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Dentistry — Evaluation of antibacterial activity of dental restorative materials, luting cements, fissure sealants and orthodontic bonding or luting materials

Médecine bucco-dentaire — Évaluation de l'activité antibacterial des produits pour restauration dentaires

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Contents

Page

Foreword

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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).

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

For an explanation of 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 www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 106, *Dentistry*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Due to the general applicability of in vitro tests for antibacterial activity and their widespread use in evaluating a large range of dental restorative materials, it is the purpose of this document to define a scheme for testing which requires decisions to be made in a series of steps rather than to specify a single test. This should lead to the selection of the most appropriate test for a respective dental restorative material to be evaluated.

Two categories of test are listed: extract test and direct contact test.

The choice of one or more of these categories depends upon the nature of the material to be evaluated, the potential site of use and the nature of the use of the respective material. Extract tests are primarily directed to substances leaching out from materials, whereas direct contact tests are directed to both, effects from leachables and surface effects. The choice of test then determines the details of the preparation of the samples to be tested, the preparation of the cultured bacteria or biofilms, and the way in which the bacteria or biofilms are exposed to the samples or their extracts.

Both categories of tests are intended to be first conducted toward planktonic cultures of bacteria and then, in case of positive results, toward bacterial biofilms.

This document proposes measurement of reduction of bacterial ability to replicate as the main method to assess antibacterial effects. Additionally, bacterial membrane damage can be assessed in order to further verify bacterial cell death, and reductions in bacterial metabolic activity can be investigated as another measure of bacterial viability.

There are several means of producing results in each of these test categories. The investigator should be aware of the test categories and into which category a particular technique fits, in order to ensure the comparability with other results on similar materials both at the intra- and interlaboratory level.

Examples of quantitative test protocols for assessing reduction of bacterial ability to replicate by colony forming units (CFU) assay, and additionally for assessing bacterial membrane damage by flow cytometry and for investigating reductions in bacterial metabolic activity by MTT assay are given in this document along with guidance for the interpretation of the results.

Dentistry — Evaluation of antibacterial activity of dental restorative materials, luting cements, fissure sealants and orthodontic bonding or luting materials

1 Scope

This document specifies test methods for the evaluation of dental restorative materials, luting cements, fissure sealants and orthodontic bonding or luting materials that are claimed by their respective manufacturers to exert "antibacterial" effects.

NOTE Materials for pulp capping (e.g. calcium hydroxide formulations), endodontic filling materials, dental implants or implant systems, nightguards, and additive manufactured (e.g. 3D-printed) materials are not covered in this document.

This document does not cover tests on the effectiveness of sterilization or disinfection procedures, nor shall it be used to demonstrate lack of microbial contamination of medical devices used in dentistry.

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 4049, *Dentistry — Polymer-based restorative materials*

ISO 6344-1, *Coated abrasives — Grain size analysis — Part 1: Grain size distribution test*

ISO 7405, *Dentistry — Evaluation of biocompatibility of medical devices used in dentistry*

ISO 10993-1, *Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process*

ISO 10993-5, *Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity*

ISO 10993-12, *Biological evaluation of medical devices — Part 12: Sample preparation and reference materials*

ISO 10993-18, *Biological evaluation of medical devices — Part 18: Chemical characterization of medical device materials within a risk management process*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 1942, ISO 7405, ISO 10993-1 and ISO 10993-5 apply.

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

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
dental restorative material
material or combination of materials specially formulated and prepared for use in the practice of dentistry and/or associated procedures for restoring lost integrity of teeth or for replacing teeth

3.2
positive control material
well characterized material and/or substance that, when evaluated by a specific test method, demonstrates the suitability of the test system to yield a reproducible, appropriately positive or reactive response in the test system

[SOURCE: ISO 7405:2018, 3.3]

3.3
negative control material
well characterized material and/or substance that, when evaluated by a specific test method, demonstrates the suitability of the test system to yield a reproducible, appropriately negative, nonreactive or minimal response in the test system

Note 1 to entry: In practice, negative control materials include materials lacking the active component that is responsible for antibacterial activity, or materials used in clinical practice with no antibacterial activity.

[SOURCE: ISO 7405:2018, 3.4, Note 1 modified]

3.4
antibacterial material
material exhibiting antibacterial activity as compared to the negative control material.

4 Requirements

4.1 General

The material claiming to be antibacterial shall meet one of the following requirements.

4.2 Extract

For tests on extract, an antibacterial material shall exhibit a median reduction of bacterial ability to replicate of at least 99,9% (3 $\log_{10}$ steps) as compared to the negative control material when tested in accordance with section 7.1.

NOTE This requirement is in accordance with the definitions of the American Society of Microbiology (https://aac.asm.org/content/abbreviations-and-conventions) [1, 2].

4.3 Direct contact

For tests by direct contact, an antibacterial material shall exhibit a median reduction of bacterial ability to replicate of at least 99% (2 $\log_{10}$ steps) as compared to the negative control material when tested in accordance with section 7.2.

NOTE This requirement is in accordance with the definitions outlined in JIS Z 2801 [3].

5 Sample preparation and control material preparation

5.1 General

The tests described in this document shall be performed on

a) an extract of the sample

and/or

b) the sample itself.

Assessment of antibacterial properties shall be made on the material prepared according to the manufacturer's instructions. Before testing antibacterial properties of dental restorative materials according to this standard, the physical and chemical properties of the material (and extracts) shall be assessed according to ISO 10993-1 and ISO 10993-18. Before testing antibacterial properties of polymer-based restorative materials, the physical behavior of the material should be characterized according to ISO 4049.

Negative and positive control materials shall be included in each assay. If appropriate and possible, controls should be prepared by the same procedure as the sample (see 5.2 to 5.5). In all cases, controls shall resemble the dimensions and other material properties such as roughness of the test materials. For direct contact tests, test materials and controls shall have a circular shape with a diameter of 10 mm to be used in 48-well plates (see 7.3).

For tests on extracts, 0,2% chlorhexidine digluconate shall be used as a positive control [4]. Additionally to the extracts from the negative control material, nutrient broth used for bacterial culture in the respective set of experiments (see Annex A for examples) shall be used as further negative control to ensure experimental validity.

For tests by direct contact, copper plates (circular shape; diameter 10 mm; purity ≥ 99%; absence of visible surface impurities) shall be used as positive control [5]. These plates shall be ground with a P2000 paper in accordance with ISO 6344-1 in order to provide similar roughness as compared to the samples.

All test or control samples shall be stored in sterile water at (37±1) °C after mixing/curing/milling as described by the manufacturer for 24 h prior to testing, e.g. for allowing leaching of monomers in polymers. After these initial 24 h, all test or control samples shall be tested at once, and additionally after 10 consecutive elution cycles (see 5.6.4) to provide an indication on long-term antibacterial activity [6]. If antibacterial activity is still observed after 10 elution cycles, a further test after 20 elution cycles should follow in order to demonstrate a plateau (= persisting effect) of the antibacterial activity.

NOTE Chemical analysis of the extracts should be additionally performed according to ISO 10993-18.

5.2 General recommendations for sample preparation

Sample preparation shall be in accordance with ISO 7405, ISO 10993-12, and ISO 4049.

For the preparation of samples, consult the respective product standards and/or the manufacturer's instructions, and follow those descriptions as closely as possible. Justify any deviation from the manufacturer's instructions. A detailed description of the sample preparation shall be included in the test report. Sample preparation shall take into account the following factors.

a) temperature;

b) humidity;

c) light exposure: samples of photosensitive materials shall be produced under the condition that ambient light does not activate them;

d) material of sample mould: ensure that the material of the sample mould and eventual lubricant used do not interfere with the setting process of the material;

e) oxygen exposure: for materials that produce an oxygen inhibition layer during hardening, both ends of the mould shall be covered with transparent oxygen barrier materials (e.g. polyester/mylar strips) during hardening;

f) samples shall be produced under aseptic conditions. In cases, where this is not possible, the samples may be sterilized by the method appropriate to the material, if necessary and possible (see 5.7.3).

5.3 Specific recommendations for light-curing materials

In accordance with ISO 7405, the following factors shall be taken into account, considering the final use of the light-curing material:

a) material of sample mould: if possible, the material of the sample mould should be in accordance with ISO 4049, i.e. stainless steel moulds with a white backing (white filter paper) at the bottom of the sample). In case this is not possible, reflection coefficients of materials used for sample moulds should be as close as possible to that of the oral surface to which the material is to be applied in order to simulate the clinical situation.

NOTE Suitable sample mould materials with reflection coefficients close to dental hard tissues can be semitranslucent or white plastic materials such as polyethylene (PE) or polytetrafluoroethylene (PTFE).

b) light exposure: light-curing shall be done to simulate clinical usage as closely as possible. This will often require curing from one side only but will sometimes entail a two-sided cure. The cure method is material and/or process specific. In the case of one-component materials, there shall be no voids, clefts or air-bubbles present when viewed without magnification. To provide the same level of curing as would be the case in clinical usage, follow the instructions for use of the material manufacturer including the recommended powered polymerization activator, which shall include the emission wavelength region(s), the irradiance (≥ 1 000 mW/cm^2), and the exposure time. This information shall be documented in the Test Report. Care shall be taken to ensure that the light source is in a satisfactory operating condition and the use of a radiometer before using the light source is recommended to verify the irradiance from the unit;

c) oxygen exposure: for materials that produce an oxygen inhibition layer during light-curing, both ends of the mould shall be covered with transparent oxygen barrier materials (e.g. polyester/mylar strips) during light-curing.

d) sample surface treatment: if the material is recommended by the manufacturer for surface finishing after curing, the sample surfaces shall be ground and polished using the recommended clinical procedures. If there are no such instructions and if required for testing, the samples shall be ground on both ends, with a P2000 paper in accordance with ISO 6344-1, after first being set against the transparent oxygen barrier material.

5.4 Specific recommendations for chemically setting materials

In accordance with ISO 7405, the following factors shall be taken into account, considering the final use of the chemically setting material:

a) mixing: mix sufficient material to ensure that the preparation of each sample is completed from one batch. Prepare a fresh mix for each sample. The mixing shall be performed in accordance with the respective product standards, if applicable;

b) oxygen exposure: for materials that produce an oxygen inhibition layer during chemical curing, both ends of the mould shall be covered with oxygen barrier materials (e.g. polyester/mylar strips) during curing.

c) sample surface treatment: if the material is recommended by the manufacturer for surface finishing after curing, the sample surfaces shall be ground and polished using the recommended

clinical procedures. If there are no such instructions and if required for testing, the samples shall be ground on both ends, with a P2000 paper in accordance with ISO 6344-1, after first being set against the transparent oxygen barrier material.

5.5 Specific recommendations for CAD/CAM milled or subtractive manufactured materials

The following factors shall be taken into account, considering the final use of the CAD/CAM milled or subtractive manufactured material:

a) sample surface treatment: if the material is recommended by the manufacturer for surface finishing after CAD/CAM milling or subtractive-manufacturing, the sample surfaces shall be ground and polished using the recommended clinical procedures. If there are no such instructions and if required for testing, the samples shall be ground on both ends, with a P2000 paper in accordance with ISO 6344-1.

5.6 Preparation of liquid extracts of material

5.6.1 Principles of extraction

Preparation of extracts shall be performed after a 24 h-storage in sterile water at (37±1) °C following mixing/curing as described by the manufacturer, and additionally after 10 consecutive elution cycles (see 5.6.4) to provide an indication on long-term antibacterial activity [6].

If antibacterial activity was still observed for the extract after the 10th elution cycle, a further test on an extract after 20 elution cycles should follow in order to demonstrate a plateau (= persisting effect) of the antibacterial activity.

NOTE Chemical analysis of the extracts should be additionally performed according to ISO 10993-18.

Extracting conditions should attempt to simulate or exaggerate the clinical use conditions so as to determine the potential antibacterial activity without causing significant changes in the sample, such as fusion, melting or any alteration of the chemical structure, unless this is expected during clinical application. Due to the nature of certain materials (e.g. biodegradable materials), alteration of the chemical structure may occur during the extraction procedure.

NOTE The concentration of any endogenous or extraneous substances in the extract, and hence the amount exposed to the test bacteria, depends on the interfacial area, the extraction volume, pH, chemical solubility, diffusion rate, osmolarity, agitation, temperature, time and other factors.

5.6.2 Extraction vehicle

The choice of the extraction vehicle(s) taking into account the chemical characteristics of the sample shall be justified and documented. One or more of the following vehicles shall be used:

a) nutrient broth used for bacterial culture in the respective set of experiments (see Annex A for examples);

b) phosphate-buffered saline (PBS);

c) serum (for extraction of lipids);

The choice of vehicle should reflect the aim of the extraction. Nutrient broth is the preferred extraction vehicle because of its ability to support bacterial growth as well as and extract both polar and non-polar substances.

NOTE It is important to recognize that proteins from protein-rich or serum-containing nutrient broths are known to bind, to some extent, extractables.