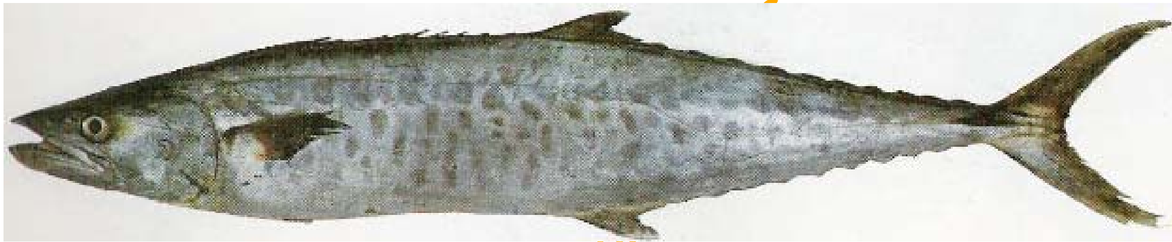
CD/K/562:2010
ICS 67.120.30

EAST AFRICAN STANDARD

Fresh seer fish (*Scomberomorus spp.*) — Specification



EAST AFRICAN COMMUNITY

Draft for comments only — Not to be cited as East African Standard

© EAC 2010 First Edition 2010

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.

© East African Community 2010 — All rights reserved*

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

Draft for comments only — Not to be cited as East African Standard

* © 2010 EAC — All rights of exploitation in any form and by any means reserved worldwide for EAC Partner States' NSBs.

 © EAC 2010 — All rights reserved

Introduction

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

IS 6123:1971(R2005), *Specification for Seer Fish (Scomberomorus spp.), Fresh*

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.

Draft for comments only — Not to be cited as East African Standard

Contents

1 Scope ... 1
2 Normative references ... 1
3 Types ... 2
4 Grades ... 3
5 Requirements ... 3
6 Packing and marking ... 4
6.1 Packing ... 4
6.2 Marking ... 4
7 Sampling and methods of analysis ... 4
7.1 Sampling of fresh seer fish (*Scomberomorus* spp.) ... 4
7.2 Determination of flavor and texture ... 5
Annex A (normative) Determination of histamine ... 6
Annex B (normative) Determination of formaldehyde ... 8
Annex C (normative) Determination of indole ... 9
Annex D (normative) Determination of faecal streptococci count ... 10

Draft for comments only — Not to be cited as East African Standard

 © EAC 2010 — All rights reserved

Fresh seer fish (*Scomberomorus spp.*) — Specification

1 Scope

1.1 This standard prescribes the requirements and the methods of sampling and test for seer fish *(Scomberomorus* spp.), fresh.

1.2 The term 'seer fish' shall apply to the following commercial species:

a) *Scomberomorus guttatum;*

b) *Scomberomorus commersonii;*

c) *Scomberomorus lineotatus;*, and

d) *Scomberomorus kuhillii.*

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*

Draft for comments only — Not to be cited as East African Standard

© EAC 2010 — All rights reserved

Draft for comments only — Not to be cited as East African Standard

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 6887-3, *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, *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, *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 Types

Fresh seer fish shall be of the following two types:

a) Whole, round; and

b) Eviscerated, head-on.

© EAC 2010 — All rights reserved

4 Grades

4.1 The material shall be of the following three grades:

Grade designation	Weight in kg	
	Whole	Eviscerated
Large	Above 5	Above 3.75
Medium	2 to 5	1.50 to 3.75
Small	Below 2	Below 1.50

5 Requirements

5.1 The material shall be clean, wholesome and fresh and shall not show any sign of spoilage. The material shall be handled and transported under sanitary conditions complying with CAC/RCP 52.

5.2 The material shall be washed in clean potable water to remove all adhering impurities and shall be iced immediately in suitable containers. The washed seer fish, preferably precooled, shall be properly and adequately iced in suitable containers. Eviscerated fish shall be prepared by removing the entrails, air bladder and membrane of the gut cavity and finally washing well with potable water containing 5 ppm of chlorine.

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

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

5.5 The material may also satisfy the microbiological and heavy metal limits given in Table 2.

NOTE The microbiological limits are of recommendatory nature only.

Table 1 — Requirements for fresh seer fish (*Scomberomorus* spp)

Characteristic	Requirement	Method of test
(1)	(3)	(4)
i) Colour of the fish	Characteristic colour	—
ii) Colour and appearance of eyes	Bright	—
iii) Gills	Bright red and almost free from discoloured mucous	—
iv) Appearance of skin	Clean	—
V) Colour of flesh	Characteristic colour	—
vi) Meat and stomach portion	Firm, shall not leave a mark when lightly pressed with finger	—
vii) Odour	Characteristic odour	—
viii) Flavour on cooking	Characteristic flavour	Annex A
ix) Texture on cooking	Firm, soft and non-fibrous	Annex A

Table 2 — Microbiological and heavy metal limits for fresh seer fish

Characteristic		Method of test
	Fresh	
(1)	(2)	(4)
i) Total bacterial count/g, in the finished product, Max	100 000	ISO 4833
ii) *Escherichia coli* count/g, Max	20	ISO 7251
iii) *Salmonella*, per 25 g	Absent	ISO 6579
xi) Heavy metals:		
a) Mercury, mg/kg, Max	0.5	EAS 41
b) Copper, mg/kg, Max	20.0	EAS 41
c) Zinc, mg/kg, Max	50.0	EAS 41
f) Arsenic, mg/kg, Max	0.1	EAS 41
e) Lead, mg/kg, Max	0.3	EAS 41

Draft for comments only — Not to be cited as East African Standard

© EAC 2010 — All rights reserved

Draft for comments only — Not to be cited as East African Standard

6 Packing and marking

6.1 Packing

The material shall be packed in containers such as polythene lined dealwood boxes of sufficient strength to withstand the stress and strain during transportation. The seer fish shall be adequately covered with crushed ice all-round, with an additional layer on top. Insulated containers may be preferably used for transportation in uninsulated carriers. The containers shall be of such material that it does not impair the quality of the material in any way.

6.2 Marking

The container shall be marked or labelled with the following particulars:

a) Name, type and grade of the material;

b) Name and address of the vendor;

c) Date of packing;

d) Net weight of fish in kilograms; and

e) Lot number and code.

7 Sampling and methods of analysis

7.1 Sampling of fresh seer fish (*Scomberomorus* spp.)

7.1.1 General

7.1.1.1 The samples after subjecting to the test given in 5.3 shall be stored in such a manner that there is no deterioration of the material during storage.

7.1.1.2 The samples shall be protected against adventitious contamination.

7.1.1.3 The sampling instruments shall be clean, dry and sterile.

7.1.2 Scale of sampling

7.1.2.1 Lot — All the containers in a single consignment of the material packed on the same day and of the same grade shall constitute a lot. If the consignment is declared to consist of material packed on different dates, the material shall be separated datewise and the containers of the same type and grade shall be grouped to constitute a lot.

Samples shall be tested from each lot for ascertaining conformity of the material to the requirement of this specification.

7.1.2.2 The number of containers to be selected from a lot shall depend on the size of the lot and shall be in accordance with col 1 and 2 of Table 3.

7.1.2.3 The containers shall be selected at random. In order to ensure randomness of selection, a random number table shall be used. If such tables are not available the following procedure may be adopted:

Starting from any container in the lot, count them as 1, 2, 3, . . . , etc, up to r in one order, where r is equal to the integral part of the value of N/n, N being the total number of containers, and n the number of containers to be chosen (see Table 3). Every rth container thus counted shall be separated until the requisite number of containers is obtained from the lot to give samples for test.

 © EAC 2010 — All rights reserved

7.1.2.4 From each of the selected containers, in order to select at random the required number of fresh seer fish, the Table 3 may be applied here. Column 1 may be taken to represent the number of seer fish in a container and col2 may be taken to represent the number of fresh seer fish to be selected.

Table 3 — Selection of containers

Number of containers in the lot	Sample size
(1)	(2)
2 to 15	2
16 to 40	3
41 to 65	5
66 to 110	7
111 to 180	8
181 to 300	9
301 and above	10

7.1.3 Number of tests

Each of the fresh seer fish selected in 7.1.2.4 shall be tested for all the requirements of this specification.

7.1.4 Criterion for conformity

The lot shall be declared to be in conformity with all the requirements of this specification when all the fresh seer fish selected in 7.1.2.4 satisfy the corresponding requirements (see Clause 5).

7.2 Determination of flavor and texture

7.2.1 Determine the flavour and texture of the material after cooking as given in 7.2.2.

7.2.2 Place a transverse section of fish in boilable film pouch with some salt for taste. Immerse in boiling water and cook for internal temperature of the muscle reaches 70% in about 20 minutes. Cool and determine the flavour and texture.

Fresh seer fish

Draft for comments only — Not to be cited as East African Standard

© EAC 2010 — All rights reserved

Draft for comments only — Not to be cited as East African Standard

Annex A
(normative)

Determination of histamine

A.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.

A.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.

A.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, A.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.

© EAC 2010 — All rights reserved

(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.

A.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.

A.5 Determination

Extract prepared sample with 75% (v/v) methanol. Pass 4–5 ml H_2O through column, A.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 A.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 . . .".

A.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.

Draft for comments only — Not to be cited as East African Standard

© EAC 2010 — All rights reserved

Annex B
(normative)

Determination of formaldehyde

B.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.

B.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.

B.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.

B.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.

B.5 Calculations

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

Draft for comments only – Not to be cited as East African Standard

© EAC 2010 — All rights reserved

Draft for comments only — Not to be cited as East African Standard

Annex C
(normative)

Determination of indole

C.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.

C.2 Apparatus

a) Spectrophotometer, and

b) Refrigerated centrifuge.

C.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.

C.4 Procedure

C.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, transfer acid layer (lower) to second 250 ml separatory funnel.

C.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.

C.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.

C.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.

C.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.

© EAC 2010 — All rights reserved

Annex D
(normative)

Determination of faecal streptococci count

D.1 Medium

D.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

D.1.1.1 Dissolve the agar in 750 ml of the distilled water by steaming. All ingredients mentioned above (D.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.

D.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.

D.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.

Draft for comments only – Not to be cited as East African Standard

© EAC 2010 — All rights reserved

Draft for comments only — Not to be cited as East African Standard

© EAC 2010 — All rights reserved