INTERNATIONAL STANDARD

**ISO 10993-15**

Second edition
2019-11

# Biological evaluation of medical devices —

## Part 15: Identification and quantification of degradation products from metals and alloys

*Évaluation biologique des dispositifs médicaux —*

*Partie 15: Identification et quantification des produits de dégradation issus des métaux et alliages*



Reference number
ISO 10993-15:2019(E)



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

Published in Switzerland

# Contents

Page

# Foreword

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

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

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

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

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

This document was prepared by Technical Committee ISO/TC 194, *Biological and clinical evaluation of medical devices.*

This second edition cancels and replaces the first edition (ISO 10993-15:2000), which has been technically revised.

The main changes compared to the previous edition are as follows:

a) the document now considers materials designed to degrade in the body as well as materials that are not intended to degrade;

b) the information on test methods has been amended to consider nanomaterials and relevant material specific standards;

c) the test solution (electrolyte) has been specified more;

d) the sample shape has been specified more;

e) the immersion test procedure has been expanded;

f) the status of Annex C in the previous edition has been changed and now included as Annex A.

A list of all parts in the ISO 10993 series can be found on the ISO website.

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



# Introduction

One of the potential health hazards resulting from medical devices can be due to the interactions of their electrochemically induced degradation products with the biological system. Therefore, the evaluation of potential degradation products from metallic materials by methods suitable for testing the electrochemical behaviour of these materials is a necessary step in the biological performance testing of materials.

The body environment typically contains cations of sodium, potassium, calcium, and magnesium, and anions of chloride, bicarbonate, phosphate, and organic acids generally in concentrations between $2 \times 10^{-3}$ mol/l and $150 \times 10^{-3}$ mol/l. A range of organic molecules such as proteins, enzymes, and lipoproteins are also present, but their concentrations can vary to a great extent. Earlier studies assumed that organic molecules did not exert a significant influence on the degradation of metallic implants, but newer investigations indicate that implant–tissue interactions should be taken into account. Depending on a particular product or application, altering the pH of the testing environment may also need to be considered.

In such biological environments, metallic materials may undergo a certain degradation, and the different degradation products can interact with the biological system in different ways. Therefore, the identification and quantification of these degradation products is an important step in evaluating the biological performance of medical devices.

# Biological evaluation of medical devices —

# Part 15: Identification and quantification of degradation products from metals and alloys



## 1 Scope

This document specifies general requirements for the design of tests for identifying and quantifying degradation products from final metallic medical devices or corresponding material samples finished as ready for clinical use.

This document is applicable only to those degradation products generated by chemical alteration of the final metallic device in an *in vitro* degradation test. Because of the nature of *in vitro* tests, the test results approximate the *in vivo* behaviour of the implant or material. The described chemical methodologies are a means to generate degradation products for further assessments.

This document is applicable to both materials designed to degrade in the body as well as materials that are not intended to degrade.

This document is not applicable to evaluation of degradation which occurs by purely mechanical processes; methodologies for the production of this type of degradation product are described in specific product standards, where available.

NOTE Purely mechanical degradation causes mostly particulate matter. Although this is excluded from the scope of this document, such degradation products can evoke a biological response and can undergo biological evaluation as described in other parts of ISO 10993.

Because of the wide range of metallic materials used in medical devices, no specific analytical techniques are identified for quantifying the degradation products. The identification of trace elements ($<10^{-6}$ w/w) contained in the specific metal or alloy is not addressed in this document, nor are specific requirements for acceptable levels of degradation products provided in this document.

This document excludes the biological activity of the degradation products. (See instead the applicable clauses of ISO 10993-1 and ISO 10993-17).

## 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3585, *Borosilicate glass 3.3 — Properties*

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

ISO 8044, *Corrosion of metals and alloys — Basic terms and definitions*

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

ISO 10993-9, *Biological evaluation of medical devices — Part 9: Framework for identification and quantification of potential degradation products*

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

ISO 10993-13, *Biological evaluation of medical devices — Part 13: Identification and quantification of degradation products from polymeric medical devices*

ISO 10993-14, *Biological evaluation of medical devices — Part 14: Identification and quantification of degradation products from ceramics*

ISO 10993-16, *Biological evaluation of medical devices — Part 16: Toxicokinetic study design for degradation products and leachables*

## 3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 8044, ISO 10993-1, ISO 10993-9, ISO 10993-12 and the following apply.

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

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

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

**3.1**
**alloy**
material composed of a metallic element with one or more addition(s) of other metallic and/or non-metallic elements

**3.2**
**electrolyte**
medium in which electric current is transported by ions

**3.3**
**open-circuit potential**
potential of an electrode measured with respect to a reference electrode or another electrode when no current flows to or from it

**3.4**
**passive limit potential**
$E_a$
electrode potential of the positive limit of the passive range

Note 1 to entry: See Figure 1.

**3.5**
**breakdown potential**
$E_p$
critical electrode potential above which localized or transpassive corrosion is found to occur

Note 1 to entry: See Figure 1.

**3.6**
**absorb**
action of a non-endogenous (foreign) material or substance passing through or being assimilated by cells and/or tissue over time

**3.7**
**potentiodynamic test**
test in which the electrode potential is varied at a preprogrammed rate and the relationship between current density and electrode potential is recorded

**3.8**
**potentiostatic test**
test in which the electrode potential is maintained constant and the current is recorded as a function of time

# 4 Degradation test methods

## 4.1 General

To identify and quantify degradation products from metals and alloys in medical devices, two procedures are described. The choice of test procedure shall be justified according to the function of the medical device.

The first procedure described is a combination of a potentiodynamic test and a potentiostatic test. The second procedure described is an immersion test.

The potentiodynamic test is used to determine the general electrochemical behavior of the material under consideration and to determine certain specific points ($E_a$ and $E_p$) on the potential/current density curve.

The potentiostatic test is used to electrochemically degrade the test material at a constant potential above the breakdown potential to generate degradation products to be analyzed.

The immersion test is used to chemically degrade the test material to generate degradation products to be analyzed.

If there is the possibility of the loss of a coating from a metallic substrate due to degradation, the potential degradation products from the substrate material shall be considered, as well as the coating itself. In addition, if a metallic substrate coated with a non-metallic material is to be tested, the requirements of ISO 10993-13 and/or ISO 10993-14 shall be used in order to determine the potential degradation products of the coating.

The identified and quantified degradation products form the basis for evaluation of biological response. If appropriate, toxicokinetic studies in accordance with ISO 10993-16 shall be used.

For those medical devices composed of or containing nanoscale materials, and for those instances where metallic degradation products are within the nanoscale size range (approximately 1 nm to 100 nm), the user is referred to ISO/TR 10993-22 when creating their risk assessment documents.

If the medical device is made using a metal or metal alloy designed to be absorbed by the body, the user is directed to relevant material specific standards (see bibliography) for methods and specific considerations (e.g. electrolyte, atmosphere, etc.) appropriate for this class of materials.

## 4.2 Prerequisites

The rates of electrochemical degradation reactions are sensitive to small variations in test conditions, instrumentation, sample conditions, and preparation. Therefore, electrochemical degradation testing shall be carried out in an appropriately equipped laboratory by experienced and qualified personnel. This includes proper maintenance and calibration of the test equipment. The methods and operating conditions of the equipment shall also be validated.

Fulfilment of electrochemical test conditions for stability, warm-up time, etc., can be demonstrated by conformance to Reference [1].

# 5 Reagent and sample preparation

## 5.1 Sample documentation

The general composition of the material(s) under test shall be documented.

## 5.2 Test solution (electrolyte)

The test solution (electrolyte) to be used shall be appropriate for the intended use of the medical device. All chemicals shall be of analytical grade and dissolved in water of grade 2 in accordance with ISO 3696.

The first choice for the electrolyte shall be an aqueous solution of 0,9 % sodium chloride.

Dependent on the composition and corrosion mechanism of the metal or alloy being tested, other electrolytes may be used, such as artificial saliva or artificial plasma. Examples of electrolyte compositions are given in Annex A, but other more material and physiologically relevant electrolyte solutions and test conditions may be utilized. Possible effect of implant–related protein interactions should be taken into account.

NOTE Formulations for artificial sweat, gastrointestinal fluids, and lung fluids have been used (see Bibliography).

In the test report, the choice of electrolyte shall be justified. If other than an aqueous solution of 0,9 % sodium chloride is used, the pH of the electrolyte shall be specified.

## 5.3 Preparation of test samples

### 5.3.1 Test samples

The sensitivity of chemical degradation testing is related to variation in material composition, to material processing, and to surface-finishing procedures. The sampling procedure, sample shape, and surface preparation are critical. In addition, confined spaces within or around the test article can result in crevice corrosion and defects in coatings can cause pit corrosion, both of which shall be taken into consideration. The samples shall be representative of the final devices.

### 5.3.2 Sampling

For each chemical test, multiple test samples shall be prepared as specified in ISO 10993-12. If substantial differences in the test results are found, the reasons for the difference shall be determined, and more samples shall be tested. The number of samples shall be justified.

If the metallic sample has anisotropic properties due to manufacturing conditions, tests involving single-surface exposure should include samples cut parallel to both the transverse and longitudinal manufacturing directions.

### 5.3.3 Sample shape

Standard samples (e.g. circular- or rectangular-section bars, flat coupons, one single free surface) may be used for degradation testing if they are prepared in a manner comparable to the final medical device. Samples of actual device components may be of any shape and condition; however, the testing shall be carried out under well-controlled conditions which shall be reported.

The surface area of the sample exposed to the electrolyte shall be determined to ±10 % of the total geometrical area to assure an accurate and repeatable determination of the degradation rates.

If representative samples are used, consideration shall be made regarding whether the differences between the representative sample and the final medical device or component could affect the results of the test. Testing of representative samples instead of the final medical device shall be supported by a description of any differences between the representative sample and the final device. The report shall



contain a detailed rationale for why each difference is not expected to alter the biocompatibility of the final device.

#### 5.3.4 Sample surface condition

Since the surface condition of a material can affect its electrochemical behaviour, the surface condition of the test sample shall be identical to the final medical device and shall be described in the test report.

## 6 Electrochemical tests

### 6.1 Apparatus

**6.1.1 Test cells** of borosilicate glass, in appropriate sizes, in accordance with ISO 3585, with a means of controlling the bath temperature within ±1 °C.

**6.1.2 Scanning potentiostat** with a potential range ±2 V and a current output range from $10^{-9}$ A to $10^{-1}$ A.

**6.1.3 Potential-measuring instrument** with a high input impedance (>$10^{11}$ Ω) and a sensitivity and accuracy to detect a change of 1 mV over a potential range between ±2 V.

**6.1.4 Current-measuring instrument** capable of measuring a current to ±1 % of the absolute value over a current range between $10^{-9}$ A and $10^{-1}$ A.

**6.1.5 Working electrode** (test sample).

**6.1.6 Counter-electrode(s)** such as platinum (grid, plate, or wire) or vitreous carbon with an area at least 10 times that of the working electrode.

**6.1.7 Reference electrode** which has a known electrode potential and is stable.

**6.1.8 pH-meter** with a sensitivity of ±0,1.

A schematic diagram of the electrochemical measurement circuit which can be used as a system with variable potential is given in Annex B, Figure B.1.

A schematic drawing of an electrolytic cell is given in Annex C.

### 6.2 Sample preparation

Mount the test sample in a watertight electrode holder so that only the test surface is in contact with the electrolyte. Take care to avoid the creation of conditions where crevice corrosion can occur due to the formation of a crevice between the mounting and the sample. Before testing, clean the sample ultrasonically for 10 min to 15 min in ethanol, carefully rinse the sample with water of grade 2 in accordance with ISO 3696, and immediately transfer the sample into the test cell.

### 6.3 Test conditions

Fill the test cell with the test solution (electrolyte). If the electrochemical behavior is temperature sensitive in the range of 10 °C to 50 °C, maintain the electrolyte cell at (37 ± 1) °C. Reduce the oxygen level in the electrolyte by bubbling oxygen-free nitrogen or argon at a rate of approximately 100 $cm^3$/min for not less than 30 min prior to the start of the test. The electrolyte shall be agitated either by the bubbling gas or mechanical means to avoid concentration gradients. If gas agitation is used, take care not to have any gas bubbles adhering to the active test surface.

Magnetic stirrers often interfere with electrochemical test cells. If they are used, their effect on the test cell shall be determined as part of the validation of test equipment (see 4.2).

## 6.4 Potentiodynamic measurements

Measure the open-circuit potential not less than 2 h after the immersion of the working electrode. This potential shall be the starting potential for potentiodynamic measurements. The sweep rate shall be 1,0 mV/s, except in tests where the sweep rate has little effect, where the test may be accelerated by increasing the sweep rate to 10 mV/s. Record the potential/current density curve up to a maximum of 2 000 mV or a maximum current density of 1,0 mA/cm$^2$, whichever comes first, to evaluate the transpassive range of the sample (see Figure 1). To ensure consistency, reverse the scan and continue back at least to the open-circuit potential. Then repeat the test back to 2 000 mV or 1,0 mA/cm$^2$. If the curves are not reproducible, then continue cycling 5 to 10 times. If consistent potential/current density curves are not achieved after 5 to 10 cycles, investigate possible causes such as test setup, electrode function, innate material properties, etc. The log current density/potential curves should also be recorded (see Figure 2). Record the breakdown potential ($E_p$) from the last cycle taken (see Figure 1).

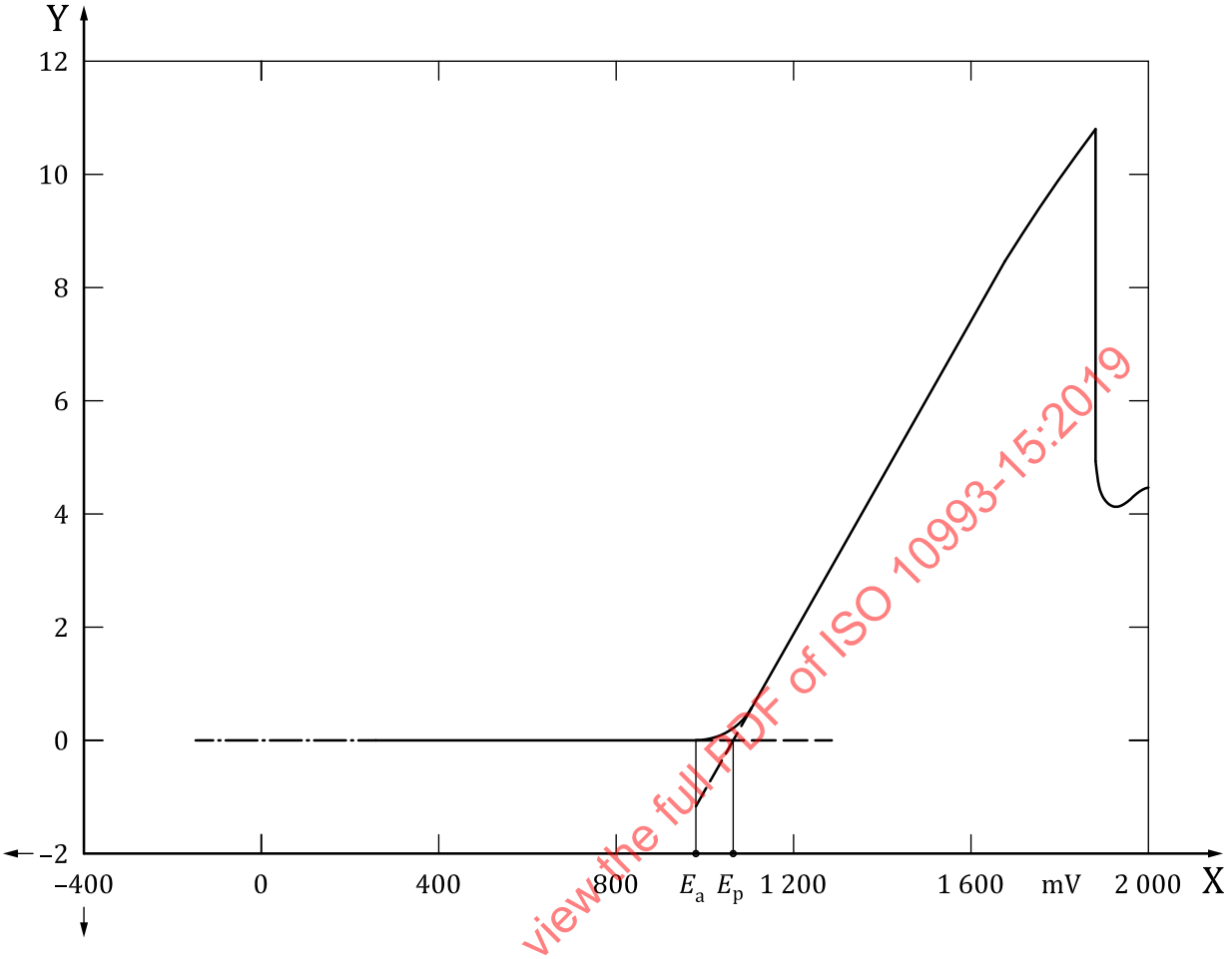
Noble metals can behave differently from passivating metals during an electrochemical test. Therefore, take care in determining the breakdown potential ($E_p$) for different metal systems.

Since some regulatory bodies can require testing according to different potentiodynamic measurement standards, the user is encouraged to check with the relevant authorities to assure use of appropriate test methods and parameters.

NOTE This method can or cannot be applicable to absorbable metals.





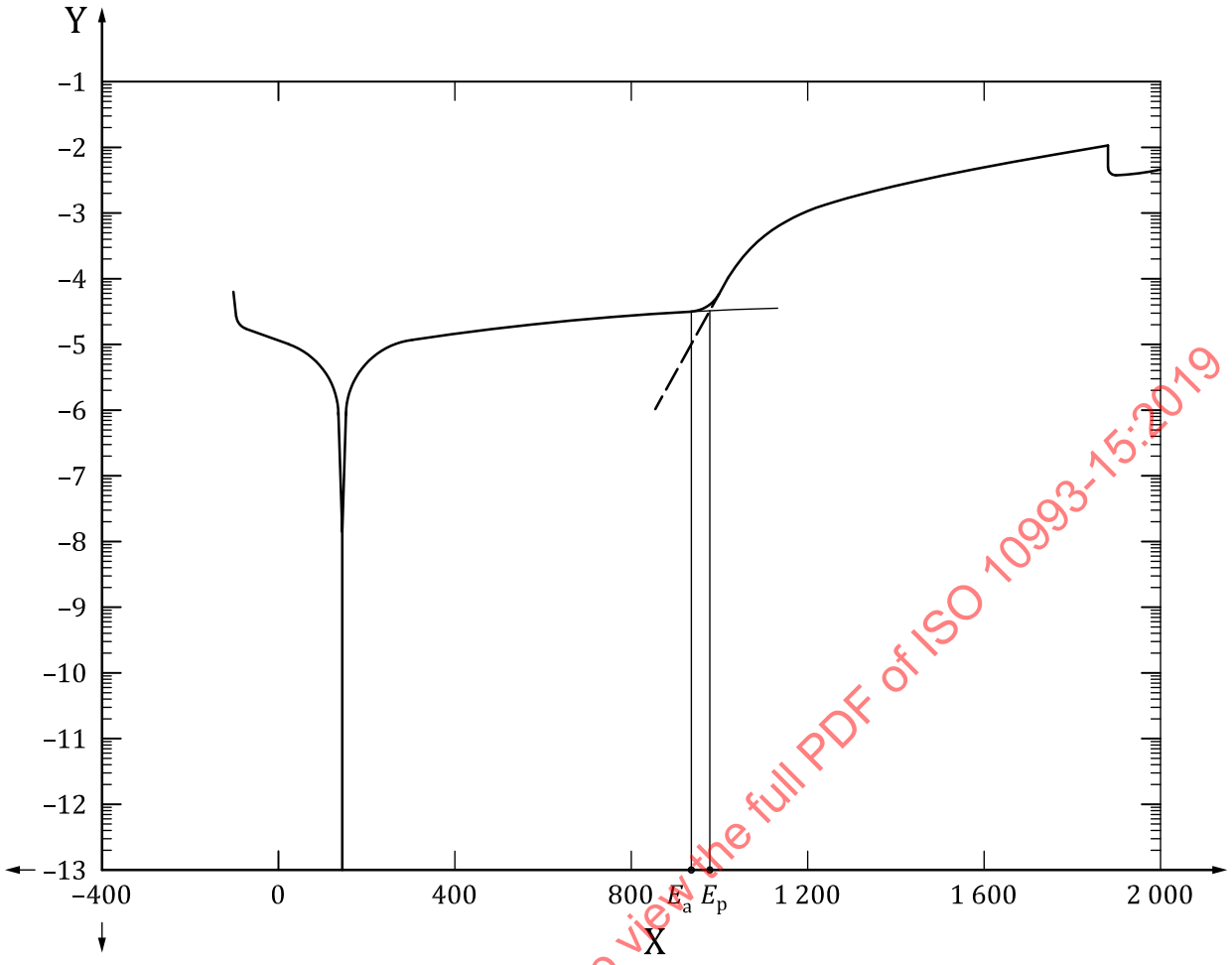

**Key**

X potential (mV)

Y current density (mA/cm$^2$)

NOTE $E_p$ is determined by extrapolation of the linear part of the oxidation curve to zero current density.

**Figure 1 — Plot of current density versus potential, showing the start of corrosion current at $E_a$ and breakdown potential, $E_p$**




**Key**

X potential in millivolts (mV)

Y log current density

**Figure 2 — Log current density versus potential plot showing the breakdown potential, $E_p$, at the inflection point of the curve**



## 6.5 Potentiostatic measurements

This method permits qualitative and quantitative determination of degradation products which might be dissolved in the electrolyte.

Hold a new test sample at a constant electrode potential during the test time, and record the current density/time curve. The potential used to determine the degradation products should be the breakdown potential ($E_p$) +50 mV. If a different potential is used, it shall be reported and justified. Depending upon the material studied, the polarization duration shall be either 1 h or 5 h and shall be reported. Measure and record the volume of the electrolyte for use in future calculations.

NOTE This method can or cannot be applicable to absorbable metals.

# 7 Immersion test

## 7.1 Apparatus

**7.1.1 Test cells** of borosilicate glass, of appropriate sizes, in accordance with ISO 3585, with a means of controlling the bath temperature within ±1 °C.

**7.1.2 pH-meter** with a sensitivity of ±0,1.

## 7.2 Sample preparation

The test sample shall be placed in a separate glass container. The size of the glass container should be selected so that an electrolyte volume of less than 1 ml/cm$^2$ of sample surface shall completely cover the sample(s). The test sample shall be totally covered by the electrolyte.

Do not risk compromising long-term data through biological (e.g. bacterial, fungal) contamination. For example, the utilized containers may need to be sterile and electrolyte may need to be prepared under aseptic conditions.

The surface area and volume of electrolyte should be sufficient for the intended method of analysis (see Clause 8).

Care should be taken such that the samples do not touch the glass surface except in a minimum support line or point. If the test sample is small, the proper surface area/volume ratio might not be attainable with a single test sample. Therefore, if the test sample shall be made up of two or more pieces, the pieces shall not touch each other.

For test samples with roughened or irregular shapes and therefore difficult to determine the surface area, the user is referred to the discussion in ISO 10993-12 and ISO/TR 10993-22 concerning how such differences can impact the risk assessment.

## 7.3 Immersion test procedure

Measure the pH of the electrolyte containing the test sample at the start of the test. Then tightly close the test cell to prevent evaporation and maintain at (37 ± 1) °C for (7 ± 0,1) d. Then remove the sample and measure the pH of the residual electrolyte. Certain materials can call for the use of tissue culture (i.e. sterile filtered) grade phosphate buffered saline as the immersion electrolyte.

It can be necessary to conduct immersion tests for a longer period of time if all of the following conditions apply:

a) the alloy is used in a permanent implant

b) the alloy contains constituents that are soluble in the use environment

c) the soluble constituents are present at potentially hazardous levels within the device

d) no additional information exists to demonstrate the stability of the constituents of concern (e.g. established surface process and acceptable corrosion resistance)

For devices that meet the above criteria, the release of potentially hazardous constituents shall be quantified over time to determine what short- and long-term exposure to the alloy will present for consideration in the risk analysis. To quantify the time-dependent release rate, successive immersion testing shall be conducted on the same test sample. After each test interval, the immersion procedure should be repeated by removing the test sample from the container, sampling the electrolyte for analysis, and placing the test sample into a container with fresh electrolyte. The pH of the electrolyte should be measured at the beginning and end of each sampling interval. Sampling intervals should be sufficiently frequent such that key release characteristics can be captured (e.g. more frequent sampling at earlier time points to capture the initial bolus of release). For example, sampling intervals for

nitinol implants might include at least days 1, 2, 4, 7, 14, 21, and 28 for the first month of cumulative exposure time, and at least bi-weekly thereafter. In a similar manner, time-dependent ion release has been seen from titanium test samples. A justification shall be provided for concluding the testing based on the release of degradation products approaching equilibrium, attaining a steady-state rate and/or falling below a predetermined rate of toxicological concern. The user of this document is alerted to the observation that variations in material lots and manufacturing lots can affect testing results. In addition to the analytical instrumentation, validation of the test protocol over the time frame of the testing shall also be conducted.

For absorbable metals, control of pH, dissolved gases, and fluid flow during testing should be considered, along with mass loss and degradation products. In the case of Mg-based products, it is also important to consider assessment of $H_2$ gas generation.

## 8 Analysis

For both electrochemical and immersion tests, observe and record the condition of the test sample under low-power microscopy (>50×) and report any significant changes to the surface. More detailed analysis of the surface may be undertaken if appropriate.

After each experiment, perform a qualitative and quantitative analysis of the electrolyte using a method of adequate sensitivity for the intended purpose (e.g. atomic absorption, ICP, or mass spectroscopy). Report compositional constituents detected above the limits of quantification. If potentially biologically hazardous constituents are identified but not quantified, other analytical analyses may be necessary. In addition, any deposits on the counter-electrode shall be analysed as well.

## 9 Test report

The test report shall contain at least the following details:

a) complete identification of the test sample, including the chemical composition;

b) ratio of the exposed surface area of the sample to the volume of the electrolyte;

c) composition and pH (with an uncertainty of ±0,1) of the electrolyte and a description of the natural or reference electrode for the electrochemical test;

d) composition and initial and final pH of the electrolyte for the immersion test;

e) temperature of the electrolyte;

f) for potentiodynamic testing: current density vs. potential curve(s), optionally the log (current density) vs. potential curve for comparison;

g) open-circuit potential;

h) breakdown potential $E_p$ and the current density at the breakdown potential;

i) sweep rate;

j) current density vs. time curve(s) and total test time;

k) brief comments on the curves (e.g. hysteresis, peaks);

l) description of any significant changes of the sample surface and/or of the electrolyte;

m) results of analysis of degradation elements in the electrolyte, including degradation rate, reported in micrograms per square centimetre per hour (μg/cm$^2$/h) for the electrostatic test;

n) method of chemical analysis of electrolyte;

o) type of reference electrode [all potentials should be referenced to the normal hydrogen electrode (NHE)];

p) for immersion testing, report results of analysis of degradation elements including total cumulative mass released per device (e.g. µg), as well as mass release rate (e.g. µg/day). In addition, if results are compared between devices or samples with different geometries, results should also be normalized by device surface area;

q) name of investigator;

r) date(s) of investigation;

s) signature of the investigator.