BS EN 16421:2014

BSI Standards Publication

Influence of materials on water for human consumption — Enhancement of microbial growth (EMG)

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National foreword

This British Standard is the UK implementation of EN 16421:2014.

The purpose of this standard is to describe three European test methods that can be applied to determine the ability of nonmetallic materials to enhance microbial growth in drinking water.

Each method uses different performance characteristics, which allows its use for specific materials or product types, but all three methods have limitations. For example, multi-layer pipes cannot currently be tested with the Method 1 and Method 3, and small components cannot easily be analysed using Method 2. Harmonized product standards will specify the particular method to follow, taking into account material of construction and type of components. Pass/fail criteria is not included in this standard and will be specified by the individual Member State.

At the time of publication, this standard is not currently included in the (UK) product testing to be used for materials in contact with drinking water as all the methodology in BS 6920, together with the pass/fail criteria, has not been replaced by European Standards. Studies to establish the degree of agreement of the results obtained using the three methods on a wide range of specific materials types will enable the establishment of comparative pass/fail criteria. To ensure the correct methodology is being used, refer to the National Approval schemes for the UK before undertaking any testing. Further information can be obtained from UK approvals bodies, such as the Drinking Water Inspectorate and the Water Regulations Advisory Scheme.

The UK participation in its preparation was entrusted to Technical Committee EH/6, Effects of materials on water quality.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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Influence of materials on water for human consumption - Enhancement of microbial growth (EMG)

Influence des matériaux sur l'eau destinée à la consommation humaine - Stimulation de la croissance microbienne (SCM)

Einfluss von Materialien auf Wasser für den menschlichen Gebrauch - Förderung des mikrobiellen Wachstums

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Foreword

This document (EN 16421:2014) has been prepared by Technical Committee CEN/TC 164 “Water supply”, the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by June 2015, and conflicting national standards shall be withdrawn at the latest by June 2015.

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

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

Water intended for human consumption comes into contact with construction products during storage, transportation and distribution, including water systems inside buildings. The materials used in these products are selected on the basis of technical requirements and criteria regarding their influence on the water quality, e.g. release of substances and effects on odour, flavour or colour of the water. However, water quality problems may also arise when such materials enhance the multiplication of micro-organisms.

A test method to determine the enhancement of microbial growth is required as organic substances present in non-metallic materials (either as ingredients, contaminants or process by-products) are capable of being utilized by micro-organisms and can give rise to a noticeable deterioration in the organoleptic, physical or microbiological quality of the water with which they are in contact. Microbial growth may occur in the water itself or at the material/water interface.

Materials with the potential of supporting microbial growth do not necessarily lead to a deterioration in water quality in every situation due to the influence of various environmental factors, e.g. microbial quality of the water, temperature, presence of residual disinfectant or other growth limiting factors.

The purpose of this standard is to describe three European test methods that can be applied to determine the ability of non-metallic materials to enhance microbial growth in drinking water.

a) Method 1 determines the Biomass Production Potential (BPP) by using changes in ATP concentrations as a surrogate measure for active biomass. This method, developed by the Dutch, has been further enhanced as part of the CPDW project 2003 and 2006.

b) Method 2 uses a volumetric measurement of the biofilm. This, German method, was first published as DVGW W 270 in 1984 and is used for certification purposes with limit values established for many years.

c) Method 3 uses dissolved oxygen depletion in water as a surrogate measure of microbial activity (Mean Dissolved Oxygen Difference – MDOD). This British method, first issued as BS DD82 in 1982 and published as BS 6920 Section 2.4 (1988 and 2000), is used for materials approval with limit values.

Each method thus uses different performance characteristics, which allows its use for specific materials or product types but also has limitations. For example, multi-layer pipes cannot currently be tested with the BPP (Method 1) and the MDOD-method (Method 3), and greases or lubricants cannot currently be tested with the BPP (Method 1) and Volumetric-method (Method 2). Harmonised product standards will provide the specific methodology to be followed; this will take into account material of construction and type of components.

All three methods use natural mixtures of aquatic organisms to assess the enhancement of growth by the sample of material. The natural flora comprises many strains that are adapted to living in a relatively hostile environment like drinking water and the results of tests using natural floras have been shown to correlate well with growth on materials in practice. The numbers, types and growth requirements of harmless micro-organisms present in drinking water vary considerably and no single cultural technique exists to enumerate all the aquatic micro-organisms that may be present in a sample of water. Therefore, overall numbers of micro-organisms are generally assessed by using simple indirect measurements of their activity.

The technique for assessing enhanced microbial growth is different in each of the test methods described in this European Standard. In the BPP method described in Method 1 surface and planktonic microbial growth is determined using adenosine triphosphate (ATP) as a surrogate method for active biomass determination. In the Volumetric method (DVGW) described in Method 2, the sum of both active and non-active biofilm on the surface of the test material (living and dead micro-organisms as well as extracellular polymeric substances) is determined volumetrically. In the MDOD method described in Method 3 the measurement of dissolved oxygen uptake is used as a surrogate measure of the growth of both biofilm and planktonic aquatic micro-organisms (most of the organisms which give rise to appreciable microbial growth respire aerobically and exert an influence on the concentration of oxygen dissolved in the water in the test systems).

A variety of factors may influence the capacity of living organisms to respond in a predictable manner and thus validation procedures are an essential part of any biological assay. In all three methods validation is achieved through the use of reference materials.

It is important to note that none of the three methods allows conclusions to be made on the physical (including surface roughness), chemical or toxicological behaviour of materials nor on their resistance to detergents or disinfectants. Additionally, none of the methods provides information on the pathogenicity of any micro-organisms whose numbers may be increased by nutrients leaching from the test material.

WARNING – The tests described in this document should only be carried out in laboratories with suitable facilities and by suitably qualified persons with an appropriate level of chemical and microbiological expertise. Standard microbiological procedures should be followed throughout.

1 Scope

This European Standard specifies three methods for determining the ability of non-metallic materials to enhance the growth of micro-organisms.

This European Standard is applicable to those materials destined to be used under various conditions for the transport and storage of water intended for human consumption.

The standard allows for the testing of a single type of material, or a product in which only one material is in contact with water. It is unsuitable for use with assembled products where more than one material is exposed to water.

NOTE The results given by each method are not directly comparable.

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.

EN 901, *Chemicals used for treatment of water intended for human consumption — Sodium hypochlorite*

prEN 1254-1:2007, *Copper and copper alloys — Plumbing fittings — Part 1: Fittings with ends for capillary soldering or capillary brazing to copper tubes*

EN 1484, *Water analysis — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)*

EN 10088-1, *Stainless steels — Part 1: List of stainless steels*

EN 14944-1, *Influence of cementitious products on water intended for human consumption — Test methods — Part 1: Influence of factory made cementitious products on organoleptic parameters*

EN ISO 3696:1995, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987)*

EN ISO 5814, *Water quality — Determination of dissolved oxygen — Electrochemical probe method (ISO 5814)*

EN ISO 7393-2, *Water quality — Determination of free chlorine and total chlorine — Part 2: Colorimetric method using N, N-diethyl-1, 4-phenylenediamine, for routine control purposes (ISO 7393-2)*

EN ISO 9308-1, *Water quality — Enumeration of Escherichia coli and coliform bacteria — Part 1: Membrane filtration method for waters with low bacterial background flora (ISO 9308-1)*

EN ISO 10012, *Measurement management systems — Requirements for measurement processes and measuring equipment (ISO 10012)*

EN ISO 10523, *Water quality — Determination of pH (ISO 10523)*

EN ISO 13385-1, *Geometrical product specifications (GPS) — Dimensional measuring equipment — Part 1: Callipers; Design and metrological characteristics (ISO 13385-1)*

EN ISO 13385-2, *Geometrical product specifications (GPS) — Dimensional measuring equipment — Part 2: Calliper depth gauges; Design and metrological characteristics (ISO 13385-2)*

EN ISO 16266, *Water quality — Detection and enumeration of Pseudomonas aeruginosa — Method by membrane filtration (ISO 16266)*

ISO 2016, *Capillary solder fittings for copper tubes — Assembly dimensions and tests*

3 Terms and definitions

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

3.1
ATP
adenosine tri-phosphate is the compound produced in the cells of all living organisms which, in the context of this method, is used to measure the amount of active biomass in water and on the surface of a test piece

3.2
Attached Biomass (AB) concentration
micro-organisms producing ATP, attached to a surface

3.3
biofilm
aggregate of micro-organisms that adhere to surfaces in contact with aqueous liquids that are usually embedded in a self-produced matrix of extra-cellular polymeric substances (EPS)

Note 1 to entry: Includes living and non-living organisms

3.4
Biomass Production (BP)
sum of Attached Biomass (3.2) and Suspended Biomass (3.14)

3.5
Biomass Production Potential (BPP)
mean of Biomass Production (3.4) measurements after having taken into account the mean of the growth associated with the negative control

3.6
controls
specific test pieces with known properties with respect to micro biological growth

Note 1 to entry: Controls are used to demonstrate the satisfactory performance of the test

3.7
drinking water
water intended for human consumption conforming to the EC Directive 98/83/EC (on the quality of water intended for human consumption)

3.8
material
formulation of one or more single substances with specified composition and specified production procedure

3.9
negative control
specific test pieces made of materials that are known not to enhance microbial growth

3.10
positive control
specific test pieces made from materials that are known to enhance microbial growth

3.11
product
manufactured item in its finished form

Note 1 to entry: Examples of types of products:

— single material products.

— assembled products. These products comprise two or more components, possibly of different materials.

— multi-layer products. These products comprise two or more layers bonded together to form a single item.

— formulated products. These products are created by chemical processing after which the original constituents are no longer identifiable, e.g. lubricants and glues.

— site applied products. Products such as coatings and linings placed on the market as ingredients that will be mixed and applied on site.

3.12
purified water
purified water complying with Grade 3 of EN ISO 3696:1995

3.13
surface colonisation
micro-organism, attached on any surface in contact with drinking water, which can only be determined by contact cultures, swabs or by other suitable microbiological test procedures

3.14
Suspended Biomass (SB) concentration
micro-organisms producing ATP present in the test water

3.15
test piece
specimen(s) or portion(s) of the test sample which is (are) conditioned, treated or otherwise prepared to be tested to obtain a single test result

3.16
test sample
item(s) drawn from a batch or lot, as received for testing

3.17
test water
drinking water (3.7) together with any additional test method specific requirements

3.18
trace elements
specified substances that need to be present in the test water to ensure consistent microbial growth

4 Influence of materials on water for human consumption – Enhancement of microbial growth (EMG) – Method 1: Measured by Biomass Production Potential (BPP) measured by ATP

4.1 General

The Annexes A to E are a part of Method 1: Measured by (BPP).

This method describes the Biomass Production Potential (BPP) test which determines the growth of micro-organisms in the presence of a material incubated for a specified period of time in biologically stable water.

The Biomass Production Potential (BPP) is a method for testing the ability of a material to promote the growth of microorganisms that is based on a combination of two principles, as follows:

a) batch-based test conditions with replacement of the test water every seven days, and

b) determination of the amount of active biomass by measurement of adenosine tri-phosphate (ATP). Information on ATP and its measurement are given in Annex C.

4.2 Principle

Representative samples of the material to be tested are incubated in appropriately amended drinking water and inoculated with a mixture of naturally occurring micro-organisms derived from a surface source of water. The test pieces are incubated for a specified period of time (16 weeks) at a constant surface area to volume ratio of 0,16 cm^{-1}. This ratio is kept constant adjusting the volume of water in the test containers when a test piece is removed for biomass measurement.

The test water is replaced once every 7 days.

Formation of biomass on the test piece and in the water is determined with adenosine triphosphate (ATP) measurements after 56, 84 and 112 days of incubation. The amount of ATP is used as a measure for the amount of active biomass. The biomass associated with a defined surface area of the test piece is calculated from the amounts of attached and Suspended Biomass.

Each test is validated by the satisfactory performance of the positive and negative controls under equivalent conditions.

The test procedure is shown schematically in Annex E.

4.3 Apparatus

4.3.1 Requirements

Cleaning and sterilisation – all apparatus shall be cleaned and sterilised using procedures that render it suitable for use.

Apparatus and reagents specifically for measurement of ATP are listed separately in Annex B.

4.3.2 Vessels, containers, stoppers and connectors and general laboratory apparatus

Vessels, containers, stoppers and connectors and general laboratory apparatus shall consist of a material, such as glass and stainless steel that is inert under the specified test conditions. PTFE can be used for connectors and stoppers provided that it does not come into contact with the test water. PTFE is unsuitable for larger areas such as containers.

4.3.3 Cleaning

General laboratory glassware, stainless steel plates, test containers and test tubes for dilution water shall be cleaned with a procedure that removes all organic carbon to render it suitable for the requirements of the test.

NOTE A possible way would be by washing with a biodegradable laboratory detergent, followed by rinsing with hydrochloric acid solution, prepared by slowly adding (0,5 ± 0,01) litre of concentrated hydrochloric acid (concentrated, 30 % mass per volume, analytical reagent grade) to (0,5 ± 0,01) litre of purified water and by thoroughly rinsing with purified water. Drain and dry glassware, plates and test containers in a hot air cabinet. Drain the test tubes, cover with caps and heat at 150 °C to 175 °C for 4 h.

4.3.4 Gloves

Gloves for handling test pieces, to avoid contamination from ATP and organic carbon. Powdered latex gloves should be avoided.

4.3.5 Plates

Plates shall be of an appropriate material to be covered completely with the test material. Plates shall be cleaned according to the cleaning procedure described in 4.3.3.

4.3.6 Test containers

Test containers include screw topped glass jars with a volume of 1 000 ml, an internal neck diameter of 57 mm, provided with a lid having a PTFE inlay. Test containers shall be cleaned according to the cleaning procedure described in 4.3.3.

4.3.7 Test tubes

Test tubes shall be sterile glass tubes for preparing dilutions or to contain test pieces during sonication, and fitted with sterile caps. Test tubes shall be cleaned according to the cleaning procedure described in 4.3.3.

4.3.8 Incubator (or hot room)

Incubators shall be capable of maintaining the test temperature of (30 ± 2) °C (in the absence of light), and shall be free from volatile organic compounds.

NOTE Volatile organic compounds in the air cause microbial growth in the test containers, masking the growth attributable to the test pieces.

4.3.9 Sterilisation ovens

Sterilising ovens shall be capable of maintaining temperatures of (160 ± 10) °C, (250 ± 10) °C and (550 ± 20) °C.

4.3.10 Membrane filters

Membrane filters shall have a pore size of 1,2 µm – used for filtering the inoculum (4.4.6).

4.3.11 Autoclave

Autoclave shall be a pressure vessel capable of maintaining (121 ± 3) °C degree Celsius and a gauge pressure of 103 kPa.

4.4 Reagents

4.4.1 Test water

Test water shall be free from harmful effects on bacteria, and meet the quality criteria specified in Table 1. This can be drinking water that meets the additional requirements in Table 1.

Table 1 — Quality criteria for test water

Quality criteria	**Concentration** (mg/l)	**Comment**
Hydrogen ion concentration (Log_{10}) (pH)	6,5 – 8,5	Concentration to fall within the range specified. If pH is outside the range of Table 1 then adjust the pH of the water using either dilute hydrochloric acid or sodium hydroxide solutions as needed.
Free chlorine concentration	< 0,02	If a disinfectant residual is present, this should be neutralised by filtration though granular activated carbon.
Oxygen concentration	> 6,5	
PO_4^{3-}P concentration	2,0 – 6,7	Add potassium dihydrogen orthophosphate (4.4.3) if concentration is below 2 mg/l.
NO_{3-}-N concentration	5 – 11,3	Add potassium nitrate (4.4.4), if concentration below 5 mg/l.
Ammonia-N concentration	< 0,05	
Copper concentration	< 0,05	
Silver concentration	< 0,01	
Total dissolved organic carbon concentration	< 2	
Biostability (average maximum concentration of ATP concentration	< 10 (ngATP/l)	To be tested according to Annex A.
Trace elements (3.18)	–	To be added if needed for waters low in trace elements (See Annex A)

4.4.2 Dilution water

Dilution water is autoclaved purified water (3.12).

4.4.3 Potassium dihydrogen orthophosphate solution

Composition:

— potassium dihydrogen orthophosphate (KH_2PO_4) (790 ± 10) mg;

— purified water (3.12) 100 ml.

Preparation: dissolve the potassium dihydrogen phosphate in purified water and autoclave this solution at (121 ± 3) ºC for 15 min. Store in tightly closed containers at (4 ± 2) °C for a maximum period of 1 month. If the final PO_4^{3-} concentration is outside the range of Table 1, use ATP-free pipettes to add solutions of potassium di-hydrogen orthophosphate.

4.4.4 Potassium-nitrate solution

Composition:

— potassium nitrate (KNO_3), (3,25 ± 0,05) g;

— purified water (3.12), 100 ml.

Preparation: dissolve the potassium nitrate in purified water and autoclave this solution at (121 ± 3) ºC for 15 min. Store in tightly closed containers at (4 ± 2) °C for a maximum period of 1 month. If the final NO_3^- concentration is outside the range of Table 1, use ATP-free pipettes to add solutions of and potassium nitrate.

4.4.5 Sodium acetate solution

Composition:

— sodium acetate (water free), (25,6 ± 0,1) g;

— purified water (3.12), 100 ml.

Preparation: Dissolve the sodium acetate in the purified water. Add (1 ± 0,1) ml of this solution to (9 ± 0,1) ml of autoclaved drinking water contained in a clean and sterile culture tube with stainless steel cap and place this tube in water at (60 ± 2 °C) for 30 min.

Add (0,4 ± 0,05) ml of this solution with a 1 ml pipette to a container containing 600 ml of test water.

This solution shall be used within (6 ± 2) h and a fresh solution prepared for each test.

4.4.6 Inoculum

The inoculum shall consist of freshly collected water taken from a surface source that is suitable for abstraction of water intended for human consumption. The quality of the surface water and its microbiology will differ dependent on source.

The water shall have a hydrogen ion concentration equivalent to a pH of 5,5 to 9,0 and a copper concentration less than 0,05 mg/l.

Preparation: the water is pre-treated before use to remove any organisms that could interfere with the test.

Assemble a membrane filtration unit with the membrane filter (4.3.10). Flush the membrane filter with about 250 ml of purified water (3.12) to remove soluble organic carbon compounds from the membrane – discard this purified water. Filter the freshly collected surface source water through the membrane and collect the filtrate in a sterile flask.

Store the filtrate in the absence of light at (4 ± 2) °C for a maximum of 7 days.

Very turbid surface water (after filtration) or water visibly green with algal growth should not be used as an inoculum.

4.4.7 Specifications for controls

4.4.7.1 Positive control

A test piece made from plasticised PVC.

For each test the positive reference material shall give a mean Biomass Production Potential (BPP) value (mean of 3 measurements taken after 56, 84 and 112 days) at or above 1 0.000 pg ATP/cm^2.

If the result for the positive reference material is below this value of 10.000 pg ATP/cm^2 the entire test shall be stopped and repeated using new test pieces and fresh inoculum.

4.4.7.2 Negative control

A test piece made from borosilicate glass.

For each test the negative reference material shall give a mean biomass production (BP) value (mean of 3 measurements taken after 56, 84 and 112 days) at or below 100 pg ATP/cm^2.

If the result for the negative reference material is above this value of 100 pg ATP/cm^2 on any occasion, the entire test shall be stopped and repeated using new test pieces, fresh inoculum and apparatus (4.3).

4.5 Test samples and controls

4.5.1 Sampling, transport and storage of test samples

The test sample should originate from a batch, produced under typical manufacturing conditions, and be representative of the intended use of the material when in contact with drinking water.

Products such as washers and O-rings can be tested whole provided that they are of suitable size. Large products (e.g. pipes) should be cut to produce test pieces of the desired surface area and size.

Ensure that the surface of test pieces, intended to come into contact with test water, shall be free from adhesive tape, labels, ink or pencil marks. Take care to ensure that the transport and storage conditions shall not influence the test results.

Test samples shall be stored in the absence of light at (23 ± 5) ^{o}C, in stainless steel containers, tissue-paper, glassware or other materials, which do not influence the results of the test, except where the supplier of the test samples provides alternative written storage instructions which are those that the materials/products are subject to in practice. Materials should not be stored for longer than one month before testing.

Storage envelopes or pockets shall not be sealed, dusting powder shall not be used and cleaning shall not be carried out unless any of these procedures form part of the usual production procedures. Storage containers shall be cleaned using the same procedures as are used for the test containers.

4.5.2 Test piece preparation

4.5.2.1 General

Prepare test pieces (3.15) from the test sample (3.16) in such a way that only the surface intended to come into contact with drinking water is exposed to the test water (4.4.1).

The ratio of the surface area of the test piece to the volume of test water shall be (0,16 ± 0,02) cm^{-1}; if necessary to achieve this, a test piece shall be made up of more than one test portion.

Where practical this shall be achieved by having three specimens or portions for one test piece, each of (50 ± 5) cm^2, immersed in (900 ± 20) ml of test water.

For homogeneous materials it is acceptable to expose the whole test piece to the test water, including surfaces not intended to come into contact with drinking water.

If a homogeneous material has to be cut to obtain the required test piece size, this shall be done in a manner that ensures the area of the cut edges is as small as possible.

For non-homogeneous materials, special test pieces should be manufactured where only the surface intended to come into contact with water is exposed during the test.

4.5.2.2 Factory-applied linings

Test samples with factory-applied linings shall be prepared by the manufacturer in accordance with the standard factory procedure. Test panels/plates shall be fully coated in accordance with the standard factory procedure. If this is impractical, then a method giving an equivalent material surface to that produced by the standard factory procedure shall be used.

The test sample shall be accompanied by all necessary information on material and chemical safety.

4.5.2.3 Site-applied materials

Test samples with site-applied linings shall be prepared by the manufacturer or a contractor in accordance with the manufacturer's written instructions under supervision of the responsible body. Test panels/plates shall

be fully coated in accordance with the standard factory procedure. If this is impractical, then a method giving an equivalent material surface to that produced by the standard factory procedure shall be used.

If transportation of test samples to the test laboratory is necessary, then this period of time shall be part of the cure conditions (e.g. time and temperature). The test samples shall be delivered within the curing period.

Care shall be taken to ensure that materials and test samples are not contaminated during transport.

Start testing as soon as the curing period has been completed.

The test samples shall be accompanied by all necessary information on material and chemical safety.

4.6 Pre-treatment of test pieces

4.6.1 General

Only the procedures specified in this section should be used for cleaning the test pieces, unless specific procedures are required as part of the normal installation and/or commissioning for the particular material/product.

Test pieces are pre-treated prior to testing by procedures involving flushing, stagnation and pre-washing.

4.6.2 Flushing

Flush test pieces with drinking water in a vessel, (e.g. a beaker), having a flow of water from the bottom upwards such that the calculated speed with regard to the upper and open surface of the vessel is 1 to 3 m/min for (60 ± 5) min.

Test pieces are then left under non-flowing conditions according to 4.6.3.

4.6.3 Stagnation

Test pieces shall be immersed in test water (4.4.1) for a period of (24 ± 1) h at (23 ± 2) °C. After this period the test pieces are removed and prewashed (4.6.4).

4.6.4 Prewashing

The test pieces are flushed again as in 4.6.2 for (60 ± 5) min and then rinsed with test water (4.4.1) for (2 ± 0,5) min.

4.7 Test procedure

4.7.1 Overview

For a test on one material two containers are prepared each containing three test pieces (or the number of specimens or portions to make up the test pieces), immersed in the required volume of test water together with the inoculum. Two containers are also prepared with three pieces (or the number of specimens or portions to make up the test pieces), for each of the positive and negative controls with the required volume of water and the inoculum. All containers are incubated under the same conditions.

The test water is changed in all containers every seven days throughout the duration of the test. At days 56, 84 and 112 and for all containers, samples are taken for determining the amount of Suspended Biomass and Attached Biomass by ATP measurement before the test water is changed. For determining the amount of Attached Biomass, measurement is performed on one test piece and one positive and negative control piece, which is then discarded. The volume of test water is subsequently adjusted to account for the removal of each test piece.

The test is terminated at day 112.

The timing and sequence of the overall process, including the critical steps, are illustrated in Table 2. For further details see Annex E.

Table 2 — Summary of timing and sequence of test procedure

Day	Test water	Additions		ATP
	(± 20) ml	inoculum	Supplements	determinations
0	900	Yes	As needed	–
7 to 49 inclusive	900	No	As needed	–
56	600	No	As needed	Yes
63 to 77 inclusive	600	No	As needed	–
84	300	No	As needed	Yes
91 to 105 inclusive	300	No	As needed	–
112	End of test			Yes

4.7.2 ATP contamination

Ensure that all apparatus (4.3) is thoroughly cleaned and sterilised before use in this test.

Take precautions to avoid contamination of the test pieces and the apparatus with ATP; therefore prevent direct contact between skin and test pieces, apparatus, and reagents by using un-powdered polyethylene gloves and forceps. Do not handle with gloves, use forceps and use separate gloves and forceps for each type of material.

4.7.3 Preparation of the test container

Add 900 ml of test water (4.4.1) to the containers required for the test, using a 1 litre graduated measuring cylinder (4.3.2). The test water shall conform to the criteria in Table 1.

Add 9 ml of the inoculum to each test container (4.3.6), using a graduated or calibrated pipette (4.3.2). Ensure that only one inoculum (4.4.6) batch is used to set up a series of test piece containers and their corresponding controls.

4.7.4 Test pieces

Add three pre-treated test pieces, each test piece with a total surface area of 50 cm^2, to each of two prepared test containers (4.7.3) with the test water and inoculum. The test pieces should be kept separate on the base of the container.

If the density of the three test pieces are less than that of water, ensure the test pieces are kept submerged in the test water for the duration of the test by using stainless steel weights cleaned in accordance with 4.3.3.

4.7.5 Incubation

Incubate the test containers with test pieces at (30 ± 2) °C in the absence of light (4.3.8).

4.7.6 Positive and negative controls

For the negative control add three negative test pieces, each test piece with a total surface area of 50 cm^2, to each of two containers (4.7.3) with the test water and inoculum.

For the positive control add three positive test pieces, each test piece with a total surface area of 50 cm^2, to each of two containers (4.7.3) with the test water and inoculum.

Incubate the test containers with positive and negative control test pieces at (30 ± 2) °C in the absence of light (4.3.8).

4.7.7 Changing of test water

After every seven days of incubation, remove containers with test pieces and controls, from the incubator (4.3.8). For summary and timings of procedure see Table 2.

Gently swirl the water in each container without disturbing any biofilm present on the surface of the test pieces.

Open each container and discard the water. Add the required volume of fresh test water see Table 2. Close each container and return to the incubator.

4.7.8 Measurement of ATP concentrations

4.7.8.1 General

Before changing the test water during days 56, 84 and 112, determine the ATP concentrations in the test water and on the surface of one of the three test pieces from each duplicate in each test container and one from each duplicate container of the positive and negative control test pieces, in accordance with Annex B.

4.7.8.2 Suspended Biomass

At each of the three test periods of 56, 84 and 112 days, remove the containers from the incubator and gently swirl the water in the container without disturbing any biofilm present on the surface of the test pieces. Remove a sufficient quantity of water from each container for the ATP determination in accordance with Annex B. Determine the ATP concentration of the total water volume obtained; this ATP concentration is used in the calculation of biomass production (4.8.1).

4.7.8.3 Attached Biomass

At each of the three test periods of 56, 84 and 112 days remove one test piece (3.15) from each of the test and control containers and determine the Attached Biomass (pg ATP/cm^2) in accordance with Annex B. Discard the test pieces and positive and negative control test pieces after ATP measurement.

Advice on choice of method for removing the Attached Biomass from the test piece is given in Annex D.

4.8 Calculation of test results

4.8.1 Biomass Production (BP)

4.8.1.1 General

The mean ATP concentrations of the test pieces and test waters are determined from the duplicate attached biofilm and suspended biofilm measurements and used to calculate the BP value for each material (pg ATP/cm^2), see Table 3 for details of the sampling and measurement programme.

Key

— AB = Attached Biomass = (pg ATP/cm^2) (3.2);

— SB = Suspended Biomass (pg ATP/ml) in test water (3.17);

— BP = Biomass Production (pg ATP/cm^2) (3.4);

— BPP = Biomass Production Potential (pg ATP/cm^2) (3.5);

— V = volume of test water (ml);

— S = surface area of exposed test pieces (cm^2).

NOTE AB (pg ATP/cm^2) = ATP (pg/ml) x volume of sonicate (ml) / surface area of test piece (cm^2).

Table 3 — Sampling and measurement programme

	Container 1	**Container 2**	**Container 3**
Samples	3 Test pieces (A1-A3)	3 Test pieces (A4-A6)	3 positive references (P1-P3)
Test period day 56	A1-$AB_{day\ 56}$	A4-$AB_{day\ 56}$	P1-$AB_{day\ 56}$
	A1-$SB_{day\ 56}$	A4-$SB_{day\ 56}$	P1-$SB_{day\ 56}$
Test period day 84	A2-$AB_{day\ 84}$	A5-$AB_{day\ 84}$	P2-$AB_{day\ 84}$
	A2-$SB_{day\ 84}$	A5-$SB_{day\ 84}$	P2-$SB_{day\ 84}$
Test period day 112	A3-$AB_{day\ 112}$	A6-$AB_{day\ 112}$	P3-$AB_{day\ 112}$
	A3-$SB_{day\ 112}$	A6-$SB_{day\ 112}$	P3-$SB_{day\ 112}$
	Container 4	**Container 5**	**Container 6**
Samples	3 positive references (P4-P6)	3 negative references (N1-N3)	3 negative references (N4-N6)
Test period day 56	P4-$AB_{day\ 56}$	N1-$AB_{day\ 56}$	N4-$AB_{day\ 56}$
	P4-$SB_{day\ 56}$	N1-$SB_{day\ 56}$	N4-$SB_{day\ 56}$
Test period day 84	P5-$AB_{day\ 84}$	N2-$AB_{day\ 84}$	N5-$AB_{day\ 84}$
	P5-$SB_{day\ 84}$	N2-$SB_{day\ 84}$	N5-$SB_{day\ 84}$
Test period day 112	P6-$AB_{day\ 112}$	N3-AB_{day112}	N6-$AB_{day\ 112}$
	P6-$SB_{day\ 112}$	N3-$SB_{day\ 112}$	N6-$SB_{day\ 112}$

Key

$A1\text{-}AB_{day\ 56}$ is Attached Biomass after 56 days for test piece A1,
(ditto for $A2\text{-}AB_{day84}$, $A3\text{-}AB_{day112}$, $A4\text{-}AB_{day56}$, $A5\text{-}AB_{day84}$ and $A6\text{-}AB_{day112}$)

$A1\text{-}SB_{day\ 56}$ is Suspended Biomass after 56 days for sample A1,
(ditto for $A2\text{-}SB_{day\ 84}$, $A3\text{-}SB_{day\ 112}$, $A4\text{-}SB_{day\ 56}$, $A5\text{-}SB_{day\ 84}$ and $A6\text{-}SB_{day\ 112}$)

$P1\text{-}AB_{day\ 56}$ is Attached Biomass after 56 days for positive reference 1,
(ditto for $P2\text{-}AB_{day\ 84}$, $P3\text{-}AB_{day\ 112}$, $P4\text{-}AB_{day\ 56}$, $P5\text{-}AB_{day\ 84}$ and $P6\text{-}AB_{day\ 112}$)

$P1\text{-}SB_{day\ 56}$ is Suspended Biomass after 56 days for positive reference 1
(ditto for $P2\text{-}SB_{day\ 84}$, $P3\text{-}SB_{day\ 112}$, $P4\text{-}SB_{day\ 56}$, $P5\text{-}SB_{day\ 84}$ and $P6\text{-}SB_{day\ 112}$)

$N1\text{-}AB_{day\ 56}$ is Attached Biomass after 56 days for negative reference 1
(ditto for $N2\text{-}AB_{day\ 84}$, $N3\text{-}AB_{day\ 112}$, $N4\text{-}AB_{day\ 56}$, $N5\text{-}AB_{day\ 84}$ and $N6\text{-}AB_{day\ 112}$)

$P1\text{-}SB_{day\ 56}$ is Suspended Biomass after 56 days for negative reference 1
(ditto for $N2\text{-}SB_{day\ 84}$, $N3\text{-}SB_{day\ 112}$, $N4\text{-}SB_{day\ 56}$, $N5\text{-}SB_{day\ 84}$ and $N6\text{-}SB_{day\ 112}$)

4.8.1.2 Biomass calculations for the test piece duplicate measurements

— $AB_{test\ piece\ day\ 56} = (A1\text{-}AB_{day\ 56} + A4\text{-}AB_{day\ 56}) / 2$

— $AB_{test\ piece\ day\ 84} = (A2\text{-}AB_{day\ 84} + A5\text{-}AB_{day\ 84}) / 2$

— $AB_{test\ piece\ day\ 112} = (A3\text{-}AB_{day\ 112} + A6\text{-}AB_{day\ 112}) / 2$

— $SB_{test\ piece\ day\ 56} = (A1\text{-}SB_{day\ 56} + A4\text{-}SB_{day\ 56}) / 2$

— $SB_{test\ piece\ day\ 84} = (A2\text{-}SB_{day\ 84} + A5\text{-}SB_{day\ 84}) / 2$

— $SB_{test\ piece\ day\ 112} = (A3\text{-}SB_{day\ 112} + A6\text{-}SB_{day\ 112}) / 2$

— $BP_{test\ piece\ day\ 56} = AB_{test\ piece\ day\ 56} + SB_{test\ piece\ day\ 56} \times V/S$

— $BP_{test\ piece\ day\ 84} = AB_{test\ piece\ day\ 84} + SB_{test\ piece\ day\ 84} \times V/S$

— $BP_{test\ piece\ day\ 112} = AB_{test\ piece\ day\ 112} + SB_{test\ piece\ day\ 112} \times V/S$

4.8.1.3 Biomass calculations for the negative control duplicate measurements

— $AB_{negative\ day\ 56} = (N1\text{-}AB_{day\ 56} + N4\text{-}AB_{day\ 56}) / 2$

— $AB_{negative\ day\ 84} = (N2\text{-}AB_{day\ 84} + N5\text{-}AB_{day\ 84}) / 2$

— $AB_{negative\ day112} = (N3\text{-}AB_{day\ 112} + N6\text{-}AB_{day\ 112}) / 2$

— $SB_{negative\ day\ 56} = (N1\text{-}SB_{day\ 56} + N4\text{-}SB_{day\ 56}) / 2$

— $SB_{negative\ day\ 84} = (N2\text{-}SB_{day\ 84} + N5\text{-}SB_{day\ 84}) / 2$

— $SB_{negative\ day\ 112} = (N3\text{-}SB_{day\ 112} + N6\text{-}SB_{day\ 112}) / 2$

— $BP_{negative\ day\ 56} = AB_{negative\ day\ 56} + SB_{negative\ day\ 56} \times V/S$

— $BP_{negative\ day\ 84} = AB_{negative\ day\ 84} + SB_{negative\ day\ 84} \times V/S$

— $BP_{negative\ day\ 112} = AB_{negative\ day\ 112} + SB_{negative\ day\ 112} \times V/S$

4.8.1.4 Biomass calculations for the positive control duplicate measurements

— $AB_{positive\ day\ 56} = (N1\text{-}AB_{day\ 56} + N4\text{-}AB_{day\ 56}) / 2$

— $AB_{positive\ day\ 84} = (N2\text{-}AB_{day\ 84} + N5\text{-}AB_{day\ 84}) / 2$

— $AB_{positive\ day112} = (N3\text{-}AB_{day\ 112} + N6\text{-}AB_{day\ 112}) / 2$

— $SB_{positive\ day\ 56} = (N1\text{-}SB_{day\ 56} + N4\text{-}SB_{day\ 56}) / 2$

— $SB_{positive\ day\ 84} = (N2\text{-}SB_{day\ 84} + N5\text{-}SB_{day\ 84}) / 2$

— $SB_{positive\ day\ 112} = (N3\text{-}SB_{day\ 112} + N6\text{-}SB_{day\ 112}) / 2$

— $BP_{positive\ day\ 56} = AB_{positive\ day\ 56} + SB_{positive\ day\ 56} \times V/S$

— $BP_{positive\ day\ 84} = AB_{positive\ day\ 84} + SB_{positive\ day\ 84} \times V/S$

— $BP_{positive\ day\ 112} = AB_{positive\ day\ 112} + SB_{positive\ day\ 112} \times V/S$

4.8.1.5 Biomass Production Potential (BPP)

The Biomass Production Potential (BPP) is calculated as the average value of the BP values observed at 56, 84 and 112 days, minus the BP value for the negative control observed on the same days, and is expressed as pg ATP/cm^2.

$$BPP_{test\ piece} = \{(BP_{test\ piece\ day\ 56} - BP_{negative\ day\ 56}) + (BP_{test\ piece\ day\ 84} - BP_{negative\ day\ 84}) + (BP_{test\ piece\ day\ 112} - BP_{negative\ day\ 112})\} / 3$$

where:

$(BP_{negative\ day\ 56} + BP_{negative\ day\ 84} + BP_{negative\ day\ 112}) / 3 < 100$ pg ATP /cm^2 (4.8.1.3)

$(BP_{positive\ day\ 56} + BP_{positive\ day\ 84} + BP_{positive\ day\ 112}) / 3 > 10\ 000$ pg ATP /cm^2 (4.8.1.2)

4.9 Reproducibility and repeatability

Attention shall be given to reproducibility and repeatability of testing.

NOTE When two or more identical samples of a material are examined by one analyst using the same equipment within a short interval, it has been found the median value of the coefficient of variation for all materials tested is 8,1 %.

4.10 Test report

4.10.1 General

The following general information shall be included in the test report:

— a title (e.g. “Test Report”) and the date of issue of the report;

— unique identification of the test report (such as serial number), and on each page an identification in order to ensure that the page is recognised as a part of the test report, and a clear identification of the end of the test report;

— name and address of test laboratory and location where the test was carried out if different from the address of the testing laboratory;

— a reference to this standard EN 16421 (with date of publishing of EN) and Method 1 Measured by Biomass Production Potential (BPP) measured by ATP;

— name and address of client submitting the test sample;

— a statement to the effect that the test results relate only to the items tested;

— a statement that the report shall not be reproduced except in full without the written approval of the testing laboratory;

— a signature and title or an equivalent marking of person(s) accepting technical responsibility for the test report and date of issue.

4.10.2 Material/Product information

The following information shall be included in test report:

— trade name and designation/identifier of the material/product;

— name and address of the manufacturer of the material/product;

— method of manufacture (if known);

— the production place and date;

— generic composition of the material/product;

— nature of the material, e.g. nitrile rubber, polyethylene;

— comprehensive description of the test sample including material type, colour, shape/form, dimensions , appearance, opacity and component type (if appropriate);

— date test sample received from client;

— organization submitting the test sample (material/product) for testing;

— organization responsible for preparing the test sample (if different);

— description of the manufacturers sampling procedure (if known);

— condition of material on receipt by the test laboratory, including method of packaging;

— conditions of storage of material between test sample receipt and the start of testing;

4.10.3 Information on the test procedure

The following information on the test procedure shall be included in test report:

— number of specimens or portions of the test sample which constitute a test piece;

— volume of the test water (V) if applicable;

— surface area of test piece exposed to the test water (S) calculated from the actual dimensions of the test pieces;

— source of the test water and (if applicable) details of preparation;

— any deviation from the test procedure;

— any factors which may have affected the results, such as any incidents or any operating details not specified in this standard;

— special observations, during preparation of the test sample and/or during testing;

— all treatments/procedures used which are not described in the standard, and which may have affected the results;

— dates of start and completion of the test.

4.10.4 Test results

The test results shall, at least, include:

— the BP values on days 56, 84, and 112 as observed with the test pieces and the average BP value;

— the BP values of the negative control on days 56, 84 and 112 and the average BP value;

— the BP values of the positive control on days 56, 84 and 112 and the average BP value;

— the BPP value of the material tested.

5 Influence of materials on water for human consumption – Enhancement of microbial growth (EMG) – Method 2: Measured by biofilm volume

5.1 General

Annexes F and G are parts of Method 2: Measured by biofilm volume.

This method determines enhancement of microbial growth by materials by measuring the volume of biofilm produced.

5.2 Principle

Test pieces (3.15) are cleaned with disinfectant, rinsed with drinking water, and are exposed to slowly flowing test water in a test container for a defined period of time. Subsequently test pieces are removed, the biofilm is harvested by scraping and its volume is measured quantitatively after centrifugation.

After harvesting, test pieces are washed with test water and are placed once again in the test container for another period.

5.3 Basic requirements

The test samples, delivered by the producer shall comply with the finished products concerning composition and quality. Testing of semi-finished materials using this standard is not recommended. Multi-layer products shall be tested as such (see 5.5).

The test sample shall be produced under the same production manner as the finished product. Production process including applied curing procedures shall be provided by the test institute.

Test pieces shall be prepared from the test sample in such a way that only the surface intended to come into contact with drinking water is exposed to the test water.

The samples for testing shall be clearly identifiable.

5.4 Apparatus and reagents

5.4.1 Disinfectant

The sodium hypochlorite solution (NaOCl) for the pre-treatment of the test pieces shall contain (10 ± 1) g/l free chlorine (see EN 901).

5.4.2 Test water

Test water shall meet the quality of drinking water (3.7) without chlorine or other disinfectants Test water shall be free from harmful effects on bacteria, and shall meet the quality criteria specified in Table 4.

Table 4 — Quality criteria for test water

Quality criteria	Concentration (mg/l)	Comment
Hydrogen ion concentration (Log_{10}) (pH)	≥ 6,5 – ≤ 9,5	
Free chlorine concentration	< 0,02	If a disinfectant residual is present, this should be removed e.g. by filtration though granular activated carbon.
NO_3-N concentration	≤ 50	
Ammonia-N concentration	< 0,5	It shall be ensured that the condition that [nitrate]/50 + [nitrite]/3 ≤ 1, the square brackets signifying the concentrations in mg/l for nitrate (NO_3) and nitrite (NO_2), is complied with and that the value of 0,10 mg/l for nitrites is complied with ex water treatment works.
Copper concentration	≤ 2,0	
Silver concentration	< 0,01	
Total dissolved organic carbon concentration	–	no abnormal change

5.4.3 Specifications for controls

5.4.3.1 Positive control

Plates of stainless steel (size 20 cm × 20 cm × minimum thickness of 0,2 cm) covered on one side with paraffin (melting point 56 °C to 58 °C Merck No. 7337 or equivalent product) (3.10) which total to a surface area of at least 800 cm².

5.4.3.2 Negative control

Plates (size 20 cm × 20 cm × minimum thickness of 0,2 cm) made from materials according to 3.9 (e.g. glass or stainless steel, quality not less than specified in EN 10088-1 material number 1.4301) which total to a surface area at least 800 cm².

5.4.4 Test container and test module for pipes and hoses

The test container with a volume of approximately 100 litres shall be made of inert material (preferably stainless steel) with a water inlet on the bottom of one side, a water outlet on the upper opposite side and a lid to avoid water surface contamination (see Figure G1) .

The test module for pipes and hoses is designed for test pieces of pipes or hoses to be installed vertically with a controlled through flow of test water from bottom to top. The water flows under hydrostatic conditions and can be adjusted through a movable small container with fixed overflow (like a standpipe tower) (see Figure G2).

5.4.5 Scraper

Scraper with a rubber wiper (e.g. window cleaner) or made of hard plastics with a breadth of 12 cm to 15 cm.

For pipes and hoses a conical stopper of the relevant diameter (made of silicone or PVC-u) fixed on to a rod of stainless steel.

5.4.6 Centrifuge

Centrifuge with swing-out rotor and a performance of at least 3 000 g acceleration of gravity.

5.4.7 Glassware for centrifugation

Specially formed test tube with a calibrated scale at the bottom running from 0,01 ml to 0,4 ml in steps of 0,01 ml (see Figure G.3). For volumes of surface biofilm expected to be more than 0,5 ml (positive control) a conical centrifugation tube with a volume of approximately 15 ml is recommended.

5.4.8 Flowmeter

Liquid flowmeter for measuring a flow in the range of 2 l/h to 25 l/h.

5.5 Test samples and test pieces

5.5.1 Sampling, transport and storage

The test sample should originate from a batch, produced under typical manufacturing conditions, and be representative of the intended use of the material when in contact with drinking water.

If test samples have to be stored, this shall be done in the dark at room temperature, in stainless steel containers, tissue-paper, glassware or other materials which do not influence the results of the test, except where the supplier of the test samples provides alternative written storage instructions.

Either plates made of a material or finished products or parts of finished products can be used.

Ensure that the surface of test pieces, intended to come into contact with test water, shall be free from adhesive tape, labels, ink or pencil marks. Take care to ensure that the transport and storage conditions will not influence the test results.

5.5.2 Preparation of test pieces

5.5.2.1 General

Duplicate test pieces each with a surface area of 800 cm² are required for each test period; from these parallel results of each test period the arithmetic mean is calculated. In general the dimensions of a test piece is 20 cm × 20 cm × minimum thickness of 0,2 cm when both sides are being tested.

The test pieces are prepared from the test sample. The test pieces have to be pre-treated (see 5.6.5). Some test pieces require additional treatment before testing (see also 5.5.2.2, 5.5.2.3 and 5.5.2.4). If hoses or pipes are tested as such the respective lengths of test pieces depend on the inner diameter.

Large test samples (e.g. pipes) should be cut to produce test pieces of the desired surface area and size.

Test pieces shall be prepared in such a way that only the surface intended to come into contact with drinking water is exposed to the test water. For each test period (see 5.6.11) one positive control (see 5.4.3.1), one negative control (see 5.4.3.2) and duplicate test pieces each with a surface of 800 cm² are to be exposed.

5.5.2.2 Cementitious materials

Materials (concrete and mortar) shall be tested as test pieces with flat surfaces either as plates or as cubes. The preparation shall comply with the curing procedure conform EN 14944-1.

5.5.2.3 Factory applied products (multi layer, assembled)

Multi layer products (e.g. composite pipes, hoses or coatings) have to be tested as such. Test pieces with factory applied coatings or linings shall be prepared by the manufacturer or a contractor in accordance with the manufacturer's written instructions. Test pieces shall be made from stainless steel or glass and fully coated on one side in accordance with the standard factory procedure. If this is impractical, then a method providing an equivalent material surface to that produced by the standard factory procedure shall be used.

5.5.2.4 Site applied products

For site applied products, e.g. site applied coatings or linings, the manufacturer shall provide a copy of the detailed instructions for application which accompany the material(s).

The application instructions shall cover aspects such as:

— surface preparation;

— mix ratios and method of mixing;

— method of application;

— minimum cure temperature and time;

— material film thickness;

— associated materials, e.g. primers and undercoats.

The manufacturer shall provide all necessary information on material and chemical safety.

The test sample shall be prepared on site by the manufacturer/contractor. Where it is found necessary to deviate from these instructions, this shall only be done with the prior agreement of the test laboratory and the manufacturer or contractor.

If transportation of the test sample to the test laboratory is necessary, this period of time shall be part of the cure conditions (e.g. time and temperature). The test sample shall be delivered within the curing period.

Testing shall be started as soon as the curing period has been completed.

5.6 Test procedure

5.6.1 General

The test pieces are prepared from the test sample and then pre-treated (see 5.6.5).

The test pieces are exposed either in a test container (5.6.2) or in a test module for pipes and hoses (5.6.3) and removed (5.6.6) and examined (5.6.7) after each test period (5.6.11). The conditions described in 5.6.2 and 5.6.3 shall be met.

To ensure that the test runs are undisturbed, it is necessary to introduce and integrate materials with specified microbial behaviour. Positive controls shall show biofilm formation (see 5.6.7 and 5.6.9) whilst negative controls have to show no biofilm formation but surface colonisation (see 5.6.7 and 5.6.9).

Test pieces shall be prepared in such a way that only the surface intended to come into contact with drinking water is exposed to the test water. For each test period (see 5.6.11.2, 5.6.11.3 and 5.6.11.4) one positive control (see 5.4.3.1), one negative control (see 5.4.3.2) and duplicate test pieces each with a surface area of 800 cm² each are to be exposed.

5.6.2 Test procedure in test container

The test pieces are placed in the prepared test container (5.4.4) after pre-treatment. It shall be ensured, that they are constantly exposed to the flowing water and do not touch each other. Each test container shall contain a positive control and a negative control for each test period. In the test container test pieces for different exposure periods and different materials (test samples) can be exposed. The following conditions shall be met:

— the test is run in the dark (dark room or metal container with metal lid); the test pieces shall be permanently and completely immersed.

— a continuous flow rate of about (20 ± 2) l/h shall be maintained in the test container;

— temperature of the test water shall be (12 ± 5) °C;

— the test pieces shall only be removed from the test container for the purpose of examination.

5.6.3 Test procedure in test module for pipes and hoses

Pipes and hoses are exposed in the pipe and hose test module (5.4.4) and removed (5.6.6) and examined (5.6.7) after each test period (5.6.11). Testing and harvesting (5.4.4) should be performed in the absence of light and where necessary a non-light transmitting hard pipe should be used to surround the test pipe. The following conditions shall be met:

— pipes and hoses exposed in the test module for pipes and hoses shall be permanently and completely filled with test water;

— The through flow of the test module for pipes and hoses shall be 0,4 m/h up to 3 m/h to avoid measurable shear forces on the biofilm;

— temperature of the test water shall be (12 ± 5) °C;

— pipes and hoses shall only be removed from the test module of pipes and hoses for the purpose of examination.

5.6.4 Preparation of the test container or test module for pipes and hoses

Before using the test container for testing, wash it with test water (5.4.2). Rinse water through the system for (30 ± 5 min). Then fill the test container with test water and begin continuous flowing of test water through the test container at a flow rate of about (20 ± 2) l/h. Alternatively prepare the test module in a similar way and make sure that water flows undisturbed under the conditions given in 5.6.3.

5.6.5 Pre-treatment of test pieces

Before being immersed the first time in the test container, test pieces as well as positive controls and negative controls shall be exposed to flushing test water (5.4.2) for (20 ± 1) h. Then test pieces as well as positive controls and negative controls shall be disinfected with sodium hypochlorite solution (see 5.4.1) for (30 ± 5) min, followed by rinsing with test water for 2 min.

Each further exposure after harvesting as described in the following does not require a new pre-treatment.

5.6.6 Removal of test pieces

Before taking the test pieces and positive and negative controls out of the test container remove any floating layer from the water surface by careful aspiration (e.g. pipette) or by skimming, if necessary. Take the test pieces as well as the positive and negative controls out of the test container or the test module for pipes and hoses (e.g. using a stainless steel hook or a pair of tweezers). Do not touch the surface to be tested.

Let the water drip from the test pieces and the positive and negative controls for (1 ± 0,5) min.

5.6.7 Examination of test pieces

Remove the biofilm with a clean scraper (5.4.5) and completely transfer it into the glassware for centrifugation (see 5.4.7)

For each test piece without visibly recognizable biofilm (including the negative control), contact cultures/swabs are necessary: for bacteria, nutrient agar and for fungi e.g. Sabouraud-agar incubated for 5 days at 20°C. This is to demonstrate that there is a surface colonisation by micro-organisms and thus exclude the presence of biocides in the material (see 5.8).

NOTE Information on nutrient agar can be found in EN ISO 6222.

5.6.8 Re-exposure after harvesting

After the test period with harvesting, the removed test pieces, including the respective positive control and the respective negative control, are cleaned under flushing test water (5.4.2) in a way that no visible remaining biofilm is still present. After cleaning, the test pieces are re-immersed for the next test period in either the test containers or the test module for pipes and hoses as appropriate.

5.6.9 Centrifugation and determination of biofilm

Treat the collected biofilm in the centrifuge for 10 min at an acceleration of gravity g of at least 3000 m/sec^2 or for 5 min at an acceleration of gravity g = 4000 m/sec^2. Read the results of the biofilm volume from the calibrated part of the centrifugation tube.

For each 800 cm^2 the biofilm volume of the negative control shall not exceed 0,01 ml (see 5.8).

For each 800 cm^2 the biofilm volume of the positive control shall be at least 1,5 ml (see 5.8).

If the biofilm volume after centrifugation exceeds 0,4 ml for 800 cm², an exact volume does not have to be evaluated.

5.6.10 Examination of biofilm

For cases in which it is not possible to establish unambiguously whether the substance scraped off from the surface is microbial biofilm or probably inorganic material (e.g. mineral deposit) further investigations are required.

For example, the colony count, microscopic examination or determination of TOC (total organic carbon) according to EN 1484 may be helpful in deciding whether the deposit is biofilm or of another nature.

NOTE Information on colony count can be found in EN ISO 6222.

5.6.11 Test periods

5.6.11.1 General

Each material or product needs to undergo several test periods. All test periods have to be started at the same time. Each test period includes at least two test pieces and one positive control and one negative control.

— 1-monthly test period – means harvesting every 4 weeks during a 3 months incubation time. This results in 3 measurements: one after 4 weeks, one after 8 weeks and one after 12 weeks (option of an additional measurement after 16 weeks),

— 2-monthly test period - means harvesting after 8 weeks during a 2 (optional 4) months incubation time. This results in 1 measurement after 8 weeks (option of an additional measurement after 16 weeks),

— 3-monthly test periods mean harvesting after 12 weeks during a 3 months incubation time. This results in 1 measurement after 12 weeks.

The exposure times are given schematically in Table 5.

5.6.11.2 1-monthly test periods

The test pieces (2 × 800 cm² in parallel, also see 5.5.2) as well as the positive control and the negative control are exposed for 4 weeks, then removed and examined as described in 5.6.6 and 5.6.7. The test pieces are re-exposed after harvesting for 4 weeks (5.6.8). This procedure is repeated until 3 test periods (3 × 4 weeks) or optional 4 test periods (4 × 4 weeks) are finished (tests 1a, 1b, 1c and optional 1d).

5.6.11.3 2-monthly test periods

The test pieces (2 × 800 cm² in parallel, also see 5.5.2.1) as well as the positive control and the negative control are exposed for 8 weeks then removed and examined as described in 5.6.6 and 5.6.7. The test pieces are re-exposed after harvesting for 8 weeks (5.6.8). This procedure is repeated until test pieces and controls are exposed for 1 test period (1 × 8 weeks) or optional 2 test periods (2 × 8 weeks) are finished (tests 2a and optional 2b).

5.6.11.4 3-monthly test periods

The test pieces (2 × 800 cm² in parallel, also see 5.5.2) as well as the positive control and the negative control are exposed for 12 weeks, then removed and examined as described in 5.6.6 and 5.6.7 (test 3a).

5.6.11.5 Optional test periods

There is a small possibility that the result of the determination of the biofilm of the first 1-monthly test period (1a) is lower than the result of the determination of the biofilm of the second 1-monthly test period (1b). This

may be caused by slow adaptation of micro-organism to nutrients from tested material or product. In this case, optional test periods can be used.

The optional monthly results are evaluated only, if materials for the application range of joints and gaskets of larger surface and of smaller surface are tested, and where the first 1-monthly result is much lower than the second 1-monthly result (1b). It is only in such cases that the optional monthly results, the fourth 1-monthly result (1d) and the second 2-monthly result (2b), shall be determined and used for assessment. Under these circumstances the first 1-monthly result (1a) is to be neglected.

Table 5 — Exposure times and test periods

Duration of exposition per period	**number of test pieces**		**After 4 weeks**	**After 8 weeks**	**After 12 weeks**	**Optional: after 16 weeks**
1-monthly test periods (harvesting any 4 weeks)	2 × 800 cm² surface area (together with 1 positive control and 1 negative control)	Start of exposure (time 0)	harvest of biofilm (mean = result 1a) in ml positive control in ml negative control	harvest of biofilm (mean = result 1b) in ml positive control in ml negative control	harvest of biofilm (mean = result 1c) in ml positive control in ml negative control	optional: harvest of biofilm (mean = result 1d) in ml positive control in ml negative control
2-monthly test periods (harvesting any 8 weeks)	2 × 800 cm² surface area (together with 1 positive control and 1 negative control)			harvest of biofilm (mean = result 2a) in ml positive control in ml negative control		optional: harvest of biofilm (mean = result 2b) in ml positive control in ml negative control
3-monthly test periods (harvesting any 12 weeks)	2 × 800 cm² surface area (together with 1 positive control and 1 negative control)				harvest of biofilm (mean = result 3a) in ml positive control in ml negative control	

5.7 Calculation of test results

The results of the 1-monthly, 2-monthly and 3-monthly surface biofilm data are assembled in tables as shown below (see Table 6, Table 7 and Table 8).

In each test period 2 × 800 cm² of the tested material are harvested. From these parallel results of each test period the arithmetic mean is calculated. The results of the test periods are given to two decimal places. The analytical procedure has a detection limit of 0,01 ml/800 cm².

Table 6 — Results of monthly-harvested surface biomass

	Biofilm (**first duplicate** 800 cm²) in ml	Biofilm (**second duplicate** 800 cm²) in ml	Mean in ml	Result No.	Biofilm of **negative control** in ml	Biofilm of **positive control** in ml
After 4 weeks				1a		
After 8 weeks				1b		
After 12 weeks				1c		
Optional: after 16 weeks				1d		

Table 7 — Results of 2-monthly-harvested surface biomass

	Biofilm (first duplicate 800 cm²) in ml	**Biofilm (second duplicate 800 cm²) in ml**	**Mean in ml**	**Result no.**	**Biofilm of negative control in ml**	**Biofilm of positive control in ml**
After 8 weeks				2a		
Optional: after 16 weeks				2b		

Table 8 — Results of 3-monthly-harvested surface biomass

	Biofilm (first duplicate 800 cm²) in ml	**Biofilm (second duplicate 800 m²) in ml**	**Mean in ml**	**Result no.**	**Biofilm of negative control in ml**	**Biofilm of positive control in ml**
After 12 weeks				3a		

5.8 Evaluation of satisfactory test performance

Evaluation and assessment of the materials tested is only possible if the test procedure itself is satisfactory. This can be seen as fulfilled, if:

— microbial biofilm on each positive control is present (with the aforementioned paraffin at least 1,5 ml for 800 cm²);

— microbial biofilm on each negative control is absent(< 0,01ml) for 800 cm², but microbial surface colonisation is detectable through contact cultures/swabs (5.6.7).

If the test performance is not satisfactory, a repetition of the test shall be done.

Test pieces showing neither biofilm nor surface colonisation (comparison of contact cultures/swabs of test pieces with the respective negative control) do not comply with the requirements of this method for materials in contact with drinking water.

5.9 Test report

5.9.1 General

The following general information shall be included in the test report:

— a title (e.g. “Test Report”) and the date of issue of the report;

— unique identification of the test report (such as serial number), and on each page an identification in order to ensure that the page is recognised as a part of the test report, and a clear identification of the end of the test report;

— name and address of test laboratory and location where the test was carried out if different from the address of the testing laboratory;

— a reference to this standard EN (fill in the number of standard) (with date of publishing of EN) and Method 2: Measured by biofilm volume;

— name and address of client submitting the test sample;

— a statement to the effect that the test results relate only to the items tested;

— a statement that the report shall not be reproduced except in full without the written approval of the testing laboratory;

— a signature and title or an equivalent marking of person(s) accepting technical responsibility for the test report and date of issue.

5.9.2 Material/Product information

The following information shall be included in test report:

— trade name and designation/identifier of the material/product;

— name and address of the manufacturer of the material/product;

— method of manufacture (if known);

— the production place and date;

— generic composition of the material/product;

— nature of the material, e.g. nitrile rubber, polyethylene;

— comprehensive description of the test sample including material type, colour, shape/form, dimensions , appearance, opacity and component type (if appropriate);

— date test sample received from client;

— organization submitting the test sample (material/product) for testing;

— organization responsible for preparing the test sample (if different);

— description of the manufacturers sampling procedure (if known);

— condition of material on receipt by the test laboratory, including method of packaging;

— condition of storage of material between test sample receipt and the start of testing.

5.9.3 Information on the test procedure

The following information on the test procedure shall be included in test report:

— number of specimens or portions of the test sample which constitute a test piece;

— volume of the test water (*V*), if applicable;

— surface area of test piece exposed to the test water (*S*) calculated from the actual dimensions of the test pieces;

— source of the test water and (if applicable) details of preparation;

— any deviation from the test procedure;

— any factors which may have affected the results, such as any incidents or any operating details not specified in this standard;

— special observations, during preparation of the test sample and/or during testing;

— all treatments/procedures used which are not described in the standard, and which may have affected the results;

— dates of start and completion of the test.

5.9.4 Test results

The test results shall, at least, include the information identified in Table 9:

Table 9 — Test results to be given in the test report

Duration		**After 4 weeks**	**After 8 weeks**	**After 12 weeks**	**Optional after 16 weeks**
		Date	Date	Date	Date
1-monthly test periods	Start of exposure (date)	1st set… (ml) 2nd set (ml) mean 1a positive control (ml) negative control (ml)	1st set (ml) 2nd set (ml) mean 1b positive control (ml) negative control (ml)	1st set (ml) 2nd set (ml) mean 1c positive control (ml) negative control (ml)	optional: 1st set (ml) 2nd set (ml) mean 1d positive control (ml) negative control (ml)
2-monthly test periods			1st set (ml) 2nd set (ml) mean 2a positive control (ml) negative control (ml)		optional: st set (ml) 2nd set (ml) mean 2b positive control (ml) negative control (ml)
3-monthly test periods				1st set (ml) 2nd set (ml) mean 3a positive control (ml) negative control (ml)	

6 Influence of materials on water for human consumption – Enhancement of microbial growth (EMG) – Method 3: Measured by mean dissolved oxygen depletion

6.1 General

Annex H is a part of Method 3: Measured by mean dissolved oxygen depletion.

This method determines enhancement of microbial growth by non-metallic materials by measuring the depletion of the mean dissolved oxygen.

6.2 Principle

Drinking water in contact with a sample of the material is inoculated with a mixture of naturally occurring aquatic micro-organisms. The overall growth/activity of the microbial population is determined indirectly by the measurement of mean dissolved oxygen depletion in the test system and a control. Validation of the results is achieved by the assessment of reference materials tested in parallel with the test sample.

6.3 Test premises

6.3.1 General

Carry out the tests in premises as free as practicable from the presence of any volatile organic chemicals in the atmosphere, as such chemicals can dissolve in exposed water surfaces in sufficient quantities to produce abundant microbial growth which will mask similar growth due to the test samples.

NOTE The volatile chemical most frequently responsible for such slimes is ethanol, but many other volatile chemicals used in or near the test premises may produce this effect.

6.3.2 Safety

As well as statutory and general consideration of safety, particular care is needed in microbiological laboratories because the organisms present may be pathogenic. It is essential for any written safety guidance to be supplemented by thorough training and supervision.

6.4 Apparatus and reagents

6.4.1 General

Only reagents of analytical grade shall be used, except where specified otherwise.

Table 10 — Quality criteria for inoculum water

Parameter	Units	Minimum Value	Maximum Value
pH	–	5,5	9,0
Copper	mg/l	–	0,05
Total Presumptive coliforms (see EN-ISO 9308)	per 100 ml	10	–
Presumptive pseudomonads (see EN-ISO 16266)	per 100 ml	1	250

6.4.2 Inoculum water

Inoculum water consisting of a fresh sample taken from a lowland surface water suitable for abstracting for treatment for drinking water preparation, that complies with Table 10. The quality of surface water and its microbiology will differ depending on the source.

Determine the conformity of the inoculum water with the criteria in Table 10 on the basis of analysis of samples of the surface water. Perform analyses of the water in accordance with the appropriate methods in European or national standards.

NOTE Appropriate methods can be found in European Standards developed by CEN/TC 230.

Use the inoculum water within 6 h of collection. If suspended solids are present, filter (using filters of average pore size 10 micron) before use. If necessary the criteria in Table 10 can be met by dilution with test water (6.4.3).

6.4.3 Test water

6.4.3.1 General

Test water shall be of drinking water quality (3.7). Before collection of the drinking water for test purposes, flush the tap until the temperature of the flowing water does not vary by more than 1 ^{o}C over a period of 1 min and does not exceed 25 ^{o}C.

For measurements of water temperature, an instrument should be used which shows values to the first decimal place.

The test water shall exhibit the characteristics specified in Table 11 so that it can be added directly to the test container.

Determine the conformity of the test water with the specified criteria on the basis of analysis of samples of drinking water drawn from a tap in the test premises after flushing. Perform analyses of the water in accordance with European or national standards.

NOTE Appropriate methods can be found in European Standards developed by CEN/TC 230.

Either use the drinking water directly from the tap or store for up to 1 h prior to use. If storage is necessary, the storage vessel shall be made from a suitable inert material such as borosilicate glass, cleaned using the procedure described in 6.4.4.2, except that the final rinse water shall be at a temperature greater than 70 ^{o}C and be in contact with the inner surface of the storage vessel for a minimum of 10 min.

If the water is deficient in respect of total oxidised nitrogen or phosphate or has an excess of residual chlorine, then carry out the procedures described in 6.4.3.2 and 6.4.3.3 respectively to alter these characteristics.

Table 11 — Quality criteria for test water

Parameter	Units	Minimum Value	Maximum Value
Total presumptive coliforms	per 100 ml	–	< 1
Bacterial colony count after incubation at:	per 100 ml		
— 37 °C for 48 h	per ml	–	50
— 22 °C for 72 h (see EN-ISO 6222)	per ml	–	500
Phosphate	mg/l (as phosphate ion)	2,0*	6,7
Total oxidised nitrogen	mg/l (as N)	5,0	11,3
Free residual chlorine	mg/l (as Cl_2)	–	0,05
Total residual chlorine	mg/l (as Cl_2)	–	0,2
pH	–	6,5	9,5
Copper	mg/l	–	0,05
Silver	mg/l	–	0,01
Dissolved oxygen	mg/l	6,5	--
* Add potassium dihydrogen orthophosphate (4.3.5) if concentration is below 2 mg/l			

6.4.3.2 Deficiency of total oxidised nitrogen or phosphate

To rectify a deficiency of total oxidised nitrogen or phosphate, add carefully from a graduated pipette a sufficient quantity of a 10 g/l solution of potassium nitrate and/or a 2 g/l solution of potassium dihydrogen orthophosphate to achieve the minimum concentrations in the test water specified in Table 11.

6.4.3.3 Excess of free residual chlorine

Measure the free chlorine in the test water in accordance with EN ISO 7393-2. If there is an excess of free residual chlorine, add a sufficient quantity of a fresh solution of sodium thiosulfate to neutralise the measured free residual chlorine to less than 0,05 mg/l as free chlorine in the test water (see EN ISO 7393-2).

— Do not add sodium thiosulfate in excess of the required amount to the test water. Excess sodium thiosulfate could influence the outcome of the test.

6.4.4 Test containers

6.4.4.1 General

Test containers shall be cleaned, air-tight glass preserving jars of nominal capacity 1 000 ml, fitted with glass lids and seals manufactured from a medical grade of silicone rubber. Each container shall bear a permanent calibration mark for a capacity of 1 000 ml at (23 ± 3) °C.

If the surface area of a test piece is not in the range 13 000 mm^2 to 19 000 mm^2, use a test container calibrated in accordance with Table 12. The ullage of the containers shall not exceed 15 % of its total capacity.

6.4.4.2 Cleaning of test containers

Clean the containers, lids and seals with an aqueous solution of a biodegradable, laboratory detergent. Rinse the containers, lids and seals in drinking water and then once in purified water (3.12). Drain and air dry. Attach the lids and seals to the containers and store until used.

6.4.5 Dissolved oxygen meter

Meter used with an electrochemical probe for the determination of dissolved oxygen. The electrochemical probe shall be automatically temperature compensated for the solubility of oxygen in the water. Maintain the meter and probe in accordance with the manufacturer's instructions. Follow the procedures described in EN ISO 5814 for measurement of dissolved oxygen.

6.4.6 30 °C Incubator/chamber

A conventional microbiological incubator calibrated to maintain (30 ± 1) ^{o}C with fan-assisted circulation of the internal air.

6.4.7 Polyethylene bags

Bags of a type suitable for food contact. Before use these bags shall be stored in a closed container.

6.4.8 Glass plates

Plates of sand blasted glass and having a total surface area of (15 000 ± 500) mm^2. The width of the plate shall not exceed 60 mm. The plates shall be cleaned in an aqueous solution of a biodegradable laboratory detergent.

Stainless steel plates can be used in place of sand blasted glass.

They shall then be rinsed thoroughly in drinking water and then once in purified water (3.12), drained and dried in a hot air cabinet and stored in polyethylene bags (6.4.7).

6.4.9 Brass couplings

Female/female, 15 mm diameter compression brass couplings complying with ISO 2016 and with a composition meeting designations of the standard from CEN/TC 113 WG 4, work item number 00133031. Immerse the couplings in a cleaning agent (6.4.4.2) for 5 min. Rinse the couplings with purified water (3.12) and then immerse in a 1 % by volume solution of nitric acid for 1 min. Rinse in drinking water and then in distilled water again. Drain and dry in a hot air cabinet and store in polyethylene bags (6.4.7).

6.4.10 Special glass plates

Glass plates as specified in 6.4.8 except that only one side shall be sand blasted.

6.5 Preparation of test pieces

6.5.1 General

This section describes the preparation of test pieces of all types of non-metallic materials for testing for their suitability for use in contact with water intended for human consumption in terms of the growth of aquatic micro-organisms.

6.5.2 Nature of test pieces

The test pieces shall be prepared from samples of manufactured products or components of water fittings drawn from production batches that have received no other treatment, except for the exclusions given below.

6.6 Test piece and test container size

6.6.1 General

Use wherever possible a test piece with a total surface area of (15 000 ± 500) mm^2 and a test container (6.4.4) bearing a calibration mark for a capacity of 1 000 ml. If in exceptional circumstances this size of test piece cannot be obtained, use a test container calibrated for a capacity in accordance with Table 12.

Table 12 — Test container calibration mark

Sample surface area, (*a*) (mm^2)	Volume indicated by calibration mark (ml)
5 500 ≤ *a* < 9 500	500
9 500 ≤ *a* < 13 000	750
13 000 ≤ *a* < 19 000	1 000
19 000 ≤ *a* ≤ 26 000	1 500

The test piece shall be made from one or more specimens of the sample drawn from the same production batch or cut from a single larger sample. The maximum length and width of any single specimen shall be 130 mm and 70 mm respectively.

For special applications within distribution systems involving products used for joints in service reservoirs or large tanks the surface area to volume ratio used for the test shall be 1 000 mm^2/l.

During testing the test piece shall be always completely submerged in the test water. If the density of the test piece is less than that of water, ensure that the test piece is kept totally submerge for the duration of the test by using glass-encapsulated weights attached by monofilament nylon.

Determine the dimensions of all test pieces using measuring equipment calibrated in accordance with EN ISO 10012. The instruments used shall include rules and callipers complying with the accuracy requirements of EN ISO 13385-1 and EN ISO 13385-2.

If the test piece has a textured finish to the surface or the shape of the test piece is such that accurate calculation of the surface area is impracticable, then a test piece of estimated surface area shall be used, but in this case the length and width shall be recorded together with a sufficiently detailed description to enable further test pieces to be prepared that will be within ± 10 % of the surface area of the original test piece.

The supplier of the test sample should be instructed to ensure that the products or test samples represent the product as it is used in contact with drinking water. If the test sample has to be cut to obtain the required test piece area, this should be done before the test piece receives any post cure or special surface treatment and in a manner that ensures the area of the cut edges is as small as possible.

6.6.2 Test sample storage

Ensure that during the period between packaging and receipt by the laboratory test samples are protected from contamination by dirt, oil, grease, excessive heat, sunlight and volatile chemicals. Store in the laboratory in polyethylene bags (6.4.7) and in the absence of light at (21 ± 4) ^{o}C except where the individual supplying the test samples provides alternative written storage instructions which are those that the products are subject to in practice. Use a fresh bag for each test sample or set of test samples made from an identical material.

— Do not test the test samples if they have adhesive tape or labels, ink or pencil marks on them.

6.6.3 Special requirements

6.6.3.1 General

Products made from certain material types shall be subject to the additional requirements given in 6.6.3.2 to 6.6.3.7.

— Thermoplastics Products: test samples made from thermoplastics materials in any typical/suitable manufactured form using the production method for which they are designed, e.g. moulding grades - moulded test samples, extrusion grades - extruded test samples.

Do not test granules; test samples shall be manufactured products, e.g. test sheets, extruded or moulded products etc.

By their very nature the materials used to manufacture these products are designed to melt when heated and solidify when cooled; when reheated they will again melt (unlike thermosetting products which will not melt again when reheated). Provided thermoplastics products are not heated excessively (in which case pyrolysis may occur) the nature of the material should not be affected by heating and reheating. In addition the nature of the material will not normally be affected by the method of manufacture, e.g. injection moulding, extrusion, blow moulding etc.

— Thermosetting Products: test samples made from thermosetting materials in their final manufactured form.

NOTE Unlike thermoplastics materials, the performance of thermosetting materials, e.g. most rubber compounds and GRP products can vary in this test according to the conditions of final cure (together with any post cure treatments) and method of manufacture, e.g. compression moulding, extrusion, pultrusion etc.

6.6.3.2 Elastomeric products

Instruct suppliers of test samples to arrange for storage of the test samples for at least 4 weeks before dispatch to the laboratory.

Test samples of elastomeric products should not be tested within 4 weeks of manufacture otherwise the results obtained with this method may not be representative of the material as it is used in practice.

6.6.3.3 Composite products

If composite test samples are to be examined, ensure that the test piece is produced only from the water-contact material.

Provided that the test sample is not one that falls within the definition of site applied materials (see 6.6.3.7) composite products may be tested in the form of specially prepared test samples.

6.6.3.4 Ion exchange resins

Place 2 g of the resin beads in a test container (6.4.4) calibrated for a capacity of 1 000 ml.

6.6.3.5 Greases

Evenly spread 5 g of the test sample over the sand blasted surface of a special glass plate (6.4.10).

Place the plate in a test container (6.4.4) calibrated for a capacity of 1 000 ml.

Since the grease may be easily washed off the plate it is essential to ensure that the test water (6.4.3) is added so as to cause the least possible disturbance of the grease film on the test plate in the test containers, both at the commencement of each test, and also at each change of test water. Similarly, it is important to ensure the pre-test rinsing with the test waters does not disturb the grease film.

6.6.3.6 Factory applied coatings

Instruct the manufacturer or supplier to apply test samples of these products to panels of a material with similar adherent properties to the material(s) for which the coating is designed. The panels shall be resistant to rusting or corrosion under the conditions of the tests (for example, stainless steel).

Ensure that the panels are given the same number of coats (including primers and undercoats) and identical maturing and curing conditions as would be applied to articles being coated in the factory.

All surfaces and edges of the panels shall be covered completely with the coating.

Test samples should be prepared and cured by the manufacturer/supplier and tested as received without any further curing/treatment. To ensure that the test samples provided are typical of normal production they should

be drawn from the production line wherever possible and the following additional information provided – the date of preparation, the mode of preparation and the curing conditions.

6.6.3.7 Site applied materials

6.6.3.7.1 General

SAFETY WARNING – Many site applied coatings contain hazardous solvents. Observe all the manufacturer's safety warnings during the preparation of these samples.

NOTE These include all coatings, sealing compounds, soldering fluxes, anaerobic adhesives and most cementitious products intended for application on site.

The test piece shall consist of all specified component parts. Ensure that they are supplied to the test laboratory together with all relevant instructions for site application, including information on the time for complete cure relative to temperature. Test piece preparation conditions and curing shall always be achievable on site. Test pieces from samples which include paints, coatings, adhesives, sealants, epoxy resins, and cementitious materials, shall be prepared by or the preparation witnessed by the test laboratory staff, who shall then take responsibility for the subsequent pre-test curing of the test pieces.

Prepare test panels as follows using all the specified component parts of the products according to the manufacturer's instructions. Apply all relevant primers or undercoats to all surfaces of a glass plate (6.4.8) before application of the finish coat. Where the test sample consists of two or more parts, prepare these in strict accordance with the manufacturer's instructions and record the precise mixing ratio used as either mass or volume. Determine and record the wet film thickness of each coating if this is specified by the supplier.

During the application of the test sample and throughout the curing period keep the panels suspended (e.g. by using stainless steel or nylon monofilament) so as to avoid contact of the test sample with apparatus. If specialised equipment is normally required for site application of a material, then have the panels prepared by a contractor under the personal supervision of, and to the satisfaction of, a person from the testing laboratory.

6.6.3.7.2 Sealing compounds

Apply these to glass plates (6.4.8) or cast pieces for testing as sheets (2 mm to 5 mm thick) between food grade polyethylene film. If polyethylene film is used, remove it from the semi-cured sheets within 48 h and suspend the sheets as in 6.6.3.7.1 for the remainder of the cure period.

6.6.3.7.3 Curing

Immediately following completion of the preparation of the panels or sheets, cure them in accordance with the manufacturer's instructions (including conditions for application, initial cure and time before contact with drinking water, if specified) except if the material is intended for use in water undertakings' installations when the cure period shall not exceed 21 days and the atmospheric temperature of curing shall not exceed (7 ± 2) ^{o}C. If the material is intended for use within dwellings or other buildings, then the cure period shall not exceed 7 days and the atmospheric temperature of curing shall not exceed (12 ± 2) ^{o}C.

Throughout the cure period suspend the test panels in a thermostatically controlled cooled incubator with fan-assisted air circulation, and with ventilation to prevent accumulation of volatile substances.

Test the cured test piece in a test container (6.4.4) calibrated for a capacity of 1 000 ml.

6.6.3.7.4 Jointing compounds

Apply 0,25 g of the sample to both screw threads of a brass coupling (6.4.9), discard the olives and reassemble the coupling so that the compression nuts are finger tight. Rinse the re-assembled coupling in drinking water for 10 min before placing it in a test container (6.4.4) calibrated for a capacity of 1 000 ml. Do not cure but test immediately.

6.6.3.7.5 Solder fluxes

Make up a test piece as follows.

In accordance with 6.4.9 clean 2 length of (60 ± 5) mm of copper pipe with 22 mm diameter.

Clean one 22 mm diameter straight copper capillary coupling provided with internal (lead-free) solder rings and complying with prEN 1254–1:2007 (DPC 94/700007) in accordance with 6.4.9, but omit the acid wash. Apply the solder flux to the pipes in accordance with the manufacturer's instructions, assemble the joint and heat until the solder melts. Cool the joint and, after rinsing in accordance with 6.7.1.1, place the test piece in a test container (6.4.4) calibrated for a capacity of 1 000 ml. Test immediately.

If the manufacturer of the flux recommends a specific solder mix, then use a 22 mm straight copper capillary end-feed coupling and cleaned in accordance with 6.4.9. Use the recommended solder in sufficient quantity to fill the annulus around the joint, determined by visual inspection of an additional test piece after assembly.

6.6.3.7.6 Anaerobic adhesives

Apply according to the manufacturer's instructions the anaerobic adhesive to both threads of a brass coupling (6.4.9) and reassemble the coupling. Cure the assembly in accordance with the adhesive manufacturer's instructions.

Place the assembly in a test container (6.6) calibrated for a capacity of 1 000 ml.

NOTE Anaerobic adhesive cure spontaneously in the absence of oxygen, curing often being catalysed by metal ions.

6.6.3.7.7 Organic based additives or coatings for use with, or in, cementitious materials

These materials give rise to a temporary increase in the pH value of a water in which they are soaked and therefore it is essential to pre-condition the test pieces before commencing the test.

Either prepare the test piece in accordance with 6.6.3.7.1 or coat test blocks cast in pre-formed moulds, whichever represents the use of the material in practice. Immediately after completion of curing, place the test piece in a clean and dust free container of 1 000 ml capacity. Fill the container with drinking water exhibiting an aggressivity index (I) of greater than 12.0, and obtained from a tap connected directly to a service pipe at mains pressure. Calculate the aggressivity index using the following equation:

$$I = \text{pH} + \log_{10}(A H)$$

where

A is the total alkalinity expressed as mg/l of $CaCO_3$;

H is the calcium hardness expressed as mg/l of $CaCO_3$.

Measure the pH of the water after 24 h according to EN ISO 10523. Discard the water and refill the container with fresh drinking water. Continue this process of sequential soaking until the pH of the water is < 9,0 ± 0,1 on two successive occasions. Dry and store the test piece in a polyethylene bag (6.4.7) until required for testing. Do not store the test piece if the additive or coating is designed for site application but test immediately. Prepare a minimum of three test samples.

It is possible that a water having an I of about 12 but with a low alkalinity and hardness may be aggressive to the test piece and a pH value of < 9,0 may not be achieved; in this case it is necessary to use another water.

Test the test pieces in test containers (6.4.4) calibrated for a capacity of 1 000 ml.

6.6.4 Specifications for controls

6.6.4.1 General

Two reference materials shall be prepared for use with each batch of test samples. The positive and negative references are used in the validation of the results for the MDOD test (6.8.2).

6.6.4.2 Positive control

Prepare a block of paraffin wax (microscopy grade) containing between 20 and 25 carbon atoms per molecule (melting point 50 ^{o}C to 55 ^{o}C) with a total surface area of 15 000 mm^2.

6.6.4.3 Negative control

Use a glass plate, made from borosilicate glass, with a surface area of 15 000 mm^2. The surface area and dimensions of each reference material shall comply with 6.6.

6.6.5 Test water control

Prepare a cleaned test container (6.4.4); after the addition of the inoculum (6.4.2) and test water (6.4.3) this constitutes the test water control.

6.7 Test procedures

6.7.1 Preparation of the test container

6.7.1.1 General

On the same day as testing is to start, soak the test piece in test water (6.4.3) for 10 min and then rinse the test piece in flowing test water for a further 2 to 3 min.

To help ensure that optimum precision and accuracy of results are obtained in any biological test, the test should be in regular use within the test laboratory.

It is recommended that the minimum number of different test samples in any one batch should be three. In addition include with each batch of test samples one container without either test pieces or reference materials; this constitutes the test water control (6.6.5).

Place the test piece from each sample and reference materials (6.6.4.2 and 6.6.4.3) in separate test containers (6.4.4). In addition, include with each batch of test samples, one empty container which constitutes the test water control (6.6.5). Add 100 ml of the inoculum water (6.4.2) to each container, and fill with test water (6.4.3) to the calibration mark appropriate to the test piece surface area (see Table 12). If the surface area of a test piece is not in the range 13 000 mm^2 to 19 000 mm^2, use a volume of inoculum as given in Table 13.

Table 13 — Volume of inoculum

Sample surface area, (a) (mm^2)	Volume of inoculum (ml)
5 500 ≤ a < 9 500	50
9 500 ≥ a < 13 000	75
13 000 ≥ a < 19 000	100
19 000 ≥ a < 26 000	150

If the density of the test piece is less than that of water, ensure the test is kept totally submerged in the test water for the duration of the test by using glass-encapsulated weights cleaned in accordance with 6.4.4.2. Seal each container with the lid.

6.7.1.2 Cementitious test samples and test samples containing bacteriostatic or bactericidal compounds

If a cementitious test piece is included in the batch, then include an additional container for a cementitious reference test. Into this container place a paraffin wax reference material (see 6.6.4.2) and the second cementitious test piece, followed by the inoculum water and test water as in 6.7.1.1.

If there is reason to believe that a product contains a water soluble bactericidal or bacteriostatic compound, set up an additional reference container for bactericidal/bacteriostatic reference test using the test piece plus the paraffin wax reference material, as in 6.7.1.1.

NOTE These reference tests are used in the validation of the results from the MDOD test (6.8.2).

6.7.1.3 Test samples applied to metal fittings: jointing compounds, solder fluxes and anaerobic adhesives

If a test piece applied to a metal fitting is included in the batch, then include in the test an additional container for a metal fitting reference test. Into this container, place an identical metal fitting but without the test piece. Add the inoculum water and test water as in 6.7.1.1.

NOTE This reference test is used in the validation of the results from the MDOD test (6.8.2).

6.7.2 Incubation

Incubate each sealed container in the absence of light at (30 ± 1) ^{o}C for the periods of time given in 6.7.3. Use the same incubation conditions throughout the test procedure.

6.7.3 Changing the test water

Twice a week (after either 3 or 4 days of incubation) decant the water from each container and then refill to the mark with fresh test water (6.4.3), reseal and continue the incubation (6.7.2).

At the end of the four day incubation period during the fifth, sixth and seventh weeks of the test do NOT change the water until the dissolved oxygen measurement has been made (6.7.4).

6.7.4 Measurement of dissolved oxygen

6.7.4.1 General

Immediately after opening each test container and before the water is changed after the four day incubation period, during the fifth, sixth and seventh weeks of test, measure the dissolved oxygen concentration in the water in accordance with EN ISO 5814 For lubricants and lubricated materials follow the instructions given in 6.7.4.2.

First determine the dissolved oxygen concentration of the water for both the test water control (6.6.5) and negative reference (6.6.4.3). Then determine the dissolved oxygen concentration of the water for each test container showing no visible turbidity. Last, measure the dissolved oxygen concentration of the water in the containers showing visible turbidity. Between each reading, rinse the probe in purified water. Record the values obtained in accordance with EN ISO 5814, as milligrams per litre to one decimal place.

Calculate the Mean Dissolved Oxygen Difference (MDOD) (see 6.8.1) for each test piece.

Biological test procedures occasionally give individual values outside the expected range. Therefore if the MDOD value is > 1,69 mg/l but < 2,0 mg/l continue the test for a further two weeks and then calculate the final MDOD using all 5 sets of results.

At the end of this test, useful information can be obtained about the compatibility of the test material with drinking water. A visual examination of the test piece, comparing it with an untested example, can indicate

potential problem areas such as discolouration, surface blemishes, including osmotic blistering, and changes in the surface texture.

6.7.4.2 Measurement of dissolved oxygen for lubricants and lubricated materials

Some materials give rise to an oily film on the surface of the test water. If the electrochemical dissolved oxygen probe is introduced into the water through an oily film, the characteristics of its membrane are sometimes irreversibly altered. Measure the dissolved oxygen concentration of such test waters by the method described in 6.7.4.2.

Decant, with minimal disturbance, 300 ml of the water from the control and from the reference materials and test water control test containers (see 6.6.4 and 6.6.5) into separate clean 500 ml beakers. Measure the dissolved oxygen concentrations of these waters. Carefully remove 300 ml of the water from beneath the oily film of the test sample container. In order to separate the test water from the oily film, use a clean 400 ml beaker modified to allow the soak water to be drained out of the beaker from below the surface by a bottom exit tube. Transfer this to a clean 500 ml beaker and by visual inspection ensure that there is no oily film present on the surface of this water. Measure the dissolved oxygen concentration in this water as before.

Commercially available fat-separating gravy jugs have been found to fulfil these requirements. The soak water (with oil film) should be carefully transferred into the jug with minimal disturbance of the water surface. The oil-free water should be carefully drained into a clean 500 ml beaker.

6.8 Calculation of results

6.8.1 Calculation of the mean dissolved oxygen difference (MDOD)

Calculate the arithmetic mean of the three dissolved oxygen values obtained from each test piece container, each of the reference tests and the test water control. Subtract the mean of each test container from the mean of the test water control.

For a test sample applied to a metal fitting, subtract the mean of the metal fitting reference test (6.7.1.3) from the mean of the test piece.

Express the resultant value for each test or reference test as the mean dissolved oxygen difference in milligrams per litre to one decimal place. If the MDOD from the test piece container lies within the range > 1,69 mg/l to 2,9 mg/l, then test two further test pieces shall be tested. If the value is greater than 2,3 mg/l but less than 2,4 mg/l, express the result to 2 decimal places.

6.8.2 Validation of results

Each reference material shall exhibit results within the respective range of values given in Table 14.

Table 14 — Performance characteristics of reference materials

	Glass	**Paraffin Wax**
MDOD (mg/l)	0,0 ± 0,6	7,5 ± 2,5

The control shall exhibit results within the range of values given in Table 15.

Table 15 — Performance characteristic of control

Dissolved oxygen (mean value mg/l)	8,5 ± 2,5

If any reference material or the control gives results that do not comply with the values given in Tables 14 or 15 respectively, repeat the tests using fresh reagents and test pieces.

The quality characteristics of the test and inoculum waters should be carefully assessed when an 'out of-range' result occurs.

If the results for the cementitious or bactericidal/bacteriostatic reference test (6.7.1.2) fail to exhibit a MDOD that is within ± 0,6 mg/l of the MDOD obtained for the positive reference test (with paraffin wax) when tests have been done in the same batch, then report the material as having a bacteriostatic or bactericidal effect.

6.8.3 Repeatability and reproducibility

Mean dissolved oxygen difference (MDOD) – When two or more identical test samples of a material or product are examined by one analyst using the same apparatus within a short time interval, it has been found that the repeatability *r* of the arithmetic mean is 15 %. On this basis the repeatability will be exceeded on average not more than once in 20 cases in the normal and correct operation of this method.

When two or more identical test samples of a material or product are examined by two operators working in different laboratories, the reproducibility *R* of the arithmetic mean is 28 %. The reproducibility will be exceeded on average not more than one in 20 cases in the normal and correct operation of this method.

NOTE *r* and *R* have been calculated following the general principles and definitions given in ISO 5725 using 90 results obtained from the testing of one homogeneous reference material in three laboratories during 1985.

6.9 Test report

6.9.1 General

The following general information shall be included in the test report:

— a title (e.g. “Test Report”) and the date of issue of the report;

— unique identification of the test report (such as serial number), and on each page an identification in order to ensure that the page is recognised as a part of the test report, and a clear identification of the end of the test report;

— name and address of test laboratory and location where the test was carried out if different from the address of the testing laboratory;

— a reference to this standard EN (fill in the number of standard) (with date of publishing of EN) and Method 3: Measured by mean dissolved oxygen depletion;

— name and address of client submitting the test sample;

— a statement to the effect that the test results relate only to the items tested;

— a statement that the report shall not be reproduced except in full without the written approval of the testing laboratory;

— a signature and title or an equivalent marking of person(s) accepting technical responsibility for the test report and date of issue.

6.9.2 Material/Product information

6.9.2.1 General

The following information shall be included in test report:

— trade name and designation/identifier of the material/product;

— name and address of the manufacturer of the material/product;

— method of manufacture (if known);

— the production place and date;

— generic composition of the material/product;

— nature of the material, e.g. nitrile rubber, polyethylene;

— comprehensive description of the test sample including material type, colour, shape/form, dimensions , appearance, opacity and component type (if appropriate);

— date test sample received from client;

— organization submitting the test sample (material/product) for testing;

— organization responsible for preparing the test sample (if different);

— description of the manufacturers sampling procedure (if known);

— condition of material on receipt by the test laboratory, including method of packaging;

— conditions of storage of material between test sample receipt and the start of testing.

6.9.2.2 Cementitious products

For these materials/products the following information shall be included:

— details of any preconditioning given, including the Aggressivity Index of the preconditioning water, together with the pH values of each of the sequential preconditioning leachates;

— for admixtures the final report also the volume of admixture added to a specified mass of cement.

6.9.2.3 Factory Applied Products - coatings - test samples prepared by the manufacturer or supplier

For these materials/products the following information shall be included:

— method of preparation of test sample – was the product prepared in accordance with the application instructions – plus the number and nature of coats applied, cure conditions etc.

6.9.2.4 Factory Applied Products – used in assembly – greases, lubricants, solvent cements etc. – test samples prepared by the test laboratory in accordance with 2.1 of BS 6920.

For these materials/products the following information shall be included:

— method of preparation of test sample – was the test sample prepared in accordance with the application instructions;

— the cure conditions, if any, used before testing started.

6.9.2.5 Site applied products

For these materials/products the following information shall be included:

— typical uses of the product;

— batch number(s) of Site Applied Products (and other products, when known). If this information is not available this shall be stated in the final report;

— realistic cure conditions achievable on site – in the case of cure temperatures above either 7 °C or 12 °C (as appropriate) how these will be achieved reliably on site;

— method of preparation of test sample – was the test piece prepared in accordance with the user instructions - plus the number and nature of coats applied, cure conditions etc.;

— full details of test piece preparation, including component mix ratios (if appropriate), time and temperature of cure and test piece description. If non-standard cure conditions (other than those set out 6.6.3.7.3) have been used for the product then include a statement highlighting these conditions;

— for test pieces prepared at a different location to the test laboratory the following additional information;

  — location;

  — description of equipment used and the area where the test piece was prepared;

  — full description of test piece preparation, mixing ratios and batches numbers;

  — chain of custody of the test pieces, method of transfer to the test laboratory and temperature profiles of the test piece during transport to the laboratory for final curing;

  — time and temperature of final curing.

6.9.3 Information on the test procedure

The following information on the test procedure shall be included in test report:

— number of specimens or portions of the test sample which constitute a test piece;

— volume of the test water (V) if applicable;

— surface area of test piece exposed to the test water (S) calculated from the actual dimensions of the test pieces;

— source of the test water and (if applicable) details of preparation;

— any deviation from the test procedure;

— any factors which may have affected the results, such as any incidents or any operating details not specified in this standard;

— special observations, during preparation of the test piece and/or during testing;

— all treatments/procedures used which are not described in the standard, and which may have affected the results;

— dates of start and completion of the test.

6.9.4 Results

The test results shall, at least, include:

— the MDOD of the test and reference materials (in mg/l); if the test was extended by an extra two weeks to nine weeks give both MDOD values;

— the mean dissolved oxygen concentration of the control (in mg/l), and both values if the test is extended to 9 weeks;

— a description of the test sample after the completion of the test.

Annex A
(normative)

Method 1: Measured by Biomass Production Potential (BPP) measured by ATP – Assessment of the suitability of test water for its use in the BPP test (biological stability and the need for addition of trace elements)

Use the following method to prove that the biological stability of the test water meets the biostability requirement of Table 1 of this standard and if addition of trace elements are needed to ensure that biological growth is not limited by insufficient trace elements in the water.

Prepare a series of containers as given in Table A.1. Add 600 ml of the water to be assessed for its suitability for use in the BPP test, to each of 6 borosilicate flasks (1 l). The water under test should comply with the requirements given in Table 1. Before use clean the flasks, caps and liners in accordance with 4.3.3.

Add 1 ml of each of the stock solutions of trace elements (solutions 1, 2 and 3 – see Table A.2) to two of these flasks (containers 5 and 6). Add 1 ml of a freshly prepared sodium acetate solution (4.4.5) to two flasks without added trace elements (containers 3 and 4) and to two flasks with added trace elements (containers 5 and 6), to obtain a final concentration of sodium acetate of (5 ± 0,1) mg C/l. Add 1 ml of the inoculum (4.4.6) to all the containers (1, 2, 3, 4, 5 and 6) and incubate these in the absence of light at (30 ± 2) °C.

Table A.1 — Overview on the test series

Reagent	**Container number**					
	Water under test without addition of acetate and trace elements		Water under test with addition of acetate		Water under test with addition of acetate and trace elements	
	1	2	3	4	5	6
Test water	+	+	+	+	+	+
Trace elements	–	–	–	–	+	+
Sodium acetate	–	–	+	+	+	+
Inoculum	+	+	+	+	+	+

Determine the concentration of ATP after 0, 1, 3, 4 and 7 days of incubation. The test can be terminated when the concentration of ATP remains constant, or declines within this period.

Calculate the average value of the maximum concentration of ATP in each set of duplicate containers.

The test water is suited for use in the BPP test when the concentration of ATP remains below 10 ng /l in containers 1 and 2 during incubation.

Addition of trace elements to the test water is not necessary, when the level of ATP in the containers 3 and 4 is equal to the level in the containers 5 and 6. If the level of ATP in containers 5 and 6 is higher than in containers 3 and 4, an addition of trace elements is necessary.

Table A.2 — Trace elements

Solution/Compounds	Stock (mg/l)	Final concentration (mg/l)
Solution 1	5 000	5
$CaCl_2$	10	0,01
H_3BO_3	5	0,005
$CoCl_2 \cdot 6H_2O$	5	0,005
$Na_2MoO_4 \cdot 2H_2O$		
Add 1 ml to 1 000ml		
Solution 2	7 500	7,5
$MgSO_4 \cdot 7H_2O$	300	0,3
$MnSO_4 \cdot H_2O$	10	0,01
$ZnSO_4 \cdot 7H_2O$	10	0,01
$CuSO_4 \cdot 5H_2O$	300	0,3
$FeSO_4$		
Add 1 ml to 1 000ml		
Solution 3	270	0,27
KH_2PO_4	530	0,53
$K_2PO_4 \cdot 3H_2O$	800	0,8
$Na_2HPO_4 \cdot 12H_2O$		
Add 1 ml to 1 000ml		

NOTE Stocks of the above stock solutions are prepared in demineralised water and autoclaved.

Annex B
(normative)

Method 1: Measured by Biomass Production Potential (BPP) measured by ATP – Protocol of ATP analysis

B.1 Introduction

The procedures described in this Annex are applicable to the measurement of ATP concentrations obtained from materials in contact with water and water samples. The detection limit of the procedure is about 1 ng ATP/l. The detection limit for biofilms obtained from pipe and construction materials is calculated on the basis of the surface area of material processed. Excessive pH concentration, excess chloride, calcium, or copper concentrations and the colour of the sample may affect the accuracy of the ATP measurement. Other factors affecting measurement include low (< 18 °C) or high (> 25 °C) temperatures of the sample and the reagents.

B.2 Apparatus and glassware

Standard microbiological laboratory equipment, in particular includes:

— apparatus for sterilisation by steam (autoclave); sterilise glassware and drinking water for 20 min at (121 ± 3) °C;

— luminometer; with a minimum sensitivity of 1 pg ATP/ml;

— balance; with an accuracy of ± 1 mg;

— freezer; with a temperature < –65 °C.

B.3 Reagents and auxiliary substances

B.3.1 General

Prepare all solutions as prescribed by the manufacturer. Allow all reagents to reach (22 ± 5) °C before use (about 15 min).

B.3.2 Sterile water

Autoclaved drinking water with a low copper (< 0,05 mg/l) and ATP (≤ 5 ng ATP/l) concentrations.

B.3.3 ATP solutions for standard dilutions and calibration curves

B.3.3.1 Composition

For preparing a calibration curve in buffer, the dilution medium is the reconstitution buffer supplied with the reagent kit; for preparing a calibration curve in drinking water, the dilution medium is sterilised test water (4.4.1).

B.3.3.2 Preparation of ATP solutions

Pipette the dilution media (B.3.3.1) accurately into the ATP standard vial as described by the manufacturer.

— dissolve the ATP standard by mixing carefully (concentration 10^6 ng ATP/l);

— allow this solution to stand for 1 h before using (to assure homogeneity).

In undissolved form, the ATP standard is unstable at room temperature; however, the standard can be frozen and remains stable for longer at –70 °C. The solution shall not be refrozen after thawing.

Start with the ATP dilution of 10^6 ng ATP/l. Make further dilutions according to Table B.1.

Table B.1 — Concentration of standards for the calibration curve

Concentration of the standard	Volume	Dilution from standard	Blank volume
(pg ATP/ml)	(ml)	(pg ATP/ml)	(ml)
10 000	0,1	1 000 000	9,9
1 000	1,0	10 000	9,0
750	3,0	1 000	1,0
500	2,0	1 000	2,0
250	1,0	1 000	3,0
100	0,1	10 000	9,9
75	3,0	100	1,0
50	2,0	100	2,0
25	1,0	100	3,0
10	0,1	1 000	9,9
7,5	3,0	10	1,0
5	2,0	10	2,0
2,5	1,0	10	3,0
0			

To produce a calibration curve in buffer: the ‘Blank’ is reconstituted in buffer. To produce a calibration curve in drinking water the ‘Blank’ is sterile filtered (0,20 µm) autoclaved drinking water.

Aliquots (e.g. 0,3 ml) of each dilution are transferred to tubes (or containers) and stored at a temperature of ≤ –70 ºC; at this temperature the standard dilutions remain stable for a period of at least 6 months. Standard dilutions should not be refrozen after thawing.

B.3.4 Luciferin/luciferase (enzyme) solution

B.3.4.1 Composition

Composition:

— luciferin/luciferase;

— reconstitution buffer.

B.3.4.2 Preparation

Preparation:

— dissolve the Luciferin/luciferase in the reconstitution buffer as described by the manufacturer;

— dissolve the Luciferin/luciferase by gentle swirling;

— allow this solution to stand for 15 min at (22 ± 5) °C before using it further, to assure homogeneity.

— use this solution within 4 h.

B.3.5 ATP extraction reagent

Handle the ATP extraction reagent as described by the manufacturer. Allow it to reach (22 ± 5) ºC before use.

B.4 Sampling

Where possible start the measurements immediately after the ATP samples have been prepared from the test materials and test waters and always within (24 ± 1) h.

All types of sample shall be kept on melting ice until measurement is undertaken in order to prevent changes in the microbial activity of the sample. When a portion of the sample (e.g. 100 µl) is transferred to the measuring cuvette it will rapidly equilibrate to (22 ± 5) ºC. The sample should be mixed with reagents at (22 ± 5) ºC.

Prior to sub-sampling for ATP measurement homogenize the samples by Vortex mixing for 5 s.

B.5 Procedure

B.5.1 General

Use the Luminometer in accordance with the manufacturer's instructions.

B.5.2 Blank water

Measure 2 samples of the dilution medium.

B.5.3 Control dilutions

Thaw ATP solution standard dilutions and measure either a series for a calibration curve or measure at least two concentrations in duplicate, e.g. 1 000 ng ATP/l and 10 ng ATP/l.

B.5.4 Samples

Measure all samples in duplicate. After measuring the ATP concentration of the last sample, undertake further measurements, in duplicate, on the blank water (B.5.2) and control solutions (B.5.3).

B.6 Expression of results

B.6.1 Water samples

Calculate the ATP content using the following formula:

$$A = \frac{R - B}{m}$$

where

A is the ATP content in pg/ml of sample;

R is the arithmetic mean of the two RLU values of the water samples;

B is the arithmetic mean of the four RLU values of the blank water (two before the samples and two after the samples);

m is the mean gradient of the ATP calibration curves produced from historic data.

B.6.2 Material samples

Calculate the ATP content using the following formula:

$$A = \frac{R - B}{m} \times \frac{V}{S}$$

where:

A is the ATP content in pg/ml of sample;

R is the arithmetic mean of the two RLU values of the sample;

B is the arithmetic mean of the four RLU values of the blank water (two before the samples and two after the samples);

m is the mean gradient of the ATP calibration curves produced from historic data;

V is the total volume obtained of the pretreatment of the material;

S is the total water exposed surface of the sample.

B.7 Test record

The test record shall contain at least the following information:

— reference to this procedure;

— all details necessary for complete identification of the sample;

— the results expressed in accordance with B.8;

— any particular occurrence(s) observed during the course of the analyses and any operation(s) not specified in the method which may have influenced the results.

B.8 Quality control of calibration curves

The RLU response on a given measuring day depends on the activity of the enzyme and the room temperature. New standard ATP dilutions shall therefore always be calibrated against old standard dilutions. All dilutions are measured in duplicate. R^2 -values for the calibration curves shall be higher or equal to 99 % (0,990). Deviation between slopes of the old and the new calibration curve shall be within 5 %. At deviations above 5 % the new standard dilutions shall be rejected. The slopes of the calibration curves should be recorded on a control chart. Control charts shall be made of data of at least 6 calibration curves.

To minimise the effect of uncertainty with a single calibration curve, the average slope of a series of calibration curves can be used to calculate the ATP value. The average slope shall be based on historical values. Deviation between slopes of calibration curves should normally be within 5 %.

When starting up a database of values to calculate the average slope, a minimum of 10 different dilution series shall be used, made with a minimal of three different batches ATP standard solutions.

When using an average slope value for calculation of ATP-values, it shall be controlled on each measuring day that enzyme activity corresponds to the historical values. At least two standard dilutions (e.g. 10 pg ATP/ml and 1 000 pg ATP/ml) shall be measured in duplicate on each measuring day, but frozen complete calibration curves can also be used. The RLU responses shall be collected on a control chart. These control charts shall be made of at least 10 measurements each. In case the RLU exceed the chart limits, a full set of standard dilutions shall be measured on the given measuring day, and the slope of this calibration curve used for calculation of the ATP-value.

If the laboratory is not temperature regulated, it may be necessary to measure a full dilution series on all days with peak high or low temperatures (e.g. peak summer and winter periods). In these extreme situations different temperature ranges can be selected for preparing calibration curves. The corresponding calibration curve should be chosen dependant on the laboratory temperature on when samples are measured.

Annex C
(informative)

Method 1: Measured by Biomass Production Potential (BPP) measured by ATP - ATP and its measurement

Adenosine triphosphate is an energy-rich compound that is present in all living cells and consequently provides a measure of active biomass. Energy for a living cell is obtained by the hydrolysis of ATP to adenosine diphosphate (ADP) and/or to adenosine monophosphate (AMP). The chemical formula of ATP is $C_{10}H_{16}N_5O_{13}P_3$, the structure of the molecule is given below:

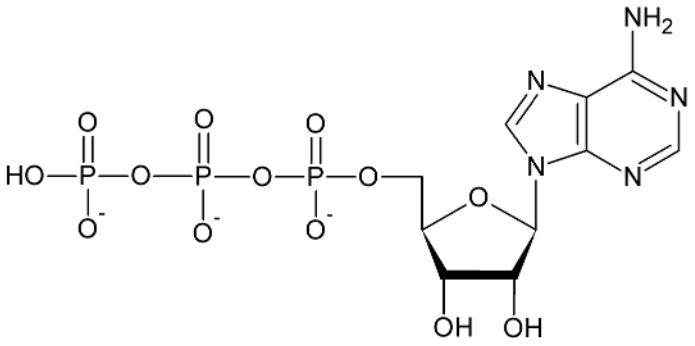

Figure C.1 — Chemical formula of ATP

At any one time, the amount of ATP in a cell will depend on its metabolic activity. Measurement of ATP is a recognised technique used to estimate the amount of active microbial biomass and hence the amount of growth that has taken place or the level of active biomass that is maintained.

ATP measurement is based on the reaction of luciferin with luciferase (originating from fireflies) which occurs in the presence of free ATP. During this process, light is produced.

Mg^{2+}

(1) Luciferin + luciferase → (luciferin-luciferase-AMP)

+ ATP + pyrophosphate

(2) (luciferin-luciferase-AMP) → Oxyluciferin + luciferase

+ ATP + CO_2 + AMP + light

Under optimal conditions, 1 photon of light is produced per molecule of ATP. The light generated is measured using a sensitive photometer and expressed in Relative Light Units (RLUs). The ATP content of the sample is then calculated with the aid of a conversion factor.

Annex D
(informative)

Method 1: Measured by Biomass Production Potential (BPP) measured by ATP – Evaluating procedures for removal of attached biomass

D.1 Introduction

A range of techniques have been studied for the removal of Attached Biomass from material surfaces. Where sonication is used biomass removal is dependent on the type of equipment and number of treatments employed. It is important that the laboratory develops a procedure that provides consistent removal of biomass. Suitable procedures are detailed below.

D.2 Procedures for removal of Attached Biomass

D.2.1 General

The method for removing biomass from materials should be capable of removing at least 90 % of the Attached Biomass, so that less than 10 % is recoverable by swabbing with a single cotton swab.

D.2.2 Swabbing

Wipe the entire surface of an equivalent test piece with one or more sterile cotton swabs, until the surface is observed to be entirely clean. Place the swab(s) in autoclaved drinking water, and sonicate for (120 ± 10) s.

D.2.3 Low energy sonication (LES)

Give three treatments of (120 ± 10) s LES, with collection of the sonicated suspension and replacement of the water following each treatment, to the glass (negative control). Give six treatments of (120 ± 10) s to all other samples. Combine the suspensions produced for each control or test sample and store them in tubes immersed in melting ice.

Measure the ATP concentration of the combined suspensions and calculate the concentration of the Attached Biomass as pg ATP/cm^2.

A total of six LES treatments is needed for the removal of biomass from soft materials. Subsequently, swabbing shall be applied. To assess the suitability of the treatments, additional swabbing should be conducted and the amount of removed biomass should be measured separately.

NOTE 1 This LES procedure is suited for use on hard materials when the contribution of ATP from swabbing is less than 10 % of the total ATP concentration obtained with LES and swabbing.

NOTE 2 LES treatment followed by swabbing is sufficient when the final swabbing yields less than 10% of the total amount removed.

D.2.4 High energy sonication (HES)

Apply the HES for (120 ± 10) s in accordance with the equipment manufacturer's instructions. During sonication keep the sample in contact with melting ice.

For hard materials one treatment is sufficient. To assess the suitability of the equipment (setting), a second sonication shall be made after replacing the test water. Less than 10 % of the concentration of Attached Biomass should be obtained from the second treatment to demonstrate that one treatment is effective. Swabbing can be applied to verify the efficacy of one HES treatment.

For soft materials two HES treatments shall be applied, with water replacement between the treatments.

The equipment and settings shall be evaluated with a sample of PVC-P (positive control) by applying a third HES treatment. This third treatment should yield less than 10 % of the total of all biomass removed. Swabbing can also be used to demonstrate the efficiency of the applied HES treatments.

D.3 Effectiveness of removal procedure

The effectiveness of the Attached Biomass removal procedure is demonstrated by removal of any remaining Attached Biomass from the material with a single cotton swab. The amount removed with the cotton swab should be less than 10 % of the total biomass removed by the main procedure. Effectiveness is determined by the formula:

$$\frac{(Y - X)}{Y} \times 100 \geq 90\ \%$$

where

X is the ATP measured by the single cotton swab;

Y is the LES or HES procedure.

Annex E
(informative)
Method 1: Measured by Biomass Production Potential (BPP) measured by ATP

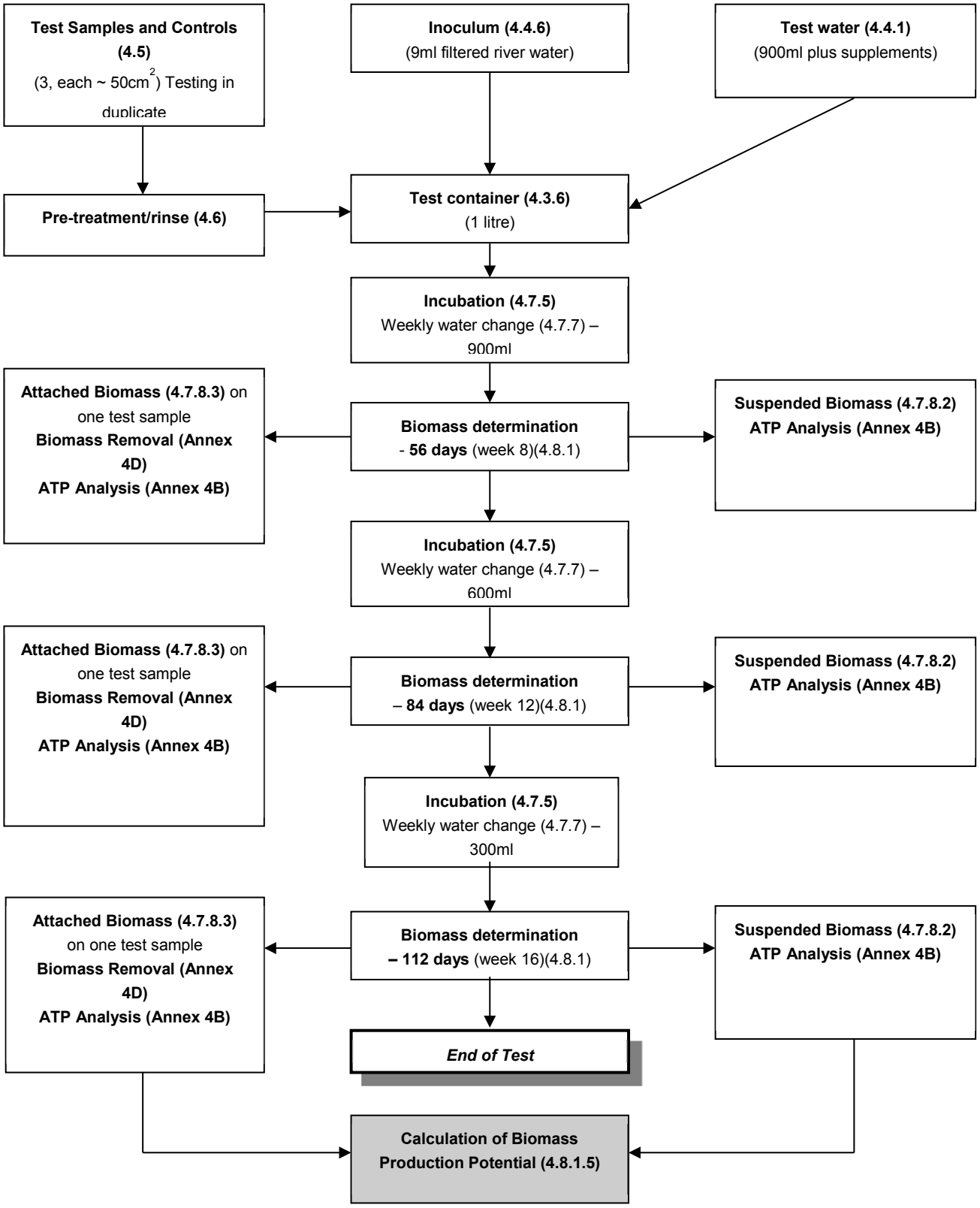

Figure E.1 — Flow diagram of test procedue – BPP

Annex F
(informative)

Method 2: Measured by biofilm volume

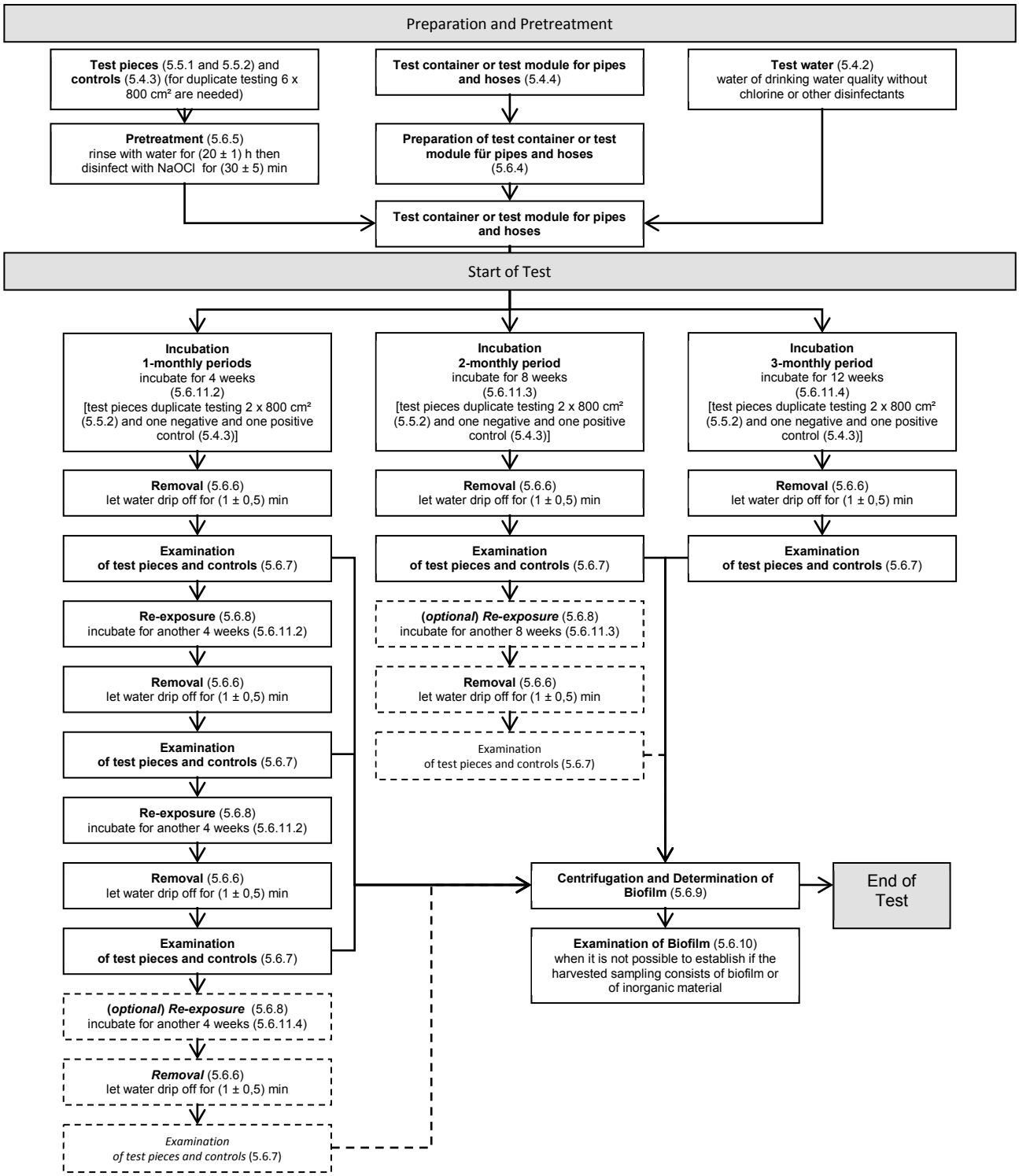

Figure F.1 — Flow diagram of test procedure – Biofilm volume

Annex G
(informative)

Method 2: Measured by biofilm volume

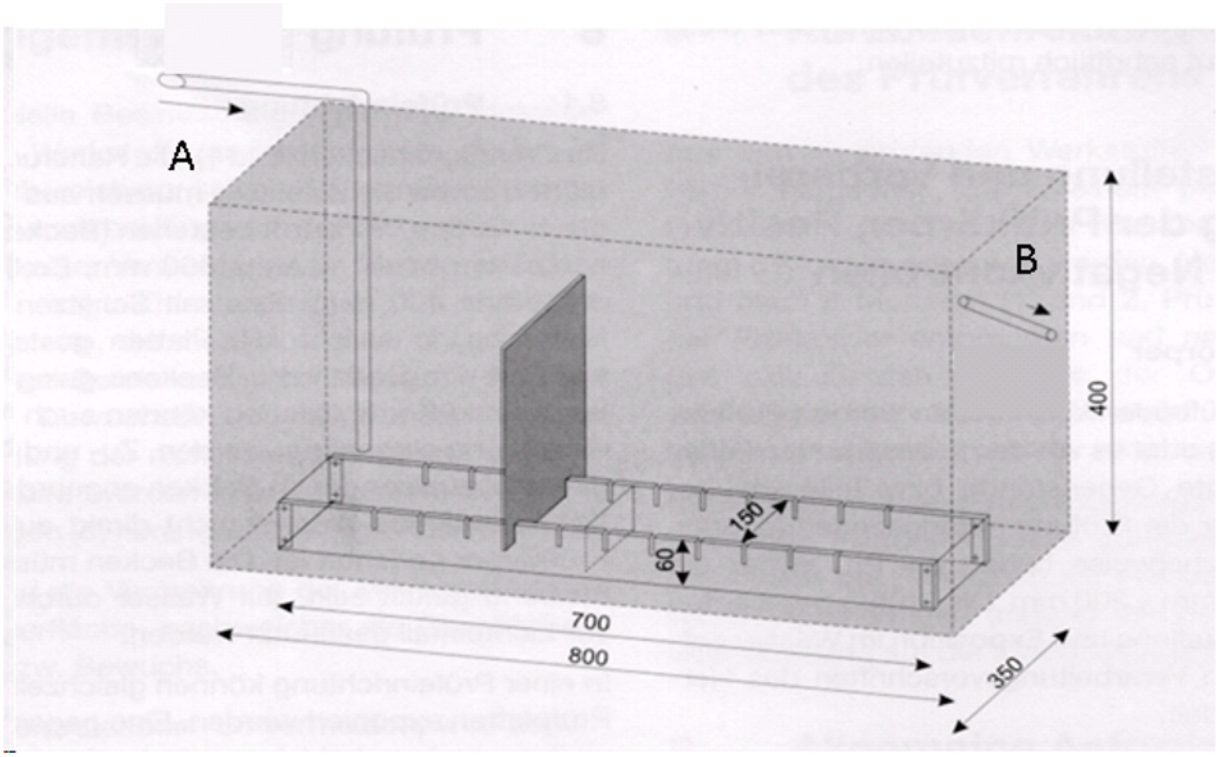

Figure G.1 — Example of a test container (possible ways to immerse test pieces and positive and negative controls: hook in or set in)

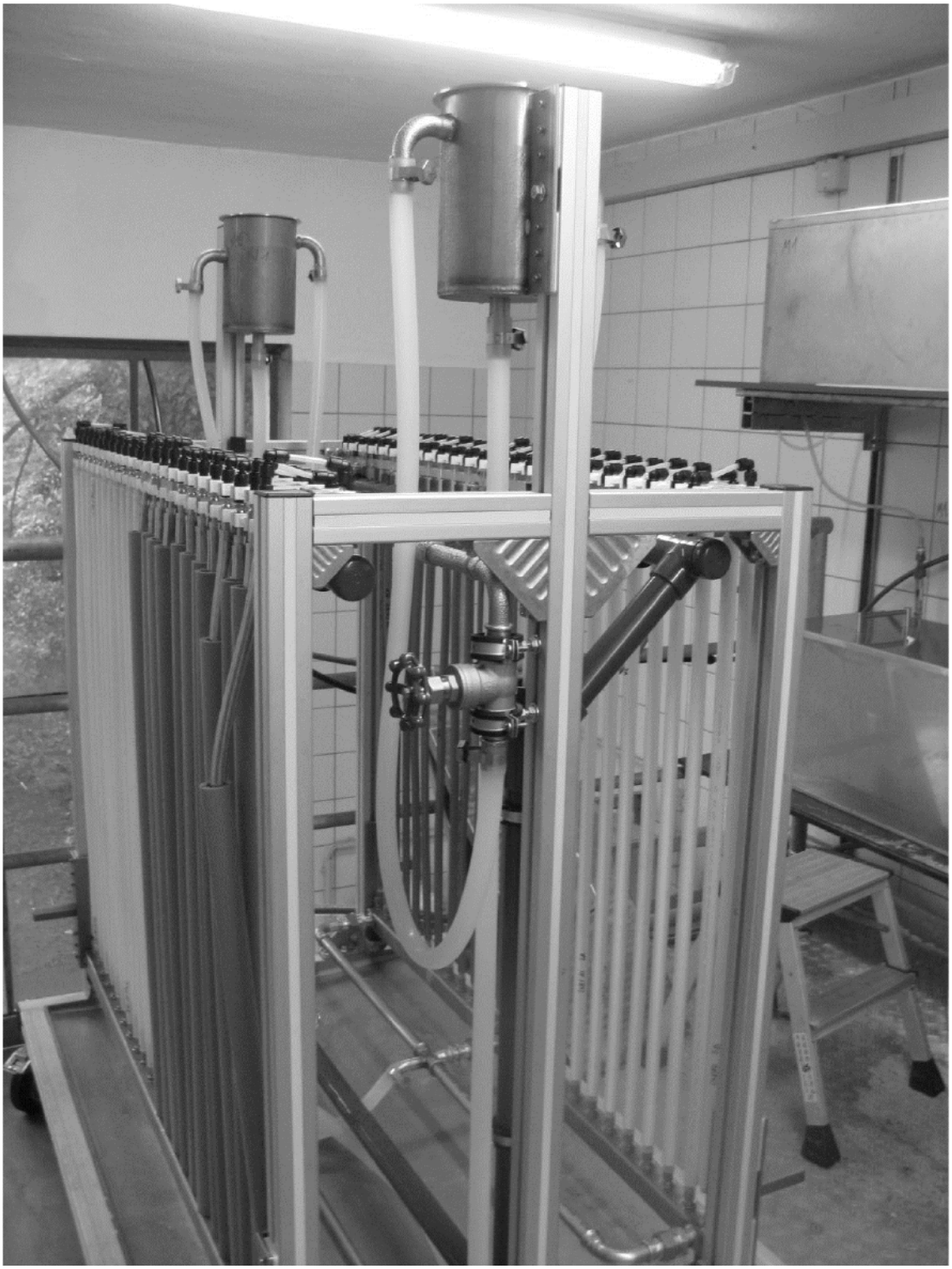

Figure G.2 — Example of a test module for pipes and hoses

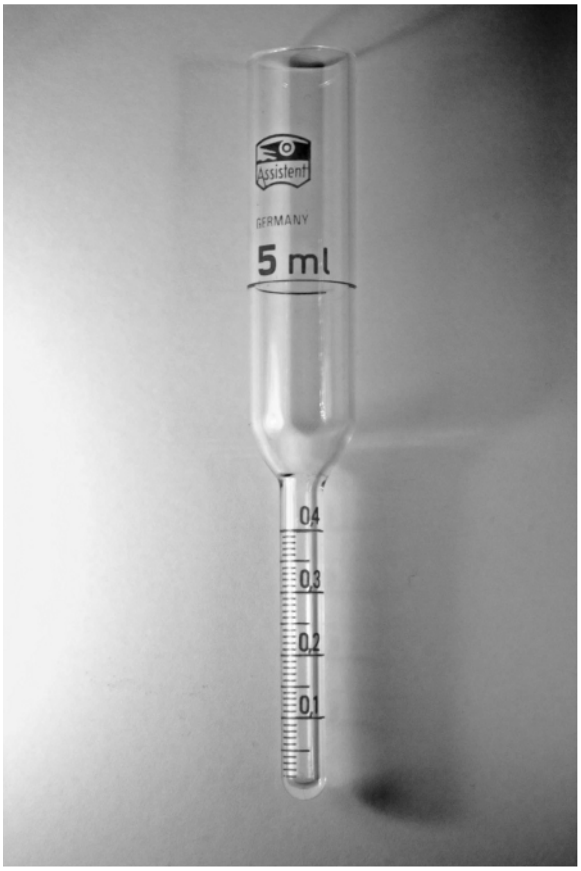

Figure G.3 — Example of glassware for centrifugation

Annex H
(informative)
Method 3: Measured by mean dissolved oxygen depletion

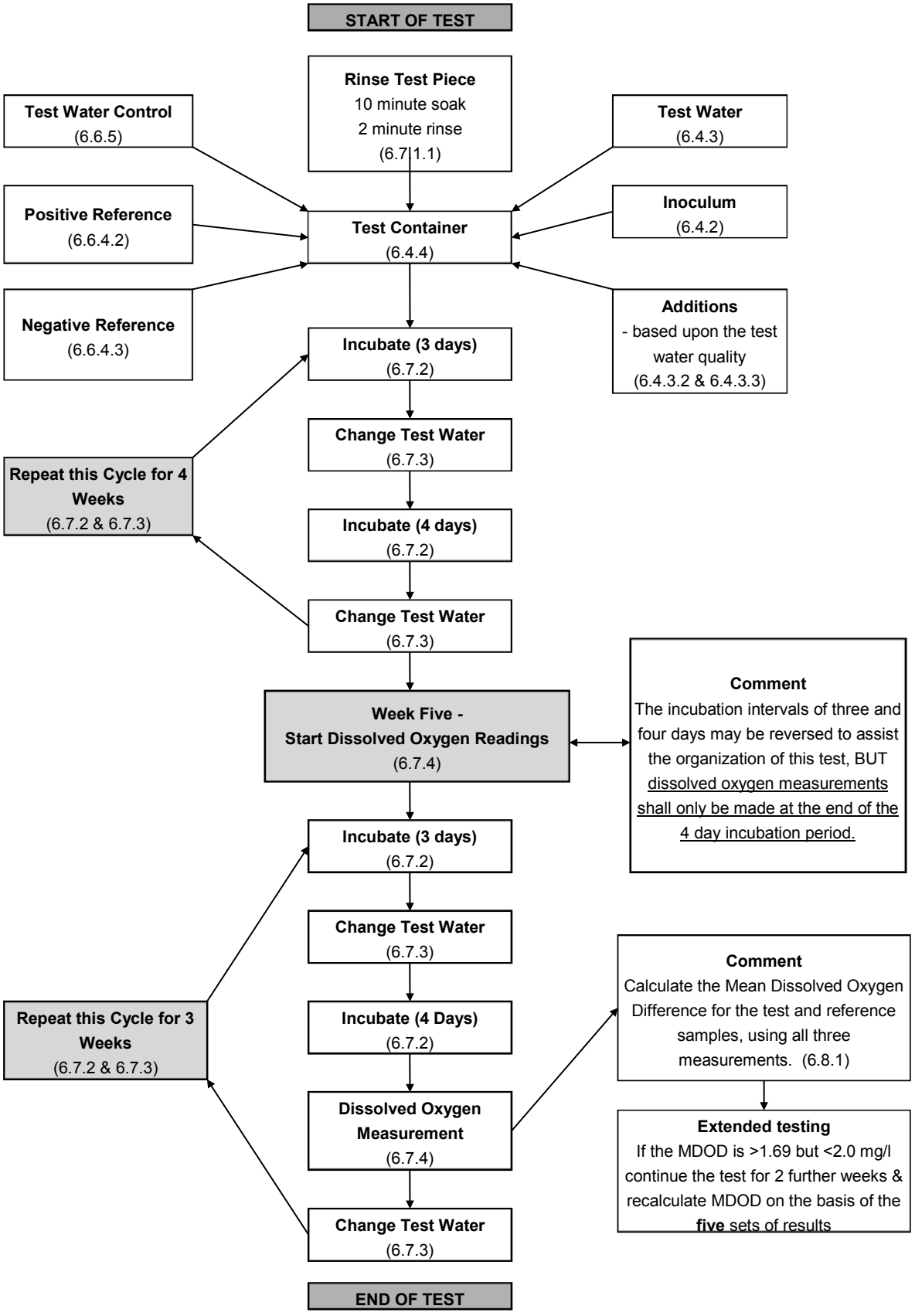

Figure H.1 — Flow diagram of test procedure – Mean dissolved oxygen depletion

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[2] ISO 5725 (all parts), *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests*

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