INTERNATIONAL STANDARD

ISO 13304-1

First edition
2013-07-01

Radiological protection — Minimum criteria for electron paramagnetic resonance (EPR) spectroscopy for retrospective dosimetry of ionizing radiation —

Part 1: General principles

Radioprotection — Critères minimaux pour la spectroscopie par résonance paramagnétique électronique (RPE) pour la dosimétrie rétrospective des rayonnements ionisants —

Partie 1: Principes généraux

Reference number
ISO 13304-1:2013(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

Page

Foreword ..... **iv**
Introduction ..... **v**
1 Scope **1**
2 Terms and definitions **1**
3 Confidentiality and ethical considerations **2**
4 Laboratory safety requirements **2**
4.1 Magnetic field 2
4.2 Electromagnetic frequency 3
4.3 Biohazards from samples 3
5 Collection/selection and identification of samples **3**
6 Transportation and storage of samples **3**
7 Preparation of samples **4**
8 Apparatus **5**
8.1 Principles of EPR spectroscopy 5
8.2 Requirements for EPR spectrometers 5
8.3 Requirements for the resonator 5
8.4 Measurements of the background signals 6
8.5 Spectrometer stability and monitoring/control of environmental conditions 6
8.6 Baseline drift 6
9 Measurements of the samples **7**
9.1 General principles 7
9.2 Choice and optimization of the measurement parameters 7
9.3 Sample positioning and loading 9
9.4 Microwave bridge tuning 9
9.5 Use of standard samples as field markers and amplitude monitors 9
9.6 Monitoring reproducibility 10
9.7 Procedure to measure anisotropic samples 10
9.8 Coding of spectra and samples 10
10 Determination of the absorbed dose in the samples **10**
10.1 Determination of the radiation-induced signal intensity 10
10.2 Conversion of the EPR signal into an estimate of absorbed dose 11
11 Measurement uncertainty **11**
12 Investigation of dose that has been questioned **12**
13 Quality assurance (QA) and quality control (QC) **13**
14 Minimum documentation requirements **14**
Bibliography **15**

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. 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. 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.

The committee responsible for this document is ISO/TC 85, *Nuclear energy, nuclear technologies, and radiological protection*, Subcommittee SC 2, *Radiological protection*.

ISO 13304 consists of the following parts, under the general title *Radiological protection — Minimum criteria for electron paramagnetic resonance (EPR) spectroscopy for retrospective dosimetry of ionizing radiation*:

— *Part 1: General principles*

Introduction

Electron paramagnetic resonance (EPR) has become an important approach for retrospective dosimetry in any situation where dosimetric information is potentially incomplete or unknown for an individual. It is now applied widely for retrospective evaluation of doses that were delivered at considerable times in the past (e.g. EPR dosimetry is one of the methods of choice for retrospective evaluation of doses to the involved populations from the atomic weapon exposures in Japan and after the Chernobyl accident) and has received attention for use for triage after an incident in which large numbers of people have potentially been exposed to clinically significant levels of radiation. Various materials may be analysed by EPR to provide information on dose. Thus, EPR is a versatile tool for retrospective dosimetry, pertinent as well for acute exposures (past or recent, whole or partial body) and prolonged exposures. Doses estimated with EPR were mainly used to correlate the biological effect of ionizing radiation to received dose, to validate other techniques or methodologies, to manage casualties, or for forensic expertise for judicial authorities. It uses mainly biological tissues of the person as the dosimeter and also can use materials from personal objects as well as those located in the immediate environment. EPR dosimetry is based on the fundamental properties of ionizing radiation: the generation of unpaired electron species (often but not exclusively free radicals) proportional to absorbed dose. The technique of EPR specifically and sensitively detects the amount of unpaired electrons that have sufficient stability to be observed after their generation; while the amount of the detectable unpaired electrons is usually directly proportional to the amount that was generated, these species can react, and therefore, the relationship between the intensity of the EPR signal and the radiation dose needs to be established for each type of use. The most extensive use of the technique has been with calcified tissue, especially with enamel from teeth. An IAEA technical report was published on the use for tooth enamel.[15] To extend the possibility of EPR retrospective dosimetry, new materials possibly suitable for EPR dosimetry are regularly studied and associated protocols established. This International Standard is aimed to make this technique more widely available, more easily applicable and useful for dosimetry. Specifically, this International Standard proposes a methodological frame and recommendations to set up, validate, and apply protocols from samples collection to dose reporting.

Radiological protection — Minimum criteria for electron paramagnetic resonance (EPR) spectroscopy for retrospective dosimetry of ionizing radiation —

Part 1: General principles

1 Scope

The primary purpose of this International Standard is to provide minimum acceptable criteria required to establish procedure of retrospective dosimetry by electron paramagnetic resonance spectroscopy and to report the results.

The second purpose is to facilitate the comparison of measurements related to absorbed dose estimation obtained in different laboratories.

This International Standard covers the determination of absorbed dose in the measured material. It does not cover the calculation of dose to organs or to the body. It covers measurements in both biological and inanimate samples, and specifically:

a) based on inanimate environmental materials, usually made at X-band microwave frequencies (8 GHz to 12 GHz);

b) *in vitro* tooth enamel using concentrated enamel in a sample tube, usually employing X-band frequency, but higher frequencies are also being considered;

c) *in vivo* tooth dosimetry, currently using L-band (1 GHz to 2 GHz), but higher frequencies are also being considered;

d) *in vitro* nail dosimetry using nail clippings measured principally at X-band, but higher frequencies are also being considered;

e) *in vivo* nail dosimetry with the measurements made at X-band on the intact finger or toe;

f) *in vitro* measurements of bone, usually employing X-band frequency, but higher frequencies are also being considered.

For the biological samples, the *in vitro* measurements are carried out in samples after their removal from the person and under laboratory conditions, whereas the measurements *in vivo* may take place under field conditions.

NOTE The dose referred to in this International Standard is the absorbed dose of ionizing radiation in the measured materials.

2 Terms and definitions

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

2.1
retrospective dosimetry (including early or emergency response)
dosimetry, usually at the level of the individual, carried out after an exposure using methods other than the conventional radiation dosimeters

2.2
electron paramagnetic resonance (EPR)
electron spin resonance (ESR)
magnetic resonance technique which is similar to nuclear magnetic resonance (NMR) but based on the net spin of unpaired electrons, such as free radicals and electron defects centers in matrices

Note 1 to entry: The terms EPR and ESR are essentially equivalent and are widely used. The term electron magnetic resonance (EMR) also sometimes is used because it is analogous to nuclear magnetic resonance (NMR).

2.3
radical/paramagnetic centre
species with unpaired electron(s)

Note 1 to entry: Paired electrons have the same quantum state except for opposite spins; unpaired electrons do not have a "partner" with the opposite spin. When the unpaired spin is on a molecule, it is usually termed a radical; when the unpaired electron is in a matrix, it often is termed a paramagnetic centre.

2.4
***in vivo* measurement**
measurement carried out within the living system, such as measurements of paramagnetic centres in teeth within the mouth

2.5
***in vitro* measurement**
measurement carried out on materials outside the organism

Note 1 to entry: The term *ex vivo* also has been used in the literature for sample measured *in vitro* but irradiated within the organism.

2.6
quality assurance
planned and systematic actions necessary to provide adequate confidence that a process, measurement, or service satisfies given requirements for quality

2.7
quality control
planned and systematic actions intended to verify that systems and components conform with predetermined requirements

3 Confidentiality and ethical considerations

All individual identifying information of persons who provided samples should not be attached to the information on the samples and kept only in a secured place. The corresponding samples should be identified by codes with indication only of parameters that are useful for scientific purposes and for making decisions. Data linking the code to the person can be kept if they are done so in a secure manner, with access limited to the persons in charge of the data.

Where appropriate, permission for obtaining and measuring the samples should be obtained under the rules of the jurisdiction where the samples are obtained.

4 Laboratory safety requirements

4.1 Magnetic field

With conventional spectrometers, the magnetic field (for signals with g-factor near 2,0, typically 350 mT for X-band and 1 200 mT for Q-band) is restricted to the region between the poles of the magnets, and therefore, there is no associated biological hazard (can affect watches or credit cards if brought very close to the gap).

The open nature of some *in vivo* EPR spectrometers (for signals with g-factor near 2,0, 40 mT for L-band) combined with large gaps between the poles has the potential to project the 0,5 mT line beyond the confines of the room. This line needs to be determined and appropriate shielding placed for areas that exceed this limit and that are accessed by the general public. The establishment of the 0,5 mT limit is based on concerns about potential effects on pacemakers, which are the only significant source of biohazards from the magnetic fields that are employed with EPR. The conventional limit is 0,5 mT (which is very conservative) and surveys should be made to confirm that this field is not exceeded where a person with a pacemaker could be positioned.

Effects of modulation fields on tissues or tooth restorations are not a significant hazard.

4.2 Electromagnetic frequency

4.2.1 *in vitro* measurement

The configurations used for *in vitro* measurements have no hazard for exposure of operators, as the spectrometer usually fully constrains the microwave to the sample with no significant amount distributed outside of the resonator.

4.2.2 *in vivo* measurement

Measurements *in vivo* have the potential hazard of local heating. The operative safety limit is that established for NMR in terms of permissible rates of energy absorption. In practice, this is a potential hazard only at high incident microwave power levels—typically > 1 W, which is at least a factor of 3 greater than that in existing instruments.

4.3 Biohazards from samples

Biological samples measured *in vitro* should be handled in conformance to the rules of the jurisdiction for routine practice for handling biological samples.

Measurements of teeth *in vivo* should follow the routines practiced for ordinary dentistry in regard to potential contamination from subjects to operators or other subjects.

5 Collection/selection and identification of samples

All samples should be collected in as uniform manner as possible and the circumstances of the collection noted, although this may not always be able to be controlled by the measuring laboratory. If prior coordination between the collecting and the measuring laboratories is possible, requirements about the sample collection, selection (of donors, location, or materials) and storage (sample holder, integrity of the sample and of the container, temperature, light, UV) should be given. If information about samples is available, keep record of them (this information can be about the location of the sample, origin or history of the sample, information about donor, etc.). All samples should have a unique identifying code associated with them.

6 Transportation and storage of samples

If sample collection is made in a place other than the measuring laboratory, then samples should be transported and stored under specified environmental conditions. These conditions should be coordinated between the collecting and the measuring laboratories. Conditions of transportation and storage of the sample may affect the integrity of the sample and also modify the quantity of paramagnetic species or the nature of the paramagnetic species in the samples. Environmental parameters such as light and other types of radiations (UV, X-rays, gamma), temperature, humidity, oxygen, sample conditionings in water or disinfectant solution, for example, contamination (e.g. dust), may significantly affect the nature and quantity of paramagnetic species in the samples. Therefore, specific attention should be taken as to the conditions of transportation and storage to avoid or limit as much as possible the influence of environmental parameters on the samples.

If possible, the influence of these parameters on the radiation-induced signal line shape and intensity should be investigated to establish the optimum conditions for transportation or storage and to avoid unnecessary precautions. When samples are known to be sensitive to one or several environmental conditions or the influence of these parameters or samples is not known, it is highly recommended that precautions are taken so as to avoid conditions that could affect the samples.

Transportation conditions, including dates, ways of transportation, and mode of control of transportation conditions, should be recorded. Appropriate sample packaging should always be used to prevent sample physical damage.

Procedures to avoid X-ray exposure of the sample during airport controls should be implemented. The dose at the X-ray hand luggage control is of the order of the microgray, so it can be considered negligible for some applications. If not, when the sample is transported in hand luggage, then authorization for X-ray exemption should be obtained in advance in order to avoid hindrance at the airport security controls. X-ray dose to the hold luggage can be higher. For shipping, appropriate labelling (including a note that the package contains radiation-sensitive dosimeters and, therefore, should not be irradiated) should be used. When this is not possible, unirradiated identical control samples or dosimeters should be placed in the package.

After the samples are received, they should be stored under stable conditions and the temperature and humidity should be monitored and recorded. Exposure to light should always be avoided.

7 Preparation of samples

Sample preparation should be performed according to an established and explicit protocol.

For *in vitro* and *ex vivo* measurements, sample preparation is usually needed to accomplish several goals, including: achieving a sample size that fits in the measurement tube; reducing anisotropy; ensuring disinfection; eliminating paramagnetic impurities from the sample; drying the sample; and stabilizing the EPR signals.

When required, preparation of the sample can be done by grinding, crushing, cutting, drilling, or other mechanical treatments. During these operations, sample overheating should be avoided using water irrigation or other cooling systems. Metal contamination of the sample can be avoided by using hard alloy tools.

As needed, sterilization, cleaning, deproteination, and/or delipidation are performed using chemical agents. Thermal treatment (annealing, freezing) can be used to accelerate or slow down recombination of the radicals. Samples with significant amounts of moisture can be dried before the EPR measurements to improve signal-to-noise ratio.

The setup of a protocol for sample preparation shall include the evaluation of the effect of the protocol on the EPR signals (lineshape and intensity) on the dose estimation, including whether it can induce EPR signals. When employing the additive dose method (see 10.2.1), it is very desirable to use protocols that do not affect the radiation sensitivity.

The protocol should be described in details in documents, including: the duration of treatment, quality of reagents, and the instrumentation used and its performance. All samples should be prepared following the same protocol. Samples used for calibration have to be treated according to the same protocol as the samples to be measured.

Any modification to the protocol should be noted and the influence of each modification evaluated (e.g. power or frequency of ultrasonic bath, reagent quality).

All details of the procedures for each sample shall be recorded in a log of the history of the sample.

For measurements *in vivo,* there are no requirements for preparation of the samples. Depending on the site that is measured, there may be a need to minimize moisture (especially when making measurements *in vivo* in teeth) or to carry out some cleaning procedures (e.g. removing obvious particulate matter from nails). Because of the limited ability to control environmental conditions fully when making measurements *in*

vivo, it is highly desirable to always utilize a standard sample that is in place and with a known relationship to the sample volume so that factors that affect the measurements (especially factors that affect the quality factor of the resonator) can be detected and accounted for in the processing of the data.

8 Apparatus

8.1 Principles of EPR spectroscopy

EPR is a technique that specifically and sensitively detects unpaired electrons. It is based on the resonant absorption of electromagnetic energy for transitions between electron spin states. A static magnetic field is applied that induces net absorption from transitions between spin states if there is a vacant level to which the spin can flip. In a magnetic field, the different spin states result in different energy levels, with the difference in the energy being proportional to the magnetic field. A transition between these two levels can be induced by an appropriate electromagnetic field.

Currently, continuous wave (CW) EPR spectroscopy is usually used for EPR dosimetry. In an EPR CW spectrometer, the resonance frequency is applied to a resonant structure and absorption of the electromagnetic waves by a sample in the resonator is detected. Typically, the resonant condition is reached by continuously changing the main magnetic field, while a fixed frequency is applied to the resonator. As a result, an EPR spectrum of absorption versus magnetic field intensity is obtained. Other methods of EPR signal detection such as pulsed EPR, fast scan EPR spectroscopy, etc. are potentially available, but to date, these have not been shown to be more effective for dosimetry application than CW EPR. So, considerations on EPR dosimetry in this International Standard are restricted to CW EPR, although most of the guidelines would be applicable to other types of EPR spectroscopy.

To improve the signal-to-noise ratio, modern EPR CW spectrometers employ high-frequency magnetic field modulation in combination with phase-sensitive detection. As a result, the original spectral line is produced not in the form of an absorption curve, but in the form of its first derivative. In modern spectrometers, the EPR signal is recorded in digital form using a dedicated computer. In most spectrometers, the computer also is used to control operation of the spectrometer, e.g. for setting measurement parameters, tuning the resonator, acquiring the signal, saving the recorded spectrum to disk, and preliminary spectra processing (such as digital filtering, baseline correction, etc.).

Depending on the magnetic field intensity and, respectively, the resonance frequency, the following band frequencies are commonly used for EPR dosimetry.

— X-band usually is used for EPR *in vitro* dosimetry because of a good compromise between sensitivity, sample size, and sensitivity to the presence of water.

— L-band is used mainly for *in vivo* tooth dosimetry because of the relatively low amount of non-resonant absorption of the microwaves due to the presence of water in biological tissues. Q-band is mainly used in research connected with investigation of spectroscopic properties of materials suitable for EPR dosimetry and has potential for being utilized for *in vitro* dosimetry. An advantage of Q-band is that only a small sample mass is required for measurements and spectral components can be better resolved in comparison with lower frequencies. On the other hand, such spectrometers are not widely available, often are more complex to use, and may have a lower signal-to-noise ratio.

8.2 Requirements for EPR spectrometers

As EPR dosimetry often deals with small sample masses and low intensity signals, the sensitivity and stability of the instruments are critical. Sensitivity and stability may be optimized by proper choice of instrumental factors (such as selection of resonator, its tuning, and minimization of the microphonic effects) and selection of the measurement parameters.

8.3 Requirements for the resonator

There are a number of different available designs in resonators, and therefore, it is important to choose the one that is optimal for the particular type of materials used for dosimetry. Critical aspects include