
**Dentistry — Dentifrices —
Requirements, test methods and
marking**

*Médecine bucco-dentaire — Dentifrices — Exigences, méthodes
d'essai et marquage*

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 106, *Dentistry*, Subcommittee SC 7, *Oral care products*.

This third edition cancels and replaces the second edition (ISO 11609:2010), which has been technically revised.

Introduction

Dentifrices should not cause any adverse reactions to the oral soft tissues when used in accordance with the manufacturer's recommendation for frequency and duration of use, nor cause any known side effects.

Guidelines on assessing the claimed or implied efficacy of dentifrices for the prevention or control of oral conditions can be found through the US Food and Drug Administration^[3], the American Dental Association^[4] and the Commission Work Project (8-95) of the FDI World Dental Federation^[16].

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Dentistry — Dentifrices — Requirements, test methods and marking

1 Scope

This document specifies requirements for the physical and chemical properties of dentifrices and provides guidelines for suitable test methods. It also specifies requirements for the marking, labelling and packaging of dentifrices.

This document applies to dentifrices, including toothpastes, destined to be used by the consumers on a daily basis with a toothbrush to promote oral hygiene.

Specific qualitative and quantitative requirements for freedom from biological and toxicological hazards are not included in this document. These are covered in ISO 7405^[1] and ISO 10993-1^[2].

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.

ISO 1942, *Dentistry — Vocabulary*

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

ISO 8601, *Data elements and interchange formats — Information interchange — Representation of dates and times*

International Nomenclature of Cosmetic Ingredients (INCI), in *International Cosmetic Ingredient Dictionary and Handbook*¹⁾

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 1942 and the following apply.

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

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <http://www.iso.org/obp>

3.1 dentifrice

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

3.2 toothpaste

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

Note 1 to entry: The product's common constituents are abrasives, humectants, binders, surfactants, flavourings, fluorides and other agents for oral health benefits.

1) Nomenclature developed by the Personal Care Products Council (formerly CTFA). Available at: <https://access.personalcarecouncil.org/eweb/DynamicPage.aspx?Site=pcpc&WebKey=4513b14e-2f75-4857-85b4-b3697be5d5d9>.

3.3

single-unit container

container of dentifrice marketed to individual consumers

3.4

primary container

container that is in direct contact with the product

4 Requirements relative to the physical and chemical properties of dentifrices

4.1 Total fluoride

4.1.1 Total fluoride concentration

The total fluoride concentration shall not exceed a mass fraction of 0,15 % when tested in accordance with one of the procedures given in [Annex C](#).

Other validated methods of similar sensitivity and accuracy may be used (see References [\[5\]](#) to [\[12\]](#), [\[28\]](#) and [\[29\]](#)).

4.1.2 Total fluoride in a single-unit container

The amount of total fluoride in a single-unit container shall not exceed 300 mg.

This requirement does not apply to containers of dentifrice to be dispensed under professionally supervised conditions or in community-based caries prevention programmes such as school toothbrushing programmes.

4.2 Heavy metals

The total maximum concentration of heavy metals shall not exceed 20 mg/kg.

Test in accordance with References [\[13\]](#), [\[14\]](#) or [\[15\]](#), or another validated method of similar sensitivity and accuracy.

4.3 pH

When tested in accordance with [5.1](#), the dentifrice shall have a pH below 10,5.

4.4 Microbiology

Testing for microbiological contamination shall be carried out according to References [\[17\]](#) to [\[22\]](#) and [\[31\]](#) to [\[38\]](#) or any other validated method of equivalent sensitivity, accuracy and specificity.

4.5 Abrasivity

The abrasivity of the dentifrice shall not exceed the following limit for dentine:

- 2,5 times that of the primary reference material, if using the procedure specified in [Annex A](#) or [B](#);

The abrasivity of the dentifrice shall not exceed the following limit for enamel:

- four times that of the primary reference material, if using the procedure specified in [Annex A](#) or [B](#).

Test in accordance with [5.2](#) or [5.3](#) or any other validated method of similar sensitivity and accuracy.

4.6 Stability

The dentifrice shall show no deterioration that may affect compliance with this document or could result in toxicological hazards after being subjected to one of the ageing procedures specified in [5.4](#) or after 30 months of storage at room temperature. If deterioration is detected, the dentifrice shall be labelled with an expiry date.

4.7 Readily fermentable carbohydrates

The dentifrice shall not contain readily fermentable carbohydrates. Compliance shall be established by the absence of such compounds in the complete formula or by performing tests in accordance with commonly used analytical methods.

5 Test methods

5.1 Determination of pH

Suspend one part by mass of the dentifrice 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.

5.2 Determination of dentine abrasivity

Determine the mean relative abrasivity compared to the primary reference sample, or any other reference material calibrated to the primary reference sample for human dentine, using one of the methods specified in [Annex A](#) or [B](#).

Other validated measurement methods on dentine of similar sensitivity and accuracy may be used, conforming to practices and principles found in References [\[39\]](#) to [\[44\]](#). For other references see, for example, References [\[23\]](#) and [\[24\]](#).

5.3 Determination of enamel abrasivity

Determine the mean relative abrasivity compared to the primary reference sample, or any other reference material calibrated to the primary reference sample for human enamel, using one of the methods specified in [Annex A](#) or [B](#).

Other validated measurement methods on enamel of similar sensitivity and accuracy may be used, conforming to practices and principles found in References [\[39\]](#) to [\[44\]](#). For other references see, for example, References [\[23\]](#) and [\[24\]](#).

5.4 Determination of stability

For the accelerated ageing procedure, the dentifrice shall be stored in its original container at $40\text{ °C} \pm 2\text{ °C}$ at $75\% \pm 5\%$ relative humidity for 3 months or at such conditions of time and temperature as will simulate storage at room temperature for 30 months [\[25\]](#). Following storage, test the product according to this document.

6 Marking and labelling

With the exception of small single units (less than 10 ml), all primary containers shall be marked with the following information:

- a) the word “dentifrice” or equivalent (see [Clause 3](#));
- b) the trade name;

- c) the name and contact information of the manufacturer or responsible distributor;
- d) the tracking code that includes an intelligible production date;
- e) a complete list of ingredients according to the International Nomenclature of Cosmetic Ingredients (INCI);
- f) the concentration and type of fluoride, if present, expressed in micrograms per gram, or percent by mass, or both;
- g) the net volume, in millilitres, or net mass in grams, or both;
- h) the expiry date, expressed according to ISO 8601, if the period of stability (shelf-life) is less than 30 months;
- i) a safety notice regarding the use, by children below 6 years of age, of dentifrices containing concentrations of fluoride of 1 000 µg/g or more.

7 Packaging

The product shall be packaged in such a way that under normal conditions of handling and transport, the container or dispensing system, or both, shall not contaminate or permit contamination of the dentifrice inside, so as to affect its compliance with this document, after being subjected to the ageing procedure described in [5.4](#).

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Annex A (informative)

Abrasivity test procedure — American Dental Association (ADA) method

A.1 General

This annex identifies the specific procedures for determination of the dentifrice abrasivity using the ADA laboratory method^[26].

A.2 Sampling

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

A.3 Procedure

A.3.1 Standard reference abrasive

The primary reference abrasive is from a specific lot of calcium pyrophosphate²⁾. An alternate, silica reference abrasive³⁾, is also available^[27]. For the procedure specified in BS 5136^[30], a chalk reference dentifrice is also available.

A.3.2 Apparatus

A.3.2.1 Brushing machine.

A cross-brushing machine is the apparatus of choice⁴⁾. 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.

2) Reference calcium pyrophosphate is available from Odontex Inc. 3030 Campfire Dr., Lawrence, KS 66040, USA, <http://www.odontexusa.com>. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

3) Alternative reference silica (Sident®) is available from Evonik, Rodenbacher Chaussee 4, 63457 Hanau Wolfgang, Germany. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

4) An acceptable product is available from Sabri Dental Enterprises, Inc., 1404 Brooke Dr., Downers Grove, IL 60515, USA, <http://www.sabridentalenterprises.com/p/about.html>. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

A.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.

A.3.3 Preparation of tooth specimens

A.3.3.1 Dentine specimens

A.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.

A.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.

A.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.

A.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.

A.3.3.2 Enamel specimens

A.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.

A.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.

A.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.

A.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.

A.3.4 Toothbrushes

The toothbrushes⁵⁾ 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 gauge⁶⁾ 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.

A.3.5 Reference diluent

The diluent is a 0,5 % carboxymethyl cellulose (7MF CMC)⁷⁾ 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.

A.3.6 Reference abrasive slurry

Using the reference material described in A.3.1, dilute 10 g of the abrasive with 50 ml of the diluent (see A.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.

A.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

5) Acceptable toothbrushes are available from Odontex Inc. 3030 Campfire Dr., Lawrence, KS 66040, USA, <http://www.odontexusa.com>. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

6) This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

7) An acceptable CMC is available from Hercules Incorporated, Aqualon Division, 1111 Hercules Road, Hopewell, VA 23860, USA, <http://www.ashland.com/industries/pharmaceutical/oral-solid-dose/aqualon-sodium-carboxymethylcellulose>. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

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.

A.3.8 Preconditioning of tooth specimens

A.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.

A.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.

A.3.9 Test design

A.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.

A.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.

A.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.

A.3.9.4 Correction factors

A.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.

A.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.

A.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 \(A.1\)](#):

$$C_f = \frac{C_r}{C_t} \quad (\text{A.1})$$

where

C_r is the mean counts for four reference samples;

C_t is the mean counts for four test samples.

A.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 [A.3.11](#), and there is no C_f term.

A.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.

A.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.

A.3.10 Calculation of abrasivity using Geiger-Müller counting

A.3.10.1 Dentine abrasivity

The dentine abrasivity, A_D , of the test dentifrices (or abrasives) is calculated as in [Formulae \(A.2\)](#) and [\(A.3\)](#):

$$C_{mr} = \frac{C_{pre} + C_{post}}{2} \quad (A.2)$$

where

C_{mr} is the mean reference net CPM;

C_{pre} is the pre-net CPM;

C_{post} is the post-net CPM.

$$A_D = \frac{C_f \times 100 \times C_{mt}}{C_{mr}} \quad (A.3)$$

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.

A.3.10.2 Enamel abrasivity

The enamel abrasivity, A_E , of the test dentifrices (or abrasives) is calculated as in [Formulae \(A.4\)](#) and [\(A.5\)](#):

$$C_{mr} = \frac{C_{pre} + C_{post}}{2} \quad (A.4)$$

where

C_{mr} is the mean reference net CPM;

C_{pre} is the pre-net CPM;

C_{post} is the post-net CPM.

$$A_E = \frac{C_f \times 10 \times C_{mt}}{C_{mr}} \quad (A.5)$$

where

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.

A.3.11 Calculation of abrasivity using liquid scintillation

A.3.11.1 Dentine abrasivity

The dentine abrasivity, A_D , of the test dentifrices (or abrasives) is calculated as in [Formulae \(A.6\)](#) and [\(A.7\)](#):

$$G_{mr} = \frac{G_{pre} + G_{post}}{2} \quad (A.6)$$

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.

$$A_D = \frac{100 \times G_{mt}}{G_{mr}} \quad (A.7)$$

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.

A.3.11.2 Enamel abrasivity

The enamel abrasivity of the test dentifrices (or abrasives) is calculated as in [Formulae \(A.8\)](#) and [\(A.9\)](#):

$$G_{mr} = \frac{G_{pre} \times G_{post}}{2} \quad (A.8)$$

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.

$$A_E = \frac{10 \times G_{mt}}{G_{mr}} \quad (A.9)$$

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 B (informative)

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

B.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 A](#)) and will be referred to as RDA-PE (Relative Dentin Abrasion – Profilometry Equivalent) and REA-PE (Relative Enamel Abrasion – Profilometry Equivalent).

B.2 Apparatus

B.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-2000⁸⁾], or a non-contact profilometer, or a similar instrument with a sensitivity to $<0,1 \mu\text{m}$ [e.g. Bruker GT-K1⁹⁾].

B.2.2 Lapping and polishing unit [e.g. Abramin automatic lapping and polishing unit¹⁰⁾], 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).

B.2.3 Brushing machine.

A cross-brushing machine is the apparatus of choice⁴⁾. 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.

8) Talyform i50 is the trade name for a product supplied by Taylor Hobson Ltd., Leicester, UK; and Surfometer is the trade name for a product supplied by Planar Products Ltd., Sunbury on Thames, UK; and Surftest SV-2000 is the trade name for a product supplied by Mitutoyo, Andover, UK. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

9) Bruker GT-K1 is the trade name for a product supplied by Bruker Co., Billerica, MA, USA. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

10) Abramin is the trade name for a product supplied by Struers Ap.S, Denmark. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

B.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 μm 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 [A.3.4](#) for an example of a suitable brush.

B.2.5 Standard reference abrasive as detailed in [A.3.1](#), being either pyrophosphate or silica abrasives.

B.3 Preparation of enamel and dentine specimens

B.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.

B.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.

B.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 \times 5 mm \times 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.

B.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.

NOTE In the case of molars, buccal, lingual, mesial and distal slices of enamel can be obtained. This is facilitated by a diamond-edged annular, circular cutting instrument.

B.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 resin¹¹⁾.

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 \times 3 mm \times 3 mm as described in [B.3.3](#).

B.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.

11) Examples of suitable epoxy resins are available from Dentsply liquid – Luciton R FasPore TM, methyl methacrylate (MMA) available from Henry Schein Dental Supply, item #167-7744 and Dentsply powder – Lucitone 199 R Powder available from Henry Schein Dental Supply item #167-7243. Alternative for “Dentsply” liquid and powder is “Durabase” liquid and powder. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

B.3.7 Measure the geometry of the specimens to ensure their quality. All specimens shall be plano-parallel, 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.

B.3.8 Measure the hardness of the specimens with Vickers indentations¹²⁾ 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.

B.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 methyl-methacrylate 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 tape¹³⁾ can be over-layered on the specimens.

B.4 Preparation of reference dentifrice

B.4.1 Reference diluent

Prepare the reference diluent as described in [A.3.5](#).

B.4.2 Reference abrasive slurry

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

B.5 Preparation of test dentifrice slurries

Prepare the test dentifrice slurries as described in [A.3.7](#).

B.6 Preparation of test brushes

B.6.1 The toothbrush used in testing shall have a rectangular head with flat-trim bristles. Brushes will be pre-conditioned 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.

12) For measurement of surface hardness, a Buehler Vicker Hardness Indenter can be used. Suitable instrumentation is available from Buehler, Lake Bluff, IL, USA. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

13) Suitable tape (1 mil polyester film tape with 1 or 2 mil silicone adhesive) can be obtained from Argon Masking Inc., Monrovia, CA, USA. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

B.7 Reference dentifrice and test dentifrice brushing procedure

B.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.

B.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.

B.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.

B.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).

B.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.

B.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).

B.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.

B.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 [B.8](#).

B.8 Profilometry method

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

B.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.

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

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

B.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 [B.7.6](#)).

B.9.2 Determination of proportional linearity

B.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.

B.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.

B.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.

B.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.

B.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.

B.9.3 Standard curve calculation

B.9.3.1 Standard curve calculation for RDA-PE

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

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

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

B.9.3.1.4 Use the Method of Least Squares to develop [Formula \(B.1\)](#):

$$y = a_{\text{RDA-PE}} x \quad (\text{B.1})$$

where

y is the mean depth of the reference standards;

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

Record the slope $a_{\text{RDA-PE}}$ of this dependence. Forcing the intercept through [0,0] further provides confidence of linear behaviour of the methodology.

B.9.3.2 Standard curve calculation for REA-PE

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

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

B.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).

B.9.3.2.4 Use the Method of Least Squares to develop [Formula \(B.2\)](#):

$$y = a_{\text{REA-PE}} x \quad (\text{B.2})$$

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.

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

B.9.4.1 Use the slope $a_{\text{RDE-PE}}$ from the correlation in [B.9.3.1.4](#). Divide the mean depth of the unknown by the slope $a_{\text{RDE-PE}}$ to calculate RDA-PE.

B.9.4.2 Use slope $a_{\text{REA-PE}}$ from the the correlation in [B.9.3.2.4](#). Divide the mean depth of the unknown by the slope $a_{\text{REA-PE}}$ to calculate REA-PE.

Annex C (informative)

A testing of total fluoride in dentifrices

C.1 General

This annex describes two different methods for testing the total fluoride content of dentifrices containing fluoride. See References [28] and [29] for other methods.

C.2 Methods

C.2.1 Total fluoride in dentifrice (paste and gel): ADA method

C.2.1.1 Background

This procedure implements a diffusion technique which extracts the fluoride as HF from the dentifrice matrix and then allows measurement of the total fluoride using an ion-specific electrode. Sample preparation is designed to account for the presence of calcium fluoride or silica-bound fluoride, or both, in the dentifrices using ethylenediaminetetraacetic acid (EDTA).

C.2.1.2 Procedure

Coat the inside of polystyrene Petri dish covers (60 mm × 15 mm) with sodium hydroxide by placing 0,3 ml of 0,5 mol/l sodium hydroxide in ethanol and allowing the alcohol to evaporate. Accurately weigh approximately 1 g of paste. Using a dilution ratio 1:10, add 10 ml of 0,1 mol/l EDTA solution with the pH previously adjusted to 8,0 by adding NaOH as necessary. Homogenize the mixture for 1 min and centrifuge 4 ml of the slurry at 14 000 r/min for 5 min using a centrifuge. Transfer 2,0 ml of the supernatant to the bottom of a Petri dish. Add 4,0 ml of 70 % HClO₄ and cover immediately with a sodium-hydroxide-coated Petri dish cover.

CAUTION — This latter step shall be done extremely carefully so that foam formed after adding HClO₄ does not wet the Petri dish cover.

Place the Petri dish in an oven at 60 °C ± 2 °C for at least 6 h.

Remove the Petri dishes from the oven and allow them to cool to room temperature. Remove the Petri dish cover and wash with 5,0 ml deionized water twice, resulting in a total volume of the solution of 10,0 ml. Transfer 1,0 ml of this solution to a 3 ml to 5 ml plastic beaker and add 1 ml of TISAB II. Analyse the solution for fluoride using an ion-specific electrode. Prepare a five-point calibration curve and use it to determine the fluoride content of each dentifrice slurry.

C.2.2 Total fluoride in dentifrice: IS 6356

NOTE This method is based on the Indian Standard IS 6356[8].

C.2.2.1 Principle

Sodium monofluorophosphate or fluoride ions are extracted with water from toothpaste and the extract is fused with sodium carbonate to convert it into sodium fluoride. The fluoride content is then determined potentiometrically with the help of a fluoride-ion-sensitive electrode.