take a suitable aliquot of the clear supernatant solution prepared as in C.2.1, add a known volume of the standard silver nitrate solution in slight excess and then add 20 ml of dilute nitric acid. Boil gently on a hotplate or a sand-bath until all solids except silver chloride dissolve (usually 15 min). Cool, add 50 ml of water and 5ml of the ferric alum indicator solution and titrate with the standard ammonium thiocyanate solution until permanent light brown colour appears.

C.3 Calculation

C.3.1 Sodium chloride, per cent by weight

$$= 5.85 \frac{(V_1 N_1 - V_2 N_2)}{W}$$

where,

V_1 = volume of the standard silver nitrate solution;

V_2 = volume of the standard potassium thiocyanate;

N_1 = normality of the standard silver nitrate solution;

N_2 = normality of the standard potassium thiocyanate; and

W = weight, in g, of the dried product taken for the test.

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Annex D
(normative)

Determination of histamine

D.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.

D.2 Apparatus

Rinse all plastic and glass containers with HCl (1 + 3) and H_2O 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 m.

D.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 m 2M NaOH/g resin to beaker. Swirl mixture and let stand <30min. Decant liquid and repeat with additional base. Thoroughly wash resin with H_2O, slurry into fluted paper and wash again with H_2O. Prepare resin fresh weekly and store under H_2O. Place glass wool plug in base of tube, D.2(a), and slurry in enough resin to form 8 cm bed. Maintain H_2O 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 H_2O before applying each extract.

(b) **Phosphoric acid** — 3.57N. Dilute 121.8 ml 85% H_3PO_4 to 1 L. For other concentration H_3PO_4, volume required for 1 L 1.19M acid = 17493/(density H_3PO_4 × percent H_3PO_4). 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.

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(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 H_2O. Swirl flask while adding H_2O.

D.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 3.57NH_3PO_4 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 H_2O 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.

D.5 Determination

Extract prepared sample with 75% (v/v) methanol. Pass 4–5 ml H_2O through column, D.2(a), and discard eluate. Pipet 1 ml extract onto column and add 4–5 ml H_2O. 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 H_2O and let elute. Follow with H_2O in larger portions until ca 35 ml has eluted. Stop column flow, dilute to volume with H_2O, stopper, and mix. Refrigerate eluate.

Pipet 5 ml eluate into 50 ml Erlenmeyer, and pipet in 10 ml 0.1M HCl. Proceed as in D.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 . . .".

D.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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Annex E
(normative)

Determination of acidity in brine

E.1 Reagents

E.1.1 Standard sodium hydroxide solution

0.1N.

E.1.2 Phenolphthalein indicator solution

Dissolve one gram of phenolphthalein in 100 ml of 95 percent (m/v) alcohol.

E.2 Procedure

E.2.1 Take a suitable aliquot of the brine solution (see C.2.1), add about 200 ml of water and titrate against the standard sodium hydroxide solution using phenolphthalein indicator solution. Calculate the percentage acidity of the brine in terms of citric acid from the relationship: 1ml of 0.1 N sodium hydroxide solution is equivalent to 0.0064 g of citric acid (anhydrous).

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Annex F
(normative)

Determination of formaldehyde

F.1 Principle

Quantitative determination of formaldehyde involves extraction of the fish muscle tissue with trichloroacetic acid for the removal of proteins and treatment with acetyl acetone — ammonium acetate reagent to form a coloured formaldehyde derivative. The absorbance is measured by spectrophotometer.

F.2 REAGENTS

a) Trichloroacetic acid (10 percent solution);

b) Sodium hydroxide (30 percent solution);

c) Acetic acid (5 percent solution); and

d) Acetyl Acetone — ammonium acetate reagent. To prepare 1 litre of the reagent, dissolve 150 g of ammonium acetate, 3 ml of glacial acetic acid and 2 ml of acetyl acetone in distilled water (*see* EAS 153) and make up volume to 1 litre.

F.3 Extract

Mince the fish muscle tissue using a homogenizer. Macerate 10 g of the fish mince in 10 percent trichloroacetic acid for 2 minutes using a mortar and pestle and then filter through a filter paper (Whatman No. 42) and make up the volume to 100 ml in a standard volumetric flask.

F.4 PROCEDURE

Place 5 ml trichloroacetic acid extract of the tissue in a 50 ml beaker. Add 10 ml of distilled water (see EAS 153) and make the solution alkaline with a few drops of 30 percent NaOH and adjust the pH to 6.0 with 5 percent acetic acid. Make up the solution to 25 ml with water and mix 5 ml of it with 5 ml of acetyl acetone — ammonium acetate reagent. Keep the mixture still for 50 minutes at 37°C and read the colour at a wave length of 410 'nm'.

For a blank, take distilled water in duplicate in the place of sample and for standard, prepare a series of tubes using 100 pg formaldehyde standard solution, concentration ranging from 0-80 pg.

F.5 Calculations

$$\text{Formaldehyde (mg, percent)} = \frac{\text{Absorbance of sample} \times \text{Concentration in standard} \times 25 \times 100 \times 100}{\text{Absorbance of standard} \times 5 \times 5 \times 10 \times 1000}$$

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Annex G
(normative)

Determination of indole

G.1 Principle

Indole is extracted with light petroleum from trichloroacetic acid — precipitated shrimp muscle. The extracted indole, soluble in light petroleum, is reacted and re-extracted with Ehrlich's reagent. Indole in the form of a rose indole complex can be determined spectrophotometrically.

G.2 Apparatus

a) Spectrophotometer, and

b) Refrigerated centrifuge.

G.3 Reagents

a) Trichloroacetic Acid (TCA) - Dissolve 6 g TCA in 100 ml distilled water.

b) Light petroleum (Boiling point 40-60 °C)

c) Ehrlich's Reagent — Dissolve 9 g paradimethylaminobenzaldehyde (PABA) in 45 ml concentrated HCl in 250 ml volumetric flask and dilute to volume with ethanol.

d) Standard Indole Solutions — Accurately prepare stock solution of 10 mg indole in 100 ml light petroleum. Use 1: 10 dilution as working solution. Refrigerate indole solutions.

G.4 Procedure

G.4.1 Homogenize 40 g shrimp with 80 ml ice-cold trichloroacetic acid solution (TCA) in a waring blender for 1 minute. Add 80 ml ice-cold light petroleum and blend for 1 minute. Transfer homogenate to 250 ml centrifuge bottle and centrifuge for 10 minutes at 10 000 rev/min. Filter supernate through Whatman No. 1 paper under suction. Transfer filtrate to 250 ml separatory funnel. After the two layers have separated, tansfer acid layer (lower) to second 250 ml separatory funnel.

G.4.2 Wash TCA

Denatured protein precipitate (separated by centrifugation) with 40 ml light petroleum and filter as described above. Transfer filtrate to second 250 ml separatory funnel already containing TCA layer from first extraction. Shake for 1 minute and let two layers separate. Transfer lower acid layer to third separatory funnel and extract for third time with 40 ml light petroleum.

G.4.3 Combine all light petroleum extracts into 1 separatory funnel. Extract indole with exactly 5 ml freshly prepared Ehrlich's reagent by vigorously shaking for 1 minute. The rose indole complex formed is quantitatively transferred to Ehrlich's reagent layer. When layers have separated, transfer lower layer to 1 cm path cell and read at 570 nm against reagent black solution.

G.4.4 Prepare standard curve as follows: Accurately measure volumes from 0.5 to 4 ml stock indole solution (working solution) and add into 80 ml TCA in a separatory funnel. Extract indole by procedures described above and construct standard curve. Rose indole complex from indole standard and from TCA-extracted shrimp is stable up to 4 h.

G.5 Calculation

With the help of the standard curve the amount of indole present in 40 g shrimp can be determined. Indole content is usually expressed as the amount of indole in microgram per 100 g shrimp muscle.

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Annex H
(normative)

Determination of faecal streptococci count

H.1 Medium

H.1.1 Sterile KF agar with the following composition shall be used:

Proteose peptone No. 3	10 g
Yeast extract	10 g
Sodium chloride AR	5 g
Sodium glycerophosphate	10 g
Maltose CP	20.0 g
Lactose	1 g
Sodium azide	0.49 g
Sodium carbonate AR	0.0636 g
Bromocresol purple	0.015 g
Agar	10 g
Distilled water (see EAS 153)	1 litre

H.1.1.1 Dissolve the agar in 750 ml of the distilled water by steaming. All ingredients mentioned above (H.1.1) except bromocresol purple and sodium carbonate are dissolved separately in 250 ml of distilled water. Mix the two solutions well. Add sodium carbonate in small portions and then filter through absorbent cotton. Add Bromocresol purple in the filterate and mix well. Distribute in appropriate quantities and sterilize in an autoclave at 121 °C for 15 minutes.

NOTE In case compounded agar medium is used, follow manufacturer's instructions for sterilization.

H.1.2 Add 1 ml of 1.0 percent solution of 2, 3, 5 triphenyl tetrazolium chloride per every 100 ml of the melted and cooled agar prior to use.

H.1.3 Pour 1 ml each of the 0.1 and 0.01 dilution sample to two separate sterile petri dishes. Add the cooled agar (nearly 10 ml). Mix it by rotating. Incubate the plates at 37 °C for 48 h. Count the red and pink colonies and compute their number per gram.

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