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

Part 2: Ex vivo human tooth enamel dosimetry

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

Partie 2: Dosimétrie ex vivo à partir de l'émail dentaire humain

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

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Foreword

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

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This document was prepared by Technical Committee ISO/TC 85, *Nuclear energy, nuclear technologies, and radiological protection*, Subcommittee SC 2, *Radiological protection*.

A list of all parts in the ISO 13304 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

Electron paramagnetic resonance (EPR) or electron spin resonance (ESR) is an approach for retrospective dosimetry of exposure to ionizing radiation in any situation where dosimetric information is potentially incomplete or unknown for an individual. EPR is a tool for retrospective evaluation of doses, pertinent as well for acute and protracted exposures in the past or recently. Doses estimated with EPR were used to correlate the biological effect of ionizing radiation to received dose, to validate other dosimetry techniques or methodologies, to manage casualties, or for forensic expertise for judicial authorities.

EPR dosimetry is based on the fundamental properties of ionizing radiation: the generation of unpaired electron species (e.g., radicals) proportional to absorbed dose. The technique of EPR specifically and sensitively detects the unpaired electrons that have sufficient stability to be observed after their generation. The amount of the detectable unpaired electrons is proportional to the total amount that were generated, and hence to the absorbed dose. These species can interact with microwaves generating the EPR signal, and therefore the relationship between the intensity of the EPR signal and the radiation dose should be established.

The most extensive use of EPR in retrospective dosimetry has been with calcified tissue, especially with enamel from teeth. 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, after the Chernobyl accident and radioactive releases of the Mayak facilities in the Southern Urals.

This document provides a guideline to perform the ex vivo measurements of human tooth enamel samples by X-band EPR for dose assessment using documented and validated procedures. The minimum requirements for reconstructing the absorbed dose in enamel, by defining precisely the technical aspects of preparing enamel samples, recording EPR spectra, assessment of radiation induced EPR signal, converting EPR yield to dose and performing proficiency tests, are described. Retrospective dose assessment using EPR has relevance in radiation effect research, validating radio-epidemiological dosimetry systems, medical management, and medical/legal requirements.

A part of the information in this document is contained in other international guidelines and scientific publications, primarily in the International Atomic Energy Agency's (IAEA) technical reports series on "Use of electron paramagnetic resonance dosimetry with tooth enamel for retrospective dose assessment"[1]. However, this document expands and standardizes the measurement and dose reconstruction procedures and the evaluation of performance.

This document is compliant with ISO 13304-1[2] with particular consideration given to the specific needs of X-band EPR dosimetry using human tooth enamel.

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

Part 2: Ex vivo human tooth enamel dosimetry

1 Scope

The purpose of this document is to provide minimum criteria required for quality assurance and quality control, evaluation of the performance and to facilitate the comparison of measurements related to absorbed dose estimation obtained in different laboratories applying ex vivo X-band EPR spectroscopy with human tooth enamel.

This document covers the determination of absorbed dose in tooth enamel (hydroxyapatite). It does not cover the calculation of dose to organs or to the body.

This document addresses:

a) responsibilities of the customer and laboratory;

b) confidentiality and ethical considerations;

c) laboratory safety requirements;

d) the measurement apparatus;

e) preparation of samples;

f) measurement of samples and EPR signal evaluation;

g) calibration of EPR dose response;

h) dose uncertainty and performance test;

i) quality assurance and control.

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/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

3 Terms and definitions

For the purposes of this document, the following terms and definitions 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/

NOTE Definitions of terms used in this document that pertain to radiation measurement and dosimetry are compatible with ICRU 60[3].

3.1
air kerma
K_a
sum of the initial kinetic energies of all the charged particles liberated by uncharged ionizing radiation per unit mass of air

Note 1 to entry: This quantity is recommended for calibrating the reference photon radiation fields and reference instruments[4].

Note 2 to entry: The unit of the air kerma is given in gray (Gy), which is equal to 1 J/kg.

3.2
absorbed dose
D
quantity of ionizing radiation energy imparted per unit mass of a specific material

Note 1 to entry: The unit of the absorbed dose is given in gray (Gy), which is equal to 1 J/kg.

3.3
background signal
BGS
signal in the EPR spectrum not generated by ionizing radiation

Note 1 to entry: The background signal (BGS) is not equivalent to the signal component of the *radiation induced signal (RIS)* (3.25), which is generated by environmental background radiation.

3.4
bias
deviation of results or interferences from the true value and the estimator

3.5
calibration curve
mathematical description of the dose response relation derived by the in vitro irradiation (3.16) of tooth enamel samples to known doses

3.6
confidence interval
range within which the true value of a statistical quantity lies, given a value of the probability

3.7
decision threshold
critical value of a measurand quantifying absorbed dose (3.2) in a sample above which exposure can be identified

3.8
detection limit
smallest true value of a measurand quantifying absorbed dose (3.2) in a sample above which irradiation can be identified with given probability

3.9
electron paramagnetic resonance
EPR
electron spin resonance
ESR
magnetic resonance technique detecting the net spin (magnetic moment) of unpaired electrons of paramagnetic centres (3.22) in matter

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

3.10
EPR peak-to-peak line width
ΔB_{pp}
difference in the applied magnetic field values between the minimum and the maximum of the first derivative of a single EPR signal

3.11
EPR signal
first derivative of the electron paramagnetic resonant microwave absorption of a specific paramagnetic centre (3.22) measured as function of the applied magnetic field

Note 1 to entry: The area under the absorption curve is proportional to the amount of unpaired spins of the paramagnetic centre. Hence, the amount of spins is proportional to the double integral of the EPR signal (EPR signal intensity) or the product of EPR signal amplitude and the square of the EPR peak-to-peak line width.

3.12
EPR signal amplitude
A
peak-to-peak amplitude of the EPR signal (3.11)

3.13
EPR signal intensity
I
quantity proportional to the amount of paramagnetic centres that generated the EPR signal (3.11)

Note 1 to entry: The signal intensity can be evaluated by numerical double integration of the EPR signal by the extension of the signal along the magnetic field. The signal intensity of a specific paramagnetic centre can also be evaluated by comparing with a reference spectrum of the specific centre using least square method. The reference spectrum may result from measurement of a sample including the specific paramagnetic centre or by mathematical simulation of the spectrum.

3.14
EPR spectrometer
apparatus to measure the resonant absorption of electromagnetic energy (microwaves) resulting from the transition of the spin of unpaired electrons between different energy levels, upon application of microwave-frequencies to a paramagnetic substance in the presence of a magnetic field

3.15
EPR spectrum fitting
linear least squares curve fitting of an EPR spectrum using a set of reference EPR spectra of specific paramagnetic centres

3.16
in vitro irradiation/measurement
irradiation/measurement carried out on tooth enamel samples outside the human body

Note 1 to entry: The term ex vivo dosimetry refers to samples measured in vitro but were irradiated within the human body.

3.17
linear energy transfer
LET
d*E*/d*l*
quotient of d*E*/d*l*, as defined by the International Commission on Radiation Units and Measurements (ICRU), where dE is the average energy locally imparted to the medium by a charged particle of specific energy in traversing a distance of d*l*

3.18
magnetic field
B
magnetic flux density (induction)

Note 1 to entry: SI unit Tesla (T) replaced the Gauss (G). 1 T = 10 000 G.

3.19
microwave bridge
apparatus to generate microwaves that are provided to the microwave resonator and to detect microwaves that were reflected at the resonator

3.20
microwave resonator
resonator for electromagnetic waves consisting of a metal box with appropriate dimensions that confines the electromagnetic fields in the microwave range and allows formation of standing waves

Note 1 to entry: For EPR measurement the sample is located inside of the microwave resonator. The term microwave cavity is equivalent to microwave resonator.

3.21
microwave resonator working volume
volume inside the resonator extending along the vertical resonator axis around the centre, within which the local sensitivity does not decrease more than 25 % relative to the maximal sensitivity at the centre

3.22
paramagnetic centre
species with unpaired electron(s)

Note 1 to entry: Paired electrons have the same quantum state but opposite spin orientation; unpaired electrons do not have a "partner" with the opposite spin. When the unpaired spin is on a molecule, it is termed a radical; when the unpaired electron is in a solid, it is termed electron or electron defect (hole) centre.

3.23
quality assurance
planned and systematic actions necessary to provide adequate confidence that a process, measurement, or service satisfies given requirements for quality

3.24
quality control
planned and systematic actions intended to verify that systems and components conform with predetermined requirements

3.25
radiation induced signal
RIS
EPR signal (3.11) resulting from paramagnetic centres (3.22) generated by ionizing radiation

3.26
reference spectrum
unit EPR spectrum of a specific paramagnetic centre (3.22) used to evaluate the intensity of the EPR spectrum of this centre in a sample under investigation

Note 1 to entry: The unit spectrum is reconstructed from EPR measurement of a sample containing the specific paramagnetic centre or by mathematical simulation.

3.27
retrospective dosimetry
dosimetry to assess dose coming from past exposures

3.28
standard sample
sample used to verify the performance stability of the EPR spectrometer

Note 1 to entry: The EPR signal of the standard sample shall be stable to allow reproducible measurements over extended periods.

3.29
tooth enamel calibration samples
tooth enamel powder samples prepared from whole teeth exposed in vitro to defined absorbed doses (3.2) or from unexposed teeth with in vitro exposure of the powder to calibrate the RIS dose response

4 Apparatus

4.1 Specifications for EPR spectrometer

The specifications of the apparatus provided by the manufacturer include

a) sensitivity,

b) range of frequency and power of the applicable microwaves,

c) range and stability, scan range and spatial homogeneity of the applicable magnetic field,

d) magnetic field modulation amplitude and frequency, and

e) unloaded quality factor (*Q* value) of the microwave resonator.

4.2 Spectrometer sensitivity

Commercial X-band EPR spectrometers have typically the sensitivity (indicated by the minimum detectable spin number/signal half-width) of less than 1×10^{14} spins/T[5]. This corresponds to the amount of CO_2^{-}-radicals generated in 100 mg of tooth enamel by absorbed radiation dose of less than 1 mGy[6].

4.3 Microwave bridge

The frequencies of microwaves provided by X-band microwave bridges from different suppliers are in the range of 9 GHz to10 GHz depending on the types of attached microwave resonators. A microwave bridge equipped with an auto frequency control (AFC) is recommended. The maximal power provided by microwave bridges lies typically in the range of 100 mW to 200 mW. For EPR measurement of tooth enamel, the microwave bridge should be able to provide microwave power from 0,5 mW to 25 mW[7].

4.4 Magnetic field

For measurement of tooth enamel, the static magnetic field (centre field) should be set to a value that is equivalent to a Landé factor of g = 2,00 (350 mT at microwave frequency of 9,8 GHz). Typical values for the magnetic field scan range from 5 mT to 10 mT[1][7].

The resolution of applied magnetic field, its stability over time and homogeneity over sample volume, determine the maximal degree of the EPR signal distortion (variation of signal line width). With up-to-date EPR spectrometers, values for field resolution, stability per hour and homogeneity over sample volume are all better than 5 µT. Hence, an EPR line with width of 0,5 mT, as e.g., the $g_{\perp}$ EPR signal component of the CO_2^{-}-radical in tooth enamel can be recorded with distortion of less than 1 % for several measurements within one hour.

EPR spectrometers exist with maximal values of the field modulation frequency of 50 kHz or 100 kHz. For measurement of tooth enamel, maximal available modulation frequency should be used with typical values of the field modulation amplitudes in the range of 0,15 mT to 0,5 mT[1][7].

4.5 Microwave resonator

A microwave resonator is characterised by its resonance frequency and the unloaded quality factor, Q, (2π·stored/lost magnetic energy), which contributes linearly to the spectrometer sensitivity. For measurement of tooth enamel, typical unloaded Q values of resonators are in the range 2 000 to 10 000[8].

The coupling of microwave power to the resonator shall be tuned before the start of each measurement.

NOTE High Q resonators containing dielectric materials can result in additional intrinsic signals. Detrimental effects of the additional signals on the RIS can be reduced by subtracting a measured empty tube spectrum from the sample spectrum prior to dose evaluation.

5 Preparation of tooth enamel samples

5.1 General

For dosimetry, tooth enamel should be prepared as powder samples. The same preparation conditions shall be used for analyzing samples in case of suspected in vivo exposure as for samples with in vitro exposure used for establishing a calibration curve.

The exact protocol for preparing tooth enamel powder samples shall be established by each laboratory considering the following aspects as listed below[1]:

a) Teeth should have been sterilized after extraction (see 13.5), to avoid infection of the operator.

b) Before cutting the crown, fat adhesion should be removed (e.g., with acetone) and, if dry teeth are used, they should be soaked in deionized water for at least one day to soften the dentine.

c) All cutting and drilling should be done with low speed and/or water cooling to avoid overheating, which can generate additional EPR signals.

d) Cutting-off the root with dental rotating saw blade and removing diseased (dark) parts from the crown surface by dental drill. Dark parts can have additional EPR signals.

e) Optional washing of the crown (e.g., with 0,1 mol/l Na_2-EDTA solution) to remove potential metal contamination on the crown surface.

f) Dentine shall be removed, by drilling or optionally by its softening and denaturation by treatment in ultrasonic cleaner with aqueous alkaline solution (5 mol/l to 10 mol/l NaOH or 2 mol/l KOH), followed by further removal with a drill. Residual dentine reduces EPR measurement accuracy.

g) Enamel fragments should be powdered by mortar and pestle to reduce EPR spectra anisotropy.

h) Optional etching of enamel grains, e.g., acetic acid with 20 % (volume fraction) to remove potential surface defects generated by grinding.

i) In order to remove water from the samples, grains should be washed with ethanol prior to drying. Residual water in the sample reduces sensitivity of EPR measurement.

j) Selecting samples with defined range of grain size by sieving, (see 5.2).

k) Storage of samples in sample containers in darkness at room temperature to avoid UV generated EPR signals.

l) After irradiation and before first EPR measurement samples shall be stored at room temperature for 15 days, or at 60 °C for 10 h, or at (90 to 95) °C for 2 h in order to eliminate transient EPR signals induced during irradiation[1][9].

NOTE Treatment of the tooth crown with aqueous alkaline solution improves visibility of the enamel-to-dentine interface and facilitates dentine removal.

