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Dentistry — Test methods for dental unit waterline biofilm treatment

Médecine bucco-dentaire — Méthodes d'essais pour le traitement du biofilm dans les conduites d'eau de l'unit dentaire

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ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

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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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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 106, *Dentistry*, Subcommittee SC 6, *Dental equipment*.

This first edition of ISO 16954:2015 cancels and replaces the first edition of ISO/TS 11080:2009, of which it constitutes a technical revision.

Dentistry — Test methods for dental unit waterline biofilm treatment

1 Scope

This International Standard provides type test methods for evaluating the effectiveness of treatment methods intended to prevent or inhibit the formation of biofilm or to remove biofilm present in dental unit procedural water delivery systems under laboratory conditions.

This International Standard does not apply to devices intended to deliver sterile procedural water or sterile solution. It also does not apply to lines, tubing, or hoses that deliver compressed air within the dental unit.

This International Standard does not establish specific upper limits for bacterial contamination or describe test methods to be used in clinical situations. It also does not establish test methods for evaluating any deleterious side effects potentially caused by treatment methods.

The test methods provided in this International Standard can be used to test other dental equipment that delivers non-sterile water to the oral cavity.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 7494-1, *Dentistry — Dental units — Part 1: General requirements and test methods*

ISO 7494-2, *Dentistry — Dental units — Part 2: Water and air supply*

ISO 10523, *Water quality — Determination of pH*

ISO 19458, *Water quality — Sampling for microbiological analysis*

IEC 60601-1, *Medical electrical equipment — Part 1: General requirements for basic safety and essential performance*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in IEC 60601-1, ISO 1942, ISO 7494-1, and ISO 7494-2 and the following apply.

3.1
biofilm
structured community of microorganisms inhabiting a self-developed extracellular biopolymeric matrix attached to a surface

3.2
dental unit
combination of interconnected dental equipment and dental instruments constituting a functional assembly for use in the provision of dental treatment

[SOURCE: ISO 1942:2009, 2.86]

3.3
dental unit procedural water delivery system
system of components of a dental unit which convey water from a supply source to one or more outlets used for dental treatment

3.4
procedural water
water supplied by the dental unit for use in the oral cavity

EXAMPLE Handpiece procedural water, multifunctional syringe water, scaler procedural water, or rinse cup water.

[SOURCE: ISO 7494-2:2003, 3.1]

3.5
surrogate dental unit water system
test apparatus which accurately recreates the procedural water delivery system of a dental unit, including design, construction, configuration, and operation of all water-bearing elements of the procedural water delivery system, but not necessarily including other dental unit components which do not directly come in contact with or control the flow of procedural water

3.6
test water
water having specified chemical and physical characteristics used for testing prior to the addition of the specified bacterial challenge suspension

3.7
bacterial challenge suspension
consortium of specified bacteria suspended in a nutrient growth medium or buffered solution used to inoculate test water

3.8
inoculated test water
prepared aqueous suspension used in testing, containing specified amounts of sterilized test water and one or more bacterial challenge suspension(s)

3.9
test apparatus for the control group
apparatus used in testing in which no treatment method is applied and no antimicrobial material is present in the waterline components

3.10
test apparatus for the test group
apparatus used in testing in which a treatment specified by the dental unit manufacturer is applied and any antimicrobial materials specified by the manufacturer are present in the waterline components, unless otherwise specified in the test requirements

4 Treatment methods

Depending upon the specific technical approach of a treatment method and its intended benefits, the performance objectives of a dental unit procedural water delivery system treatment method can include one or both of the following:

— prevention or inhibition of biofilm formation on surfaces within the dental unit procedural water delivery system;

— removal of biofilm from surfaces within the dental unit procedural water delivery system.

This International Standard specifies separate test methods for each of the above performance objectives. These requirements can be expanded upon, for example to include additional replicates or test scenarios. Additions to the test method shall follow the general principles of this International Standard and be fully described in the test report.

5 Test water and bacterial challenge suspensions

5.1 Test water

This subclause specifies the preparation of test water prior to inoculation.

5.1.1 Reagents

5.1.1.1 Water, in accordance with ISO 3696:1987, grade 3.

5.1.1.2 Calcium chloride ($CaCl_2$), or an equivalent molar quantity of a calcium chloride hydrate.

5.1.1.3 Magnesium chloride ($MgCl_2$), or an equivalent molar quantity of a magnesium chloride hydrate.

5.1.1.4 Sodium bicarbonate ($NaHCO_3$).

5.1.1.5 Tryptic soy broth (TSB), 1/3-strength, 10,0 g tryptic soy medium per litre broth.

5.1.1.6 Sodium hydroxide (NaOH), 1 mol/l.

5.1.1.7 Hydrochloric acid (HCl), 1 mol/l.

5.1.2 Preparation of hardness stock solution 1

Dissolve 74,0 g of calcium chloride (5.1.1.2) and 31,7 g of magnesium chloride (5.1.1.3) in 1,00 l water (5.1.1.1). Hardness stock solution 1 shall be sterilized by heat or filter-sterilized using a 0,2 µm microfilter and used within 24 h or stored at (5 ± 3) °C for up to 6 months.

5.1.3 Preparation of hardness stock solution 2

Dissolve 56,0 g of sodium bicarbonate (5.1.1.4) in 1,00 l water (5.1.1.1). Hardness stock solution 2 shall be filter-sterilized using 0,2 µm microfilter and used within 24 h or stored at (5 ± 3) °C for up to 6 months. Hardness stock solution 2 is not to be heat sterilized.

5.1.4 Preparation of test water prior to inoculation

For each litre of test water to be prepared, add 1,00 ml of 1/3-strength TSB (5.1.1.5) and 1,80 ml of hardness stock solution 1 (5.1.2) to 1,00 l water (5.1.1.1) and steam sterilize. After the sterilized solution has cooled, for each litre of test water add 4,00 ml of hardness stock solution 2 which has been filter-

sterilized using a 0,2 µm microfilter. Adjust the pH to 7,0 to 8,0, measured according to ISO 10523, by adding sodium hydroxide (5.1.1.6) or hydrochloric acid (5.1.1.7). The test water shall be used within 24 h or stored at (5 ± 3) °C for up to one week.

NOTE 1 The hardness of the prepared test water is approximately 1,8 mmol of calcium ions per litre (equivalent to approximately 180 mg/l as $CaCO_3$). This corresponds to the upper limit of the generally accepted range for hard water.[17]

NOTE 2 The test water has a concentration of approximately 10 mg TSB per litre, which yields a total organic carbon (TOC) level of approximately 4 mg/l, although the exact TOC level might vary somewhat. This approximate TOC level is consistent with the recommended upper limit of 4 mg/l for TOC in chlorinated drinking water[1] and is included in the test water to reduce the time for biofilm formation.

5.2 Bacterial challenge

Bacterial challenge suspensions used to inoculate the test water shall be prepared with the following bacteria from the American Type Culture Collection (ATCC) or an international authorized ATCC distributor:

a) *Pseudomonas aeruginosa* (ATCC #700888);

b) *Klebsiella pneumoniae* (ATCC #13882).

Alternate strains of *P. aeruginosa* and *K. pneumoniae* (i.e. different ATCC numbers for the same bacterium species) can be substituted if the specified strains are not available, provided that the isolation source indicated by ATCC is water or a water system.

The bacterium species shall be separately reconstituted in sterilized dilute TSB, having a concentration of 0,3 g TSB per litre. The reconstituted cultures shall be used within eight transfer passages. Cultivation of the bacteria for inoculating test water shall be performed one day before preparing the inoculated test water in accordance with 5.3.

Compliance with appropriate laboratory safety practices is critical when working with these bacteria, including the handling of waste that can be contaminated with these bacteria.

5.3 Inoculated test water

On days of test apparatus operation inoculated test water shall be prepared within two hours before the commencement of the daily flow program specified in 6.2.2 by inoculating sterilized test water (5.1) with both of the reconstituted bacterial cultures (5.2), to achieve a concentration of 5×10^1 CFU/ml to 5×10^2 CFU/ml of each of the bacteria and a total bacterial concentration of 10^2 CFU/ml to 10^3 CFU/ml in the inoculated test water. The temperature of the sterilized test water at the time of inoculation shall be (23 ± 3) °C.

To ensure accurate concentrations of each bacterium species in the inoculated test water, it can be useful to centrifuge and re-suspend each of the reconstituted bacterial cultures in sterile phosphate buffer and determine the approximate bacterial concentration using turbidimetric measurements. Using these results, the volume of each of the single-species bacterial suspensions to be added to the test water can be calculated. Alternatively, other methods for achieving the specified inoculation range can be used.

6 Test apparatus

The test apparatuses shall consist of a specified number of either dental units or surrogate dental unit water systems which closely replicate the dental unit procedural water system.

In order to achieve reproducible results, all components of the test apparatuses which are in contact with the procedural water shall be new each time the test procedure for biofilm prevention or inhibition

(7.2) or the sequence of test procedures for biofilm prevention or inhibition (7.2) and for biofilm removal (7.2) is performed.

NOTE As specified in 7.3.1, the test procedure for biofilm removal (7.3) is performed after first developing biofilm in the test apparatuses per the test procedure for biofilm prevention or inhibition (7.2).

6.1 Test apparatus design

6.1.1 General

If the test apparatuses consist of dental units, the dental units shall represent the most challenging model or configuration (when more than one model or configuration is available from the manufacturer). Length of waterlines, number of branch waterlines and likelihood of stagnation are among the factors that shall be considered in determining the most challenging model or configuration.

If the test apparatuses consist of surrogate dental unit water systems, the surrogate dental unit water systems must be able to simulate the basic clinical performance parameters of a functioning dental unit, including as described in 6.2. The surrogate dental unit water systems must represent the most challenging model or configuration of the dental unit which the surrogate dental unit water systems are intended to represent (when more than one model or configuration is available from the manufacturer). Surrogate dental unit water systems shall accurately recreate the procedural water delivery system, including design, construction, configuration and operation of the water-bearing elements of the procedural water delivery system. Other components which do not directly come in contact with or control the flow of procedural water need not be included, such as structural and decorative components. The components of surrogate dental unit water systems shall be subject to the same environmental conditions (i.e. light exposure and temperature) as components in the dental units which they represent.

Any air gap system or other backflow prevention device required to comply with the backflow prevention requirements of ISO 7494-2 for isolating the procedural water from the incoming water shall be included in the test apparatuses.

Critical elements to be recreated by the surrogate dental unit water systems includes the following:

— configuration of waterlines (placement of components, arrangement of branch lines, dead legs, etc.);

— tubing diameter(s);

— tubing length(s);

— tubing material(s);

— other components which contact procedural water or regulate flow (control blocks, valves, fittings, etc.);

— location of any water treatment devices (filters, automatic or passive treatment systems).

Dental units or surrogate dental unit water systems shall include at least one hose which in normal use supplies procedural water to a handpiece and one hose which in normal use supplies procedural water to a multifunctional (air/water) syringe. Hoses which normally supply procedural water to other instruments that are attached to the dental unit can be included in the dental units or surrogate dental unit water systems. Dental handpieces or other instruments or attachments that are normally removed from the dental unit and sterilized between patients shall be disconnected from the hose and not used in this test if practical.

EXAMPLE At point C according to ISO 14457:2012, Figure A.1.

Cuspidor bowl rinse waterlines shall be disconnected from dental units or excluded from surrogate dental unit water systems. Cuspidor cupfill waterlines can be included in dental units or surrogate dental unit water systems.

NOTE Cuspidor waterlines are permitted to be excluded based on the assumption that other waterlines will tend to be more challenging test environments for the removal, prevention, and inhibition of biofilm.

6.1.2 Considerations specific to antimicrobial materials and materials which prevent microbial adhesion

If the dental units or surrogate dental unit water systems to be tested include any antimicrobial materials or materials which prevent microbial adhesion in contact with the procedural water, the following modifications to the test apparatuses shall be implemented.

Test apparatuses for evaluating biofilm prevention or inhibition:

— Test apparatuses for the control group: Any antimicrobial materials or materials which prevent microbial adhesion in the dental units or surrogate dental unit water systems in the control group shall be replaced with materials that do not contain the antimicrobial agent(s) or adhesion preventive effect, but otherwise closely represent the antimicrobial material.

— Test apparatuses for the test group: The dental units or surrogate dental unit water systems in the test group shall include (if applicable) the antimicrobial material(s) or material(s) which prevent microbial adhesion.

Test apparatuses for evaluating biofilm removal:

— Test apparatuses for the test group: Any antimicrobial materials or materials which prevent microbial adhesion in the dental units or surrogate dental unit water systems in the test group shall be replaced with materials that do not contain the antimicrobial agent(s) or adhesion preventive effect, but otherwise closely represent the antimicrobial material. This is essential to evaluating the ability of the test treatment method to remove biofilm.

6.2 Test apparatus operation

6.2.1 Flow rates

The flow rate of the handpiece procedural water shall be adjusted to (30 ± 3) ml/min.

The water flow rate of the multifunctional (air/water) syringe(s) shall be adjusted to (60 ± 6) ml/min.

If the test apparatuses include other instruments or devices supplied with procedural water, the flow rate(s) of those instruments or devices shall be adjusted according to manufacturer recommendations.

All flow rates shall be set prior to the start of the test. Flow rates shall be checked and adjusted if necessary at least once per week throughout the test period.

6.2.2 Flow patterns (on-off cycles)

The test apparatus waterlines shall be operated five consecutive days per week and set idle with no operation for two consecutive days per week. On days of waterline operation, inoculated test water shall be freshly prepared according to 5.3 and supplied to the test apparatuses. The water flow pattern shall be controlled by an automated daily flow program consisting of 30 cycles, which periodically operate the test apparatus waterlines according to the following schedule.

— The handpiece procedural water shall be operated for 30 sec per cycle. If more than one handpiece supplying procedural water is present, only one headpiece procedural waterline shall be operated during each cycle. The selected headpiece procedural waterline shall change sequentially with each cycle, assuring that each headpiece procedural waterline is operated approximately equally throughout the daily flow program.

— The syringe water shall be operated for 30 sec per cycle. If more than one syringe is present, only one syringe shall be operated during each cycle. The selected syringe shall change sequentially with each cycle, assuring that each syringe is operated approximately equally throughout the daily flow program.

— There shall be a period of 9 min per cycle without any water flow.

If instruments other than handpieces and syringes are included in the test apparatuses, their operation shall be incorporated into the daily flow program in a manner consistent with their intended clinical use.

6.2.3 Test environment temperature and preconditioning period

The temperature of the test environment shall be maintained at (23 ± 3) °C. All apparatuses shall be conditioned in this temperature range for at least 24 h before starting the test.

7 Test procedures

7.1 Testing sequence

Testing shall be performed in the following sequence:

a) Testing to evaluate biofilm prevention or inhibition according to 7.2;

b) Testing to evaluate biofilm removal according to 7.3.

NOTE This sequence reduces the quantity of test apparatuses and procedural steps by enabling the control group apparatuses having an established biofilm upon completion of 7.2 to be used as the test group apparatuses in 7.3.

7.2 Biofilm prevention or inhibition

7.2.1 General

Figure 1 depicts the test procedure for evaluating treatment methods that are intended to prevent or inhibit dental waterline biofilm formation.

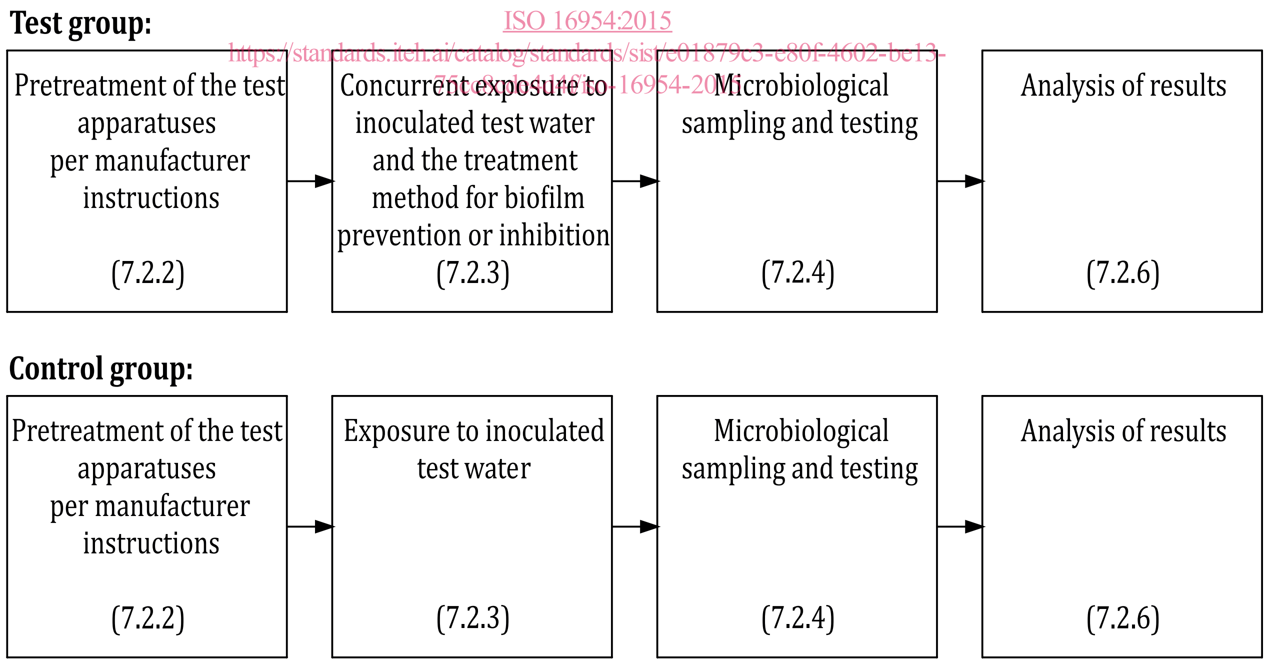

Figure 1 — Flow diagram for test method for evaluating biofilm prevention or inhibition

Since there is inherent variation in biofilm formation, testing shall be replicated on separate dental units or surrogate dental unit water systems. For the biofilm prevention and inhibition test method, at least two dental units or surrogate dental unit water systems shall be included in the test group and at least two dental units or surrogate dental unit water systems shall be included in the control group.

