



DRAFT EAST AFRICAN STANDARD

Toothpaste — 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 the East African Community. It is envisaged that through harmonized standardization, trade barriers that are encountered when goods and services are exchanged within the Community will be removed.

The Community has established an East African Standards Committee (EASC) mandated to develop and issue East African Standards (EAS). The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the public and private sector organizations in the community.

East African Standards are developed through Technical Committees that are representative of key stakeholders including government, academia, consumer groups, private sector and other interested parties. 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 Principles and procedures for development of East African Standards.

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.

The committee responsible for this document is Technical Committee EASC/TC 078, *Healthcare and medical devices*.

Attention is drawn to the possibility that some of the elements of this document may be subject of patent rights. EAC shall not be held responsible for identifying any or all such patent rights.

This second edition cancels and replaces the first edition (EAS 187:2020), which has been technically revised.

Introduction

The need for the development of an East African Standard on toothpaste has arisen from the fast expanding toothpaste industry within the region. Toothpaste assists in the removal of daily accumulation of debris and deposits from the exposed surface of the teeth without causing injury to the teeth and mucous membrane of the mouth. In addition, it may remove stain and odour but it shall not remove enamel of the teeth.

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Toothpaste — Specification

1 Scope

This Draft East African Standard specifies the requirements, sampling and test methods for fluoridated and non-fluoridated toothpaste.

This standard is not applicable to toothpaste for which therapeutic claims are made, tooth powder, toothpaste tablets, oral rinse, mouth wash and mouth rinse.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

EAS 377 (all parts), *Cosmetics and cosmetic products*

EAS 847-16, *Cosmetics Analytical methods Part 16: Determination of lead, mercury and arsenic content*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 18416, *Cosmetics — Microbiology — Detection of *Candida albican**

ISO 21149, *Cosmetics — Microbiology — Enumeration and detection of aerobic mesophilic bacteria*

ISO 21150, *Cosmetics — Microbiology — Detection of *Escherichia coli**

ISO 21392, *Cosmetics — Analytical methods — Measurement of traces of heavy metals in cosmetic finished products using ICP/MS technique*

ISO 22716, *Cosmetics — Good Manufacturing Practices (GMP) — Guidelines on Good Manufacturing Practices*

ISO 22717, *Cosmetics — Microbiology — Detection of *Pseudomonas aeruginosa**

ISO 22718, *Cosmetics — Microbiology — Detection of *Staphylococcus aureus**

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at <http://www.iso.org/obp>

— IEC Electropedia: available at <http://www.electropedia.org/>

3.1

dentine

mineralized hard tissue that forms the bulk of the tooth and develops from the dental papilla and dental pulp

3.2

dentifrice

any substance or combination of substances specially prepared for the consumers for hygiene of the accessible surfaces of teeth and surrounding tissues

3.3

enamel

highly mineralized outermost layer of the normally exposed tooth consisting primarily of hydroxyapatite in orderly crystal structures

3.4

lot

collection of product units of the same size, type and style which have been manufactured under essentially the same conditions

3.5

product unit

one unit of the final product consisting of the closed tube filled with toothpaste. The tube may be contained in a box (usually carton).

3.6

toothpaste

any semi-solid dentifrice preparation presented in the form of a paste, cream or gel

3.7

toothbrush

device, with the working end carrying filaments primarily for cleaning surfaces within the oral cavity

4 Requirements

4.1 Ingredients

4.1.1 All ingredients shall be of acceptable quality and shall meet the requirements of the relevant part of EAS 377.

4.1.2 Toothpaste shall not contain sucrose or other readily fermentable carbohydrates. Artificial sweeteners, if used, shall comply with the specifications given by FAO/WHO Joint expert committee of food additives.

4.2 General requirements

4.2.1 Consistency

Toothpaste shall be smooth and free from lumps or particles, which are palpable in the mouth as separate or discrete particles.

4.2.2 Homogeneity

The paste shall extrude from the tube at $27\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ in the form of a homogeneous mass with the application of normal force starting from one end of the tube.

4.2.3 Toxicity

Toothpaste shall not contain ingredients in sufficient concentration to cause a toxic or irritant reaction when used in the oral cavity, nor shall it be otherwise harmful in normal use.

4.2.4 Hygiene

Toothpaste shall be manufactured in accordance with ISO 22716.

4.3 Specific requirements

4.3.1 General

Toothpaste shall comply with Specific requirements given in Table 1 when tested in accordance with the test methods specified therein.

Table 1 — Specific requirements for tooth paste

S/N	Characteristic	Requirement		Test method
		Fluoridated	Non-fluoridated	
i.	pH	5.5 – 10.5		Annex A
ii.	Fluoride content as fluoride ion, mg/kg	Age below 6 years	1000 – 1250	Annex B
		Age above 6 years	>1250 – 1 500	

4.3.2 Stability

When tested in accordance with Annex C, toothpaste shall not ferment, segregate or otherwise deteriorate, during normal conditions of storage and use.

4.3.3 Effect of container

4.3.3.1 Toothpaste shall be packed in suitable tubes of material which shall not corrode, deteriorate, or cause contamination of the tooth paste during normal conditions of storage and use. The paste shall be examined visually by extruding part of the contents. The internal surface of the tube when examined in accordance with Annex D shall show no visible signs of corrosion or deterioration and the toothpaste shall show no sign of contamination.

4.3.3.2 Toothpaste shall extrude to the extent of 94 % of the nominal volume/mass, when tested in accordance with Annex E.

4.3.4 Abrasivity

4.3.4.1 The abrasivity of the toothpaste shall not exceed the limit for dentine given below:

— 2.5 times that of the primary reference material (see the procedure specified in Annex F or Annex G).

4.3.4.2 The abrasivity of the toothpaste shall not exceed the limit for enamel given below:

— 4.0 times that of the primary reference material (see the procedure specified in Annex F or Annex G).

NOTE Abrasivity values below the limits specified in this document are not intended to be used to rank the safety of toothpaste. All toothpastes beneath the abrasivity limits are recognized as safe with respect to their abrasion of human hard tissues.

4.3.4.3 When an alternative abrasivity reference to the primary reference material (calcium pyrophosphate) is used, traceability to the primary reference material shall be maintained by the testing laboratory. When any alternative abrasivity reference is used, the results shall be reported with respect to the original primary reference material (calcium pyrophosphate).

5 Heavy metals contaminants

5.1 Toothpaste shall comply with the heavy metal limits given in Table 2 when tested in accordance with test methods specified therein.

Table 2 — Heavy metals limits for toothpaste

S/N	Heavy metal	Maximum limit mg/kg	Test method
i.	Arsenic (As ₂ O ₃)	2	ISO 21392/ EAS 847-16
ii.	Lead (Pb)	0.5	ISO 21392/ EAS 847-16
iii.	Cadmium (Cd)	0.2	ISO 21392
iv.	Antimony, (Sb)	0.5	ISO 21392
v.	Mercury, (Hg)	0.01	EAS 847-16

5.2 The total heavy metal concentration in toothpaste shall not exceed 20 mg/kg.

6 Microbiological requirements

Toothpaste shall comply with microbiological requirements given in Table 3 when tested in accordance with test methods specified therein.

Table 3 — Microbiological requirements for toothpaste

S/N	Micro-organism	Limit	Test method
i.	Total viable count for aerobic mesophyllic microorganisms ^a , CFU/g or CFU/ml, max.	1 000	ISO 21149
ii.	<i>Pseudomonas aeruginosa</i> , per ml or g	Not detected	ISO 22717
iii.	<i>Staphylococcus aureus</i> , per ml or g		ISO 22718
iv.	<i>Candida albicans</i> , per ml or g		ISO 18416
v.	<i>Escherichia coli</i> , per ml or g		ISO 21150
a The total viable count for aerobic mesophyllic microorganisms of toothpaste for children below 3yrs shall not exceed 100 CFU/g.			

7 Prohibition or restrictions on clinical claims

Toothpaste shall not be claimed to control the amount of dental plaque, remove calculus, reduce dentine hypersensitivity, reduce the incidence of or treat caries or periodontal disease or any other oral diseases, unless such benefits have been clearly demonstrated by clinical trials or other scientific evidence that are acceptable to the respective Partner States National Standards Bodies.

8 Sampling, testing and compliance with the standard

8.1 Sampling

Sampling shall be done in accordance with Annex H.

8.2 Testing

Toothpaste recovered from the extrusion test shall be well-mixed into a composite sample that will be used for all relevant tests on toothpaste.

9 Packaging

9.1.1 The product shall be packaged in such a way that under normal conditions of handling, use and transport, the container or dispensing system, or both, shall not contaminate or permit contamination of the toothpaste inside, so as to affect its compliance with this standard.

9.1.2 The containers or dispensing system shall be further packed in individual secondary boxes or other protective materials.

10 Labelling

Each package shall be legibly and indelibly labelled in English and/or any other official language (French, Kiswahili, etc) used in the importing East African Partner State with the following information:

- a) name of product as "Toothpaste"

NOTE Words such as mouth gel, tooth gel, dental paste, dental cream may be used to replace the name "Toothpaste".

- b) registered trade mark if any;
- c) the name, in chemical nomenclature, of any ingredient for which special therapeutic properties are claimed, together with the concentration present in the toothpaste;
- d) nominal volume of contents or weight of contents;
- e) code number or batch number;
- f) name and physical address of manufacturer;
- g) date of manufacture;
- h) country of origin;
- i) in the case of fluoridated toothpaste, the words "Fluoride toothpaste" and its concentration shall be given;

- j) expiry date;
- k) disposal instructions;
- l) indication of age below 6 years where applicable;
- m) instruction for use and storage; and
- n) safety notice regarding the use by children below six years of age.

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Annex A (normative)

Determination of pH

Suspend one part by mass of the toothpaste into three parts by mass of water for analytical laboratory use complying with ISO 3696 (grade 3). Determine the pH of the suspension within 10 min, using a pH meter and electrode assembly.

Annex B (normative)

Determination of fluoride content

B.1 Principle

The fluorine derivative species in a sample of toothpaste (for total fluoride analysis) or in the aqueous extract of the toothpaste (for soluble fluoride analysis) is allowed to react with trimethylchlorosilane under acidic conditions to produce trimethylfluorosilane. This derivative is extracted into toluene containing n-pentane as internal standard and the organic phase is analysed by gas chromatography.

B.2 Reagents

Use only analytical reagent grade and distilled or demineralized water.

B.2.1 Hydrochloric acid, concentrated

B.2.2 n-Pentane solution in toluene (soln A), 0.6 g of n-pentane made up to 1 l with toluene

B.2.3 Standard fluoride solution (soln B), an aqueous solution of sodium fluoride containing 1.0 mg F/ml

B.2.4 Trimethylchlorosilane

B.3 Apparatus

B.3.1 Gas chromatograph

B.3.1.1 A suitable gas chromatograph equipped with a flame ionization detector, and having a column of length about 1.5 m and nominal outside diameter 6 mm packed with 10 %, methyl silicone (100 %) on 80 - 100 mesh flux calcined diatomite and acid washed.

B.3.1.2 The recommended GC instrument conditions are as follows:

- a) Temperatures - Column 50 °C
- b) Injector 80 °C
- c) Detector 100 °C
- d) Carrier gas - Nitrogen, flow rate 20 ml/min

B.3.2 Centrifuge

B.3.3 Microsyringe, 1 µl

B.4 Determination of response factor of the apparatus

B.4.1 Pipette 1 ml of the fluoride solution into a 50-ml plastics flask. Using a measuring cylinder, add 8 ml of concentrated hydrochloric acid, stopper the flask and mix the contents. Add from a burette 2.0 ml of the trimethylchlorosilane.

B.4.2 Securely stopper the flask, mix well by shaking and allow to stand for 15 min to cool. Using a safety pipette, pipette 5 ml of the toluene solution of n-pentane into the flask and stopper. Mix thoroughly by shaking vigorously for 2 min.

B.4.3 Transfer the contents of the flask to a centrifuge tube, stopper and centrifuge at 2 500 r/min for 5 min. Inject 0.6 µl of the upper layer onto the column and allow the chromatogram to develop.

B.4.4 Draw tangent base lines to the peaks of interest, and measure the heights of the trimethylfluorosilane and n-pentane peaks. (Alternatively, determine the areas of the relevant peaks).

The response factor (k) is given by the formula:

$$\frac{\text{Peak height of trimethylfluorosilane}}{\text{Peak height of n-pentane}} \times \frac{\text{grams of n-pentane in 5 ml of solution A}}{\text{gram of fluoride in 1ml of solution B}}$$

B.5 Procedure

B.5.1 For soluble fluoride

B.5.1.1 Weigh accurately 5.0 g ± 1.0 g of toothpaste into a centrifuge tube. Add approximately 15 ml of water, slurry with a glass rod and centrifuge for 10 min. Decant the supernatant liquid into a 50-ml volumetric flask. Repeat this extraction process an additional two times and bring the volume of the supernatant liquid up to 50 ml with water.

B.5.1.2 Pipette 10 ml of the above solution into a plastic centrifuge tube. Add 8 ml of concentrated hydrochloric acid and immerse in a boiling water bath for 1 min and let stand for 10 min. Then cool under running water.

B.5.1.3 Add 1 ml of trimethylchlorosilane, cap, mix and let stand for 15 min. Pipette 5 ml of n-pentane solution into the tube, mix centrifuge and inject 0.6 µL of the upper layer.

B.5.1.4 The mass of fluoride, in grams, is given by the formula:

$$\frac{\text{Peak height of trimethylfluorosilane}}{\text{Peak height of n-pentane}} \times \frac{\text{grams of n-pentane in 5 ml of solution A}}{K}$$

B.5.2 For total fluoride

B.5.2.1 Weigh accurately 1.0 g ± 0.1 g of toothpaste into a plastics centrifuge tube. Add several glass beads and 8 ml of concentrated hydrochloric acid in parts. Cap and shake, pausing several times to release pressure.

B.5.2.2 Add 8 ml of water, cap and mix. Immerse in a boiling water bath for 1 min. Mix and let stand for 2min, then cool under running water.

B.5.2.3 Add 1 ml of trimethylchlorosilane, mix and let stand for 15 min. Pipette 5 ml of n-pentane solution into the tube, mix, centrifuge and inject 0.5 µl of the upper layer.

The mass of fluoride, in grams, is given by the formula

$$\frac{\text{Peak height of trimethylfluorosaline}}{\text{Peak height of } n - \text{pentane}} \times \frac{\text{grams of } n - \text{pentane in 5 ml of solution A}}{\text{gram of fluoride in 1ml of solution B}}$$

B.6 Expression of result

Report the results of total and soluble fluorine derivative of the sample tested as milligrams per kilogram (mg/kg) of fluoride ion.

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Annex (normative)

Test for stability

C.1 General

The objective of this test method is to evaluate the ability of toothpaste to resist change or variation of its initial properties over time under stated or reasonable foreseeable conditions of storage and use

C.2 Apparatus

C.2.1 Oven

C.2.2 Glass test tubes

C.3 Procedure

C.3.1 Transfer a suitable quantity (approximately 5 g) of the composite sample of the tooth paste into each of two glass test tubes and stopper them.

C.3.2 Heat the two tubes for 72 h in an oven maintained at $45\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$. Allow to cool and examine the contents visually for homogeneity and for signs of fermentation or other deterioration or both.

C.4 Expression of results

Report the result as pass or fail.

Annex D (normative)

Determination of corrosion of the container

D.1 Procedure

D.1.1 Heat the tube of toothpaste at $45\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for ten days and then test as follows:

D.1.2 Extrude a portion of the paste and examine it for visible contamination. Slit the tube, remove the paste and examine the surfaces of the tube for signs of corrosion, chemical attack or other damage.

D.1.3 Disregard surface staining by colouring agents in the toothpaste.

D.2 Expression of result

Report the result as pass or fail.

Annex E (normative)

Determination of extrusion

E.1 Procedure

E.1.1 Maintain the tube of toothpaste at $23\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ for at least 2 h and test as follows: Remove the cap and invert the tube over a plastics beaker for which a tare has been recorded.

E.1.2 Press out the paste from the fold of the tube in the direction of the shoulder, holding the tube between thumb and index finger of the left and right hand.

E.1.3 When the tube is almost emptied, the mantle of the tube is to be pulled over an edge, removing the rest of the paste in the shoulder by nipping it off rectangular. The extrusion shall be carried out without the application of abnormal force.

E.2 Calculation and expression of results

E.2.1 Record the mass, m , of toothpaste extruded. Use a suitable method to determine the density of the toothpaste and hence calculate the volume of toothpaste extruded.

NOTE When calculating extrusion, expressed as % m/m, determination of density is not necessary.

E.2.2 The extrusion, expressed as % (v/v or m/m) of the nominal volume, is given by the formula

$$\frac{m}{\rho \times \text{nominal volume}} \times 100$$

where

m is the mass of toothpaste extruded;

ρ is the density of the toothpaste

Annex F (normative)

Abrasivity test procedure — American Dental Association (ADA) method

F.1 General

This annex identifies the specific procedures for determination of the dentifrice abrasivity using the ADA laboratory method.

F.2 Sampling

A representative sample shall be taken from at least two batches.

F.3 Procedure

F.3.1 Standard reference abrasive

The primary reference abrasive is from a specific lot of calcium pyrophosphate. An alternate, silica reference abrasive, is also available.

F.3.2 Apparatus

F.3.2.1 Brushing machine

A cross-brushing machine is the apparatus of choice. The apparatus should have eight positions for holding specimens. A toothbrush shall be positioned to pass reciprocally at a small angle (approximately 5°) over the mounted specimens, with a designated tension on the brush, while immersed in a dentifrice slurry. The distance traversed by the brush should not be longer than the brush head so that the specimen does not lose contact with the brush. The mechanism for holding the dentifrice slurry may vary with different machine designs, but should allow for easy removal of the slurry sample. It is important to have some mechanism for the agitation of the slurry while the brushing is taking place. A convenient method to accomplish this is to attach rubber mixing vanes just below the brush head. As the brushing takes place, these vanes will prevent the abrasive from settling to the bottom of the slurry container.

F.3.2.2 Radioactivity detector

The two recommended methods for the determination of the radioactivity of the used dentifrice slurries are a Geiger-Müller planchet counter and a liquid scintillation detector. The use of the Geiger counter requires that the samples be dried under defined controlled conditions. The liquid scintillation method has the advantage of reading directly from the slurry. Counting should be done for a period, expected to reduce the alpha value for counting error to less than 2 %. Counting should be performed for a minimum of 1 000 counts and for at least 1 min. The number of brushing strokes may be increased if counting times become too long.

F.3.3 Preparation of tooth specimens

F.3.3.1 Dentine specimens

F.3.3.1.1 Selection

Human root dentine of extracted permanent teeth is used as the substrate. Single-rooted teeth that were vital at extraction should be selected. An exception, because of the small size, are mandibular incisors: these should not be used. The specimen should be at least 14 mm long and 2 mm wide at the narrow end. All roots shall be caries-free and free of anatomical defects. After extraction, the roots should be stored in a neutralized solution that disinfects but does not alter the physical properties.

F.3.3.1.2 Preparation

Scrape the roots clean of all soft tissue and as much cementum as possible. Then remove the crown and the root tips using a separating disc under a flow of water.

F.3.3.1.3 Irradiation

For each set of eight specimens to be irradiated, add one or two extra roots for use in correction factors. Pack the specimens in disinfection solution and submit to a nuclear reactor for irradiation. The neutron flux should be sufficient to produce about 1 mCi of ^{32}P beta radiation after several hours. Elevated temperatures in the reactor (above 65 °C) should be avoided. A specific position shall be requested to shield the samples from fast neutrons and gamma radiation. Handling of the irradiated specimens should be done with care using good laboratory practice. The specimens should not be used during the first half-life because of excess radiation and should be used before the end of the third half-life because of lack of activity. The half-life of ^{32}P is 14.3 days so the usable life span of a set of teeth is 4 weeks.

F.3.3.1.4 Mounting of specimens

Mount the specimens individually in a mould in cold-cure methyl methacrylate resin such that either the buccal or lingual surface protrudes at least 2 mm above and parallel to the resin. Orient the mould in the brushing machine such that the direction of brushing is perpendicular to the long dimension of the root. Store the mounted specimens in a neutralized solution that disinfects but does not alter the physical properties.

NOTE The type and configuration of the mould depend on the holder of the brushing machine.

F.3.3.2 Enamel specimens

F.3.3.2.1 Selection

Selection criteria for enamel specimens are the same as for dentine. The enamel specimens should be obtained from human maxillary incisors.

F.3.3.2.2 Preparation

The entire labial surface of the specimen is used after removing the root. Clean the enamel in the same way as the root.

F.3.3.2.3 Irradiation

Irradiation of the enamel is identical to the method used with the roots. The roots and enamel specimens may be packed together for submission to the reactor.

F.3.3.2.4 Mounting

Mount the enamel specimens in the same way as the roots. The labial surface shall protrude 2 mm and be parallel to the resin surface.

F.3.4 Toothbrushes

The toothbrushes used should have nylon filaments about 10 mm in length. Filament ends should lie in a plane. Store the brushes in water overnight prior to their first use and then keep them in water until they are discarded. Use a new set of brushes for each set of teeth. Do not remove the brushes from the machine between runs but raise the tufts off the specimen so as not to bend the bristles. At the beginning of each run, set the tension of the brush on the specimen to 150 g using a Chatillon spring gauge or equivalent. This tension should be rechecked at least twice daily. The method of adjusting the tension will vary depending upon the type of mechanism on the brushing machine.

F.3.5 Reference diluent

The diluent is a 0.5 % carboxy methyl cellulose (7MF CMC) solution in 10 % glycerine. To prepare 1 l of the diluent, heat 50 ml of glycerine to 60 °C and add 5 g of CMC while stirring. When the mixture is homogeneous, add another 50 ml of heated glycerine and continue stirring for 60 min. Transfer the solution to a 1-l flask and add 900 ml of distilled water. Allow to cool but continue stirring slowly overnight. To stabilize the viscosity, allow the solution to stand overnight before using. This solution is used to make up slurries of the reference abrasive or any powder being tested.

F.3.6 Reference abrasive slurry

Using the reference material described in F.3.1, dilute 10 g of the abrasive with 50 ml of the diluent (see F.3.5). The same ratio is used for all powders. It is possible for the reference abrasive to be used as a dentifrice. If this is the case, it shall be made up as a 40 % abrasive dentifrice with the rest of the constituents being conventional dentifrice components. The slurry is then made using 25 g of reference dentifrice and 40 ml of water.

F.3.7 Dentifrice slurries

To prepare the test slurries, add 40 ml of water to 25 g of each dentifrice. For the machine, prepare eight slurries of each dentifrice. This dilution produces a final slurry volume and concentration similar to those of the reference abrasive slurry. All slurries (reference and test) should be used shortly after preparation and after vigorous mechanical stirring to prevent particles from settling.

F.3.8 Preconditioning of tooth specimens

F.3.8.1 Dentine

To reduce the variation caused by dentine surface differences, precondition the specimens prior to each use. The preconditioning treatment consists of brushing with a slurry of the reference abrasive but not taking a sample. The first time dentine specimens are used, the preconditioning should be for 6 000 strokes. Each successive daily run should begin with a shorter preconditioning brushing of 1 000 strokes. The tension of the toothbrush on the roots shall be 150 g. Discard the preconditioning slurries.

F.3.8.2 Enamel

Preconditioning of the enamel is similar to that of the dentine, except that 10 000 strokes are used prior to the first use and 1 000 strokes are given at the beginning of each day. Discard the preconditioning slurries.

F.3.9 Test design

F.3.9.1 Test design for dentine

The test design may be either a sandwich design or a Latin Square design. The sandwich design is such that a set of reference slurries is run (pre-test), followed by a set of the first test slurries. These are followed by a second set of reference slurries (post-test). This second set of reference slurries then acts as the pre-test slurries for the next test group. This continues until all the test groups are run. The Latin Square design is such that a set of reference slurries is run first. All the test groups are randomized over the eight brushing heads for the next few runs (depending on the number of test groups). Then a post-test reference set of slurries is run as the final procedure. In both test designs, the brush tension is set at 150 g and brushing is performed for 1 500 to 3 000 strokes, depending on the radioactivity level of the specimens.

F.3.9.2 Test design for enamel

The test design for enamel is identical to that for dentine, except that the number of strokes is 5 000 to 7 500 depending on the activity of the specimens.

F.3.9.3 Sampling of slurries

The sampling of the slurries following the brushing is identical for both dentine and enamel. An aliquot of each slurry is removed immediately following brushing. The size of the aliquot will depend upon the counting method and equipment, but 3 ml is usually adequate to provide a detectable level of radioactivity. A convenient method for removing the sample is a syringe fitted with a blunt needle. Take care to ensure there is no carry-over between samples. This can best be done by a complete rinsing of the syringe between samples. It is also important to remove the same quantity of sample from each slurry. Dry the sample if a planchet counter system is being used to detect the radioactivity. If drying is needed, the samples should be air-dried for a least 1 h and then dried in an oven at 60 °C with forced air overnight.

F.3.9.4 Correction factors

F.3.9.4.1 General

Correction factors are needed for both dentine and enamel abrasion tests when using the planchet counting method and are identically prepared in both methods. When testing dentifrices with abrasive systems different from the reference material, the self-absorption and backscattering characteristics of the abrasives for beta radiation may also differ. Real differences in abrasivity may then be significantly distorted. The correction factor is a means of reducing this variable. The correction factor is determined in different ways depending on the counting method used.

F.3.9.4.2 Preparation of correction factor slurries for Geiger-Müller planchet counting

Dissolve one piece of irradiated dentine (or enamel) in 5 ml of concentrated HCl. Transfer the solution to a 250-ml volumetric flask and add water to the mark. Add 1.0 ml of this radioactive solution to slurries of the reference abrasive and to each of the test abrasives prepared in the same manner as in the test. To neutralize the acid, add 1.0 ml of 0.5 mol/l NaOH. Mix the slurries thoroughly, sample, and dry the samples along with those from the test runs. Do not brush with these correction factor slurries. These samples are counted along with the test samples.

F.3.9.4.3 Calculation of correction factors

The correction factor, C_f , to be applied to all count values of the test sample is calculated as in Formula (F.1):

$$C_f = \frac{C_r}{C_t}$$

where

C_r is the mean counts for four reference samples;

C_t is the mean counts for four test samples.

F.3.9.4.3.1 Correction factors for liquid scintillation counting

The correction is determined with regard to the amount of sample mixed with the scintillation cocktail. Each sample is weighed and the net count per minute (CPM) is divided by the mass to get a net CPM per gram of slurry. These net CPM-per-gram values are then used in calculating abrasivity in place of net CPM values according to F.3.11, and there is no C_f term.

F.3.9.4.3.2 Correction factors for liquid scintillation detection

Self-absorption and backscatter are less of a concern because of the liquid medium being used. Most modern liquid scintillation equipment will automatically colour-correct, so this is not a problem. The differences in mass of the samples do need to be accounted for in the calculation. To do this, each sample taken after brushing needs to be weighed to an accuracy of 0,01 g.

F.3.9.4.3.3 Applying the correction factor

Before calculating the relative abrasion values, the net CPM of each slurry is divided by the mass of the slurry used to get a net CPM per gram of slurry. These values are then used in the calculation of relative abrasive values.

F.3.10 Calculation of abrasivity using Geiger-Müller counting

F.3.10.1 Dentine abrasivity

The dentine abrasivity, A_D , of the test dentifrices (or abrasives) is calculated as in Formulae (F.2) and (F.3)

$$C_{mr} = \frac{C_{pre} + C_{post}}{2} \quad \text{F.2}$$

where

C_{mr} is the mean reference net CPM;

C_{pre} is the pre-net CPM;

C_{post} is the post-net CPM.

$$A_D = \frac{C_f \times 100 \times C_{mt}}{C_{mr}} \quad \text{F.3}$$

where

A_D is the dentine abrasivity;

C_f is the correction factor;

C_{mt} is the mean test dentifrice net CPM;

C_{mr} is the mean reference net CPM.

F.3.10.2 Enamel abrasivity

The enamel abrasivity, A_E , of the test dentifrices (or abrasives) is calculated as in Formulae (F.4) and (F.5):

$$C_{mr} = \frac{C_{pre} + C_{post}}{2} \quad \text{F.4}$$

where

C_{mr} is the mean reference net CPM;

C_{pre} is the pre-net CPM;

C_{post} is the post-net CPM.

$$A_E = \frac{C_f \times 10 \times C_{mt}}{C_{mr}} \quad \text{F.5}$$

A_E is the enamel abrasivity;

C_f is the correction factor;

C_{mt} is the mean test dentifrice net CPM;

C_{mr} is the mean reference net CPM

F.3.11 Calculation of abrasivity using liquid scintillation**F.3.11.1 Dentine abrasivity**

The dentine abrasivity, A_D , of the test dentifrices (or abrasives) is calculated as in Formulae (F.6) and (F.7):

$$G_{mr} = \frac{G_{pre} + G_{post}}{2} \quad \text{F.6}$$

where

G_{mr} is the mean reference net CPM per mass of slurry, in grams;

G_{pre} is the pre-net CPM per mass of slurry, in grams;

G_{post} is the post-net CPM per mass of slurry, in grams.

$$A_D = \frac{100 \times G_{mt}}{G_{mr}} \quad \text{F.7}$$

where

A_D is the dentine abrasivity;

G_{mt} is the mean test dentifrice net CPM per mass of slurry, in grams;

G_{mr} is the mean reference net CPM per mass of slurry, in grams.

F.3.11.2 Enamel abrasivity

The enamel abrasivity of the test dentifrices (or abrasives) is calculated as in Formulae (F.8) and (F.9)

$$G_{mr} = \frac{G_{pre} \times G_{post}}{2} \quad \text{F.8}$$

where

G_{mr} is the mean reference net CPM per mass of slurry, in grams;

G_{pre} is the pre-net CPM per mass of slurry, in grams;

G_{post} is the post-net CPM per mass of slurry, in grams

$$A_E = \frac{10 \times G_{mt}}{G_{mr}} \quad \text{F.9}$$

where

A_E is the enamel abrasivity;

G_{mt} is the mean test dentifrice net CPM per mass of slurry, in grams;

G_{mr} is the mean reference net CPM per mass of slurry, in grams.

Annex G (normative)

Determination of relative dentifrice abrasivity to enamel and dentine by a surface profile method

G.1 General

This method is based on the determination of abraded depth after brushing, using profilometry. This method has been established to be equivalent to the radio-tracer method (see Annex G) and will be referred to as RDA-PE (Relative Dentin Abrasion – Profilometry Equivalent) and REA-PE (Relative Enamel Abrasion Profilometry Equivalent).

G.2 Apparatus

G.2.1 Contact profilometer, or a similar instrument with a sensitivity to $<0.1 \mu\text{m}$ [e.g. Talyform i50, Surfometer or Surftest SV-2000]], or a non-contact profilometer, or a similar instrument with a sensitivity to $<0.1 \mu\text{m}$ [e.g. Bruker GT-K1]].

G.2.2 Lapping and polishing unit, [e.g. Abramin automatic lapping and polishing unit], with sequential silicon carbide discs up to P1200 and ability to use diamond slurries for specimen polishing and/or their equivalent.

NOTE Other methods of polishing enamel and dentine to conform with the baseline requirements for specimens can be used (e.g. diamond powder).

G.2.3 Brushing machine as the apparatus of choice. The apparatus should have eight positions for holding specimens. A toothbrush shall be positioned to pass reciprocally at a small angle ($\approx 5^\circ$) over the mounted specimens, with a designated tension on the brush, while immersed in a dentifrice slurry. The distance traversed by the brush should not be longer than the brush head. While measured specimen must lose the contact with bristles of the brush, bristles should not lose the contact with the mounting material, i.e. dental acrylic. The mechanism for holding the dentifrice slurry may vary with different machine designs, but should allow for easy removal of the slurry sample. It is important to have some mechanism for the agitation of the slurry while the brushing is taking place. It could be accomplished by rubber mixing vanes just below the brush head, metal stirrers, or plungers that prevent the abrasive from settling to the bottom of the slurry container.

NOTE Some machines have less than eight specimen positions, but can be used provided the n value for specimens/treatment is reached.

G.2.4 Manual toothbrush. Keep brushes consistent. At a minimum, do not vary within a test the manufacturer, source, type (same brand/version), and age within any one experiment. Suitable brushes have been found to have bristles made of nylon, bristles having a uniform diameter of $200 \mu\text{m}$ and a uniform overall length of 11 mm, as measured from the face of the toothbrush. Bristles should be grouped in tufts of approximately 18 to 22 bristles per tuft, 38 tufts per toothbrush in 12 rows of tufts, with rows ranging from one tuft to four tufts per row, no tuft more than 2 mm from its nearest neighbour, and tufts occurring within a row substantially symmetric from the left to right side of the brush head. The bristles shall be no more than $\pm 200 \mu\text{m}$ differential at the flat surface. See F.3.4 for an example of a suitable brush.

G.2.5 Standard reference abrasive as detailed in F.3.1, being either pyrophosphate or silica abrasives.

G.3 Preparation of enamel and dentine specimens

G.3.1 Use human caries-free, erupted or un-erupted permanent teeth or bovine teeth that can produce specimens of required size.

NOTE Human lower incisors may be unsuitable because of their small size.

G.3.2 Remove all soft tissue remnants by scraping with a suitable instrument (e.g. curette, scalpel, etc.), sterilize and store the teeth with 0.1 % thymol or other neutralized solution that disinfects but does not alter the physical properties of the tooth.

G.3.3 Section the teeth at the amelo-cemental junction with a dental bur or disc. Use the coronal portion for enamel specimens and the radicular portion for dentine specimens resulting in the size of the specimen to be no less than 3 mm × 5 mm × 3 mm.

NOTE Depending on the size and morphology of the crown and roots, it may be possible to prepare two specimens from each, but no more than two/tooth.

G.3.4 For enamel, section the molar crown vertically in either a bucco-lingual or a mesio-distal direction resulting in two enamel specimens/crowns. For dentine, section the root portion vertically in half, so that an outer portion of root surface is available for polishing. If using bovine specimens that are larger than human specimens, you can section longitudinally in half a diamond-edged annular, circular cutting instrument.

G.3.5 Place the enamel and dentine portions, outer face down, in moulds large enough to accommodate the size of the specimen and a holder of the brushing machine. Embed specimens in the epoxy resin, and make sure that they are flat and level with the surface of the resin).

NOTE It is important to use non-heat curing epoxy as to prevent change in chemical or physical properties of dentine and/or enamel tissue while curing. Allow the resin to set for at least 24 h. The overall final dimensions of the specimens can differ slightly depending on the dimensions of the specimen-holding positions of the brushing machine used; however, the size of the embedded enamel or dentine has to be at least 5 mm × 3 mm × 3 mm as described in F.3.3.

G.3.6 Mounted dentine and enamel specimens are then ground and polished using serial grit aluminium oxide paper to a final flat surface roughness R_a no greater than 0.1 µm measured by the profilometer.

G.3.7 Measure the geometry of the specimens to ensure their quality. All specimens shall be planoparallel, with flat and/or slightly convex surface (concave/irregular specimens should be avoided completely) and checked using a profilometer. Specimen surface shall be flat within 10 µm across the dentine and/or enamel surface (assessed by profilometric inspection). The methyl methacrylate block shall meet strict rectangular criteria where individual corners of the blocks shall be level (plano-parallel) to within 100 µm when measured corner to corner and to within 100 µm when measured side to side.

G.3.8 Measure the hardness of the specimens with Vickers indentations) on damp specimens at 300 g load for 15 s. Measurements shall be made outside of the intended brush area. Acceptable specimens shall have an initial Vickers surface hardness of 30 to 70 (human and bovine dentine). This step provides a source of qualification for the dentine, thus reducing the biological variations of the tissue since specimens are randomized to treatment based on surface hardness. Enamel specimens will have surface hardness of no less than 300 and be randomized similarly as those of dentine.

G.3.9 Use at least eight enamel and eight dentine specimens for each dentifrice being evaluated and for the reference dentifrice or slurry. Prior to brushing, mask specimens using two polyester tape strips to create a window with at least 3 mm² × 6 mm² (note that some of the brushing area will be on the methylmethacrylate resin) yet providing enough coverage on the edges of the hard tissue to serve as a reference surface for profilometric evaluation of abrasive tissue loss. Keep the specimens hydrated during all preparation, abrasion and measurement procedures.

NOTE It is important that the tape adhesive can be cleanly removed from the specimen surface, but strong enough to withstand brushing. If needed, two pieces of 1 mil tape can be over-layered on the specimens.

G.4 Preparation of reference dentifrice

G.4.1 Reference diluent

Prepare the reference diluent as described in F.3.5.

G.4.2 Reference abrasive slurry

Use either the pyrophosphate or the silica reference abrasives listed in F.3.1. Prepare the slurry as described in F.3.6 using the reference diluent (see F.3.5) or as a 40 % abrasive dentifrice.

G.5 Preparation of test dentifrice slurries

Prepare the test dentifrice slurries as described in F.3.7.

G.6 Preparation of test brushes

The toothbrush used in testing shall have a rectangular head with flat-trim bristles. Brushes will be preconditioned for 20 000 strokes on the V8 brushing machine in water under standard conditions prior to use the first time. Brushes may last up to 200 000 strokes but inspect for wear frequently and change as needed. Brushes need to be soaked in water for 24 h between/prior to individual tests.

G.7 Reference dentifrice and test dentifrice brushing procedure

G.7.1 Fix specimens in the V8 brushing machine standard mounts, taking care to ensure that the dental acrylic is either above the mount base or at minimum flush to the mount base.

G.7.2 Each time a specimen is mounted, verify the load of the brush on each specimen at each station using a spring gauge and/or orthodontic gauge. The load shall be of 150 g with the head of the brush being centred over the hard tissue specimen.

G.7.3 If adjustable, set the brush stroke speed to 170 strokes per minute (acceptable variations were seen from 120 to 170 strokes per minute). Brushing strokes are tuned to have the brush head stroke across specimens for the entire brush head, with the brush losing contact and re-establishing contact with each stroke reversal with the hard tissue specimen. Ideally, some brush bristles should keep contact with the dental acrylic base

NOTE One stroke corresponds to the forward and backward movement of the brush heads over the specimens.

G.7.4 For dentine and RDA-PE, specimens shall be brushed for 4 000 strokes with the test toothpastes and the abrasivity reference standard. An internal RDA-PE 250 upper limit shall be achieved by brushing with the abrasive standard for 10 000 strokes (2.5× brushing the internal RDA 100).

G.7.5 The eight specimens shall be cycled through all eight brushing stations. When brushing RDA-PE 100 reference slurries and dentifrice samples, cycle specimens after each 500 strokes. Turn specimens by 180°, re-mix dentifrice slurries at each rotation to guard against abrasive settling, and recheck the brush load on each specimen to ensure 150 g load. Repeat until full completion of 4 000 strokes. When brushing RDA-PE 250 reference slurries, rotate, re-mix, and recheck load after each 1 250 strokes, until full completion of 10 000 strokes.

G.7.6 Enamel is significantly harder than dentine and the depth of abrasion for enamel will be significantly lower versus that of dentine. To ensure proper dynamic range of mean depth of abrasion, specimens shall be

brushed for 10 000 strokes with the test toothpastes and with the abrasivity reference standard of 10. An internal REA-PE 40 upper limit shall be achieved by brushing with the abrasive standard for 40 000 strokes (4× brushing the internal REA 10).

G.7.7 The eight specimens shall be cycled through all eight brushing stations. For REA-PE reference slurries with the assigned value of 10 and dentifrice samples, cycle specimens after each 1 250 strokes. Turn specimens by 180°, re-mix dentifrice slurries at each rotation to guard against abrasive settling, and recheck the brush load on each specimen to ensure 150 g load. Repeat until full completion of 10 000 strokes. When brushing REA-PE reference slurries with the assigned value of 40, rotate, re-mix, and recheck load after each 5 000 strokes, until full completion of 40 000 strokes.

G.7.8 Remove the specimens from the holders, wash under running tap water, remove the tape and measure the depth of abrasion by profilometry as described in G.8.

G.8 Profilometry method

G.8.1 After dentifrice slurry brushing, remove the tape from specimens and re-measure using the operating method for the particular profilometer.

G.8.2 Measure the mean depth of abrasion as abraded depth versus control masked area on the specimen surface.

- a) For two-dimensional contact profilometers, start the profile 0.5 mm from the previously taped (controlled) area of the specimen across the abraded zone and into the opposite previously taped (control) area opposite at least 0.5 mm. Total trace length then will be 4 mm, 0.5 mm on one side of the abraded area, 3 mm on the abraded area, and 0.5 mm on the opposite side of the abraded area. Follow the tracing for at least 12 traces evenly spaced across abraded area, avoiding boundary with dental acrylic by 0.1 mm. If proportional linearity is not achieved, attempt additional scans/traces up to a maximum of 36 before discarding results of the test. Calculate the mean depth of abrasion.
- b) For non-contact profilometers, create a rectangular 3D scan. In the measured rectangular 3D scan, include 0.5 mm from the previously taped (control) area, 3 mm abraded zone, and 0.5 mm of the opposite controlled area. Avoid dental acrylic/hard tissue specimen boundary at 0.1 mm. Calculate the mean depth of abrasion.

NOTE One way to leverage currently existing software for profilometers is to use “baseline subtraction” option that allows subtraction of abraded area from the baseline area.

G.8.3 Calculate the mean abrasive depth across the respective specimen group.

G.9 Calculation of relative dentine abrasivity (RDA) and relative enamel abrasivity (REA) of test dentifrices using profilometry measurements

G.9.1 General

For RDA-PE, the reference brushed for 4 000 strokes is considered to have an RDA-PE value of 100. The reference brushed for 10 000 strokes is considered to have an RDA-PE value of 250. For REA-PE, the reference brushed for 10 000 strokes is considered to have a value of 10. Reference brushed for 40 000 strokes has an REA-PE value of 40 (see H.7.6).

G.9.2 Determination of proportional linearity

G.9.2.1 Proportional linearity is used as a key quality control in the abrasion experiment that establishes if the experiment has been run as intended. It is used to make decisions on the validity of generated data.

G.9.2.2 Proportional linearity for RDA-PE is the ratio of the mean depth of abrasion of the reference brushed for 10 000 strokes divided by the mean depth of abrasion of the reference brushed for 4 000 strokes. The target proportional linearity shall be 2.5 (as the ratio of 10 000 strokes / 4 000 strokes = 2.5). The acceptable experimental proportional linearity ranges have been found to be 2.2 to 2.8.

G.9.2.3 If proportional linearity is not within the acceptable experimental range (2.2 to 2.8), then the data are not valid. There is probably a systemic error in the set-up of the brushing procedure and the experiment shall be re-run.

G.9.2.4 Proportional linearity for REA-PE is the ratio of the mean depth of abrasion of the reference brushed for 40 000 strokes divided by the mean depth of abrasion of the reference brushed for 10 000 strokes. The target proportional linearity shall be 4.0 (as the ratio of 40 000 strokes / 10 000 strokes = 4). The acceptable experimental proportional linearity ranges have been found to be 3.7 to 4.3.

G.9.2.5 If proportional linearity is not within the acceptable range (3.7 to 4.3), then the data are not valid. There may be a systemic error in the set-up of the brushing procedure and the experiment shall be re-run.

G.9.3 Standard curve calculation

G.9.3.1 Standard curve calculation for RDA-PE

G.9.3.1.1 Assign RDA-PE value of 100 to standard reference brushed for 4 000 strokes and assign RDAPE value of 250 to standard reference brushed for 10 000 strokes.

G.9.3.1.2 Plot the mean depths of reference standards versus their assigned RDA values.

G.9.3.1.3 Build a standard correlation curve using [0,0] intercept and the values of the reference standards (RDA vs. Mean Depth).

G.9.3.1.4 Use the Method of Least Squares to develop Formula (G.1):

$$Y = \alpha RA - PE^x \quad (G.1)$$

where

y is the mean depth of the reference standards;

x is the RDA-PE of the reference standard (1, 100, 250, respectively).

G.9.3.1.5 Record the slope α RDA-PE of this dependence. Forcing the intercept through [0,0] further provides confidence of linear behaviour of the methodology.

G.9.3.2 Standard curve calculation for REA-PE

G.9.3.2.1 Assign REA-PE value of 10 to a standard reference brushed for 10 000 strokes and assign REAPE value of 40 to standard reference brushed for 40 000 strokes.

G.9.3.2.2 Plot mean depths of reference standards vs. their assigned REA-PE values.

G.9.3.2.3 Build a standard correlation curve using [0,0] intercept and the values of the reference standards (REA-PE vs. Mean Depth).

G.9.3.2.4 Use the Method of Least Squares to develop Formula (G.2):

$$Y = \alpha REA - PE^x \quad G.2$$

where

y is the mean depth of the reference standards;

x is the REA-PE (0, 10, 40, respectively).

Forcing the intercept through [0,0] further provides confidence to linear behaviour of the methodology.

G.9.4 Calculation of RDA-PE and REA-PE

G.9.4.1 Use the slope α RDE-PE from the correlation in G.9.3.1.4. Divide the mean depth of the unknown by the slope α RDE-PE to calculate RDA-PE.

G.9.4.2 Use slope α REA-PE from the correlation in G.9.3.2.4. Divide the mean depth of the unknown by the slope α REA-PE to calculate REA-PE.

Annex H (normative)

Sampling

H.1 General requirements of sampling

In drawing, preparing, storing and handling samples, the following precautions and directions shall be observed.

H.1.1 Samples shall be taken in a protected place not exposed to damp air, dust or soot.

H.1.2 The sampling instrument shall be clean and dry.

H.1.3 The samples, the sampling instrument and the containers for samples shall be protected from adventitious contamination.

H.1.4 The samples shall be placed in clean and dry glass containers. The sample containers shall be of a size such that they are almost completely filled by the sample.

H.1.5 Each container shall be sealed air-tight after filling and marked with full details of sampling, batch or code number, name of manufacturer, and other important particulars of the consignment.

H.1.6 The samples shall be stored in such a manner that the temperature of the material does not vary unduly from the normal temperature and they are protected from light.

H.1.7 Sampling shall be done by a person agreed to between the purchaser and the supplier, and in the presence of the purchaser or his representative and the supplier or his representatives.

H.2 Sampling of packages

H.2.1 General

The sampling procedure for packages shall consist essentially in selecting and drawing, a sufficient number of unit packs.

H.2.2 Lot

In a single consignment, all the packages containing toothpaste of the same type and form, representing the same batch of manufacture, shall constitute a lot. If the consignment consists of packages containing toothpaste of different types or forms or batches of manufacture, then the packages containing products of the same type, form and batch of manufacture shall be grouped together; each group shall constitute a separate lot.

H.2.3 Scale of sampling

For ascertaining the conformity of a lot to the requirements prescribed in the specifications for individual toothpaste, tests shall be carried out on each lot separately. The number (n) of packages to be selected for drawing the samples shall depend on the size (N) of the lot in accordance with Table J.1.

Table H.1 — Scale of sampling for packages

S/N	Number of packages in the lot (N)	Number of packages to be selected (n)
i.	Up to 3	Each container
ii.	4 – 50	3
iii.	51 – 150	4
iv.	151 – 300	5
v.	301 – 500	6
vi.	501 and above	7

H.2.4 Selection

The packages shall be selected at random and to ensure randomness of selection, random number tables shall be used. In case such tables are not available, the following procedure may be adopted: 'Starting from any package, count all the packages in one order as 1,2,3 up to r and so on, where r is the integral part of N/n . Every r^{th} package thus counted shall be withdrawn to give a sample for purposes of test.

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