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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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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.

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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.

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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

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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/

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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).

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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*

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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

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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].

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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.

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5.2 Applicable grain size

Enamel powder samples are typically prepared with grain size in the range of 0,1 mm to 1 mm.

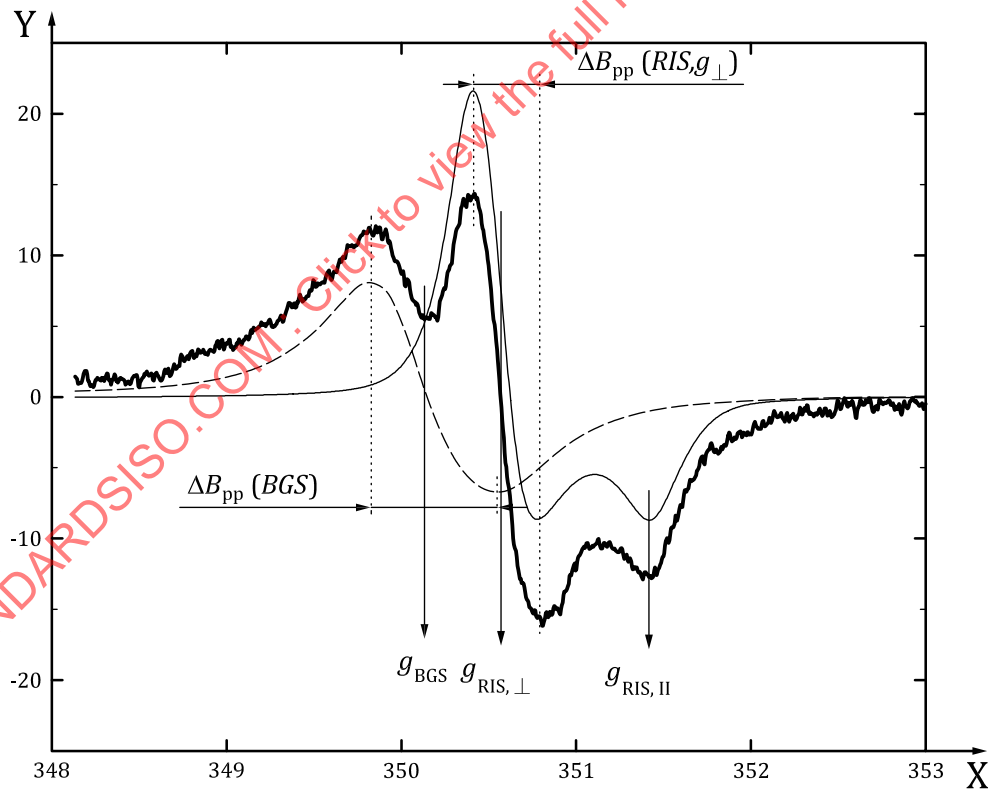
The grain size should be larger than 0,2 mm if the additive dose method is used for dose calibration or the calibration curve is reconstructed by in vitro irradiation of powder samples (see Clause 8).

NOTE The typically used range of grain size was considered as a compromise between decreasing isotropy and increasing intensity of the CO_2^- EPR signal with increasing grain size[1]. The distribution of grain size is kept constant in order to minimize variation in packing density of the enamel powder and hence in the EPR signal intensity.

6 Measurement of the EPR spectrum

6.1 Description of spectrum

The EPR spectrum of irradiated tooth enamel consists, in a first approximation, of two principal components – the background signal (BGS) and the radiation induced signal (RIS). These signals are overlapping (see Figure 1). The asymmetric RIS with $g_\perp$ = 2,001 8 and $g_{||}$ = 1,997 1 (signal maximum at g = 2,003 2 and minimum at g = 1,997 1) is derived from stable CO_2^- radicals. The peak-to-peak line width, ΔB_{pp} (RIS,$g_\perp$), of the $g_\perp$ signal component is 0,5 mT. The peak-to-peak line width, ΔB_{pp}(BGS), of the BGS at g = 2,004 6 is 0,9 mT. The intensity of RIS is proportional to absorbed radiation dose and is used for dose determination.



Key

X magnetic field, B [mT]

Y EPR absorption/dB, [a.u.]

Figure 1 — Example of an EPR spectrum of an enamel powder sample (grain size 0,1 mm to 0,6 mm) with absorbed dose in enamel of 1 Gy (bold line). The shown components of the BGS (dashed line) and the RIS (solid line) are simulated powder spectra[10]

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6.2 Applicable measurement parameters and conditions

6.2.1 General

EPR measurements shall be performed at room temperature. The selected values of the measurement parameters and conditions listed below shall be kept fixed for all measurements used for analyzing samples in case of suspected in vivo exposure as for samples with in vitro exposure used for EPR signal-to-absorbed dose calibration.

6.2.2 Microwave power

The EPR signal amplitude A increases with supplied microwave power P to the microwave resonator, which can be approximated by Formula (1), with proportionality factor c – depending on spectrometer and measurement parameters –, signal broadening parameter b – ranging from 2 for homogeneously to 1 for inhomogeneously broadened EPR signals – and microwave saturation power P_{sat} – depending on sample properties and microwave resonator quality factor Q[11].

$$A = c \cdot P^{1/2}/(1+P/P_{sat})^{b/2} \quad (1)$$

The actual values of the microwave saturation power P_{sat} of the RIS and BGS for a specific combination of sample preparation procedure and type of microwave resonator shall be determined by least-square fitting with Formula (1) of measured EPR signal-to-microwave power response curves from exposed (>1 Gy) and unexposed samples.

In dosimetry with tooth enamel, applied values of incident microwave power are generally clearly higher than saturation power for the BGS and slightly higher than the saturation power of the RIS seeking to minimize the BGS signal contribution and maximize the RIS contribution with best signal reproducibility. The optimal microwave power can be approximated as double the power at maximal BGS intensity[8].

Typical values of applied microwave power are 2 mW for high-Q cavities and 10 mW to 25 mW for other cavity types[7].

6.2.3 Magnetic centre field

The value of magnetic centre field (CF) represents the centre of the magnetic field sweep. For measurement of tooth enamel it shall coincide with the field of resonance of the $g_{\perp}$ = 2,001 8 component of the RIS and can be determined from the microwave frequency ν using Formula (2).

$$CF\ [\text{mT}] = 71{,}45 \cdot \nu\ [\text{GHz}]\ 2{,}002 \quad (2)$$

NOTE The magnetic centre field is 350 mT at microwave frequency of 9,8 GHz.

6.2.4 Magnetic field sweep width

The field sweep width (SW) shall include at least the entire tooth enamel EPR spectrum covering 5 mT.

If a Mn^{2+}:MnO standard sample is recorded simultaneously with a tooth enamel sample, SW is typically 10 mT covering the 3rd and 4th EPR lines of the Mn^{2+} spectrum.

NOTE The SW value set in the spectrometer control results with most spectrometers in a sweep of ±SW/2 around the centre field CF.

6.2.5 Magnetic field sweep time

An EPR signal is recorded with negligible distortion if the time needed to pass the peak-to-peak line width is at least 10 times longer than the time constant (TC) of the signal channel receiver low-pass filter (see 6.2.6).

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The minimal field sweep time (*ST*) for measurement of tooth enamel can be determined from selected field sweep width *SW* (see 6.2.4) and the signal channel time constant *TC* and the 0,5 mT peak-to-peak line width of the $g_{\perp}$ component of the RIS using Formula (3).

$$ST\,[\mathrm{s}] = TC\,[\mathrm{ms}]\cdot SW\,[\mathrm{mT}]/50 \tag{3}$$

Typical *ST* values are in the range of 20 s to 80 s[12].

6.2.6 Time constant of signal channel receiver

The time constant (*TC*) of the low-pass filter from the signal channel receiver reduces random (white) noise in the measurement signal. The choice of TC shall be compatible with the peak-to-peak line width of the recorded EPR signal and the choice of magnetic field sweep width (*SW*) and time (*ST*) (see 6.2.5).

Typical values for *TC* are in the range of 20 ms to 700 ms[12].

6.2.7 EPR spectrum resolution

Resolution of the EPR spectrum is given by magnetic field sweep width divided by the number of channels of the analog-to-digital converter (ADC). When 1 024 channels are used, the resulting resolution is 10 µT and 5 µT for sweep width of 10 mT and 5 mT, respectively.

NOTE The resulting spectrum resolution ensures negligible distortion of the EPR signals from tooth enamel if the resolution is better than approximately, 170 µT, one third of the 0,5 mT wide $g_{\perp}$ EPR signal component of the CO_2^-radical.

6.2.8 Conversion time of spectrum acquisition

The conversion time (*CT*) is the duration of signal acquisition for each channel of the ADC. This parameter is related with the magnetic field sweep time *ST* (see 6.2.5), which is a product of the number of channels of the ADC (see 6.2.5) and *CT*.

Typical *CT* values are in the range of 20 ms to 160 ms[1].

NOTE For some spectrometer types, *CT* is set as an independent parameter and sweep time *ST* results by multiplication with the number of ADC channels, at others *ST* is set as an independent parameter and *CT* results by division with the number of channels.

6.2.9 Magnetic field modulation amplitude

The amplitude of an EPR signal increases with the applied magnetic field modulation amplitude (*MA*). The maximal signal amplitude exists if *MA* is approximately twice the EPR signal peak-to-peak line width, but results in the approximately doubling of the line width. Negligible line broadening exists if modulation amplitude is less than one third of the peak-to-peak line width.

For dosimetry with tooth enamel, *MA* values shall not exceed the 0,5 mT peak-to-peak line width of the $g_{\perp}$ component of the RIS.

Typical *MA* values are in the range of 0,15 mT to 0,5 mT[7].

6.2.10 Number of spectrum accumulations

Signal averaging by multiple spectrum accumulation with number N of magnetic field scans reduces signal-to-noise ratio from high-frequency (white) noise proportional to $\sqrt{N}$, and additionally reduces the spectrum base line drift (low-frequency noise). The number of scans can be chosen independent of other spectrum recording parameters.

Typical numbers of scan accumulations are in the range of 20 to 120[12].

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6.2.11 Sample positioning and loading

Samples shall be measured inside of identical sample tubes made of suprasil or fused quartz glass typically with inner diameter in the range of 3 mm to 5 mm. Suprasil quartz has fewer paramagnetic impurities.

Most typical average sample mass is 100 mg. Sample mass shall range from 40 mg to 200 mg. For doses higher than 10 Gy, the sample mass can be lower.

Reproducible positioning of the sample tube in the microwave resonator is required for obtaining the best measurement reproducibility. Reproducible depth of insertion of the sample tube in the resonator can be achieved with the help of a pedestal-stick mounted from the bottom of the resonator or a mounting clip at the tube.

The insertion depth of the sample tube in the resonator shall be selected such that the centre of a sample with average sample mass coincides with the centre of the resonator in order to achieve maximal EPR signal intensity.

6.2.12 Dependence of EPR signal intensity on sample mass

The EPR signal intensity increases non-linearly with sample mass[1]. The EPR signal intensity shall be normalized by mass for use in dosimetry.

Mass normalization can be approximated linearly (division by sample mass) with deviation to linearity of less than approximately 6 % if sample volume does not exceed the working volume of the resonator (see NOTE in 6.2.12)[13]. Furthermore, linear mass normalization is appropriate if approximately same sample mass with only small variation as, e.g., (100 ± 10) mg is used for all measurements.

The exact non-linear relation $s(m)$ between EPR signal intensity (s) and sample mass (m) shall be determined for the actual resonator and sample tube in use from measurements of an exposed (>1 Gy) tooth enamel sample with increasing sample load of the glass tube[13].

Non-linear correction for linear mass normalization can be achieved by multiplying the linear normalized signal intensity by a correction factor $s(m_{ref})/s(m)$, with the signal intensity $s(m_{ref})$ at a reference mass m_{ref} of, e.g. 100 mg.

Caution is needed that the determined non-linear relation between EPR signal intensity and sample mass is valid only for the specific inner diameter of a type of sample tubes. For other diameters, specific relations shall be determined.

NOTE The microwave resonator working volume is localized in the centre of the resonator and extends for standard X-band resonators typically approximately ±5 mm from the centre along its vertical and the sample tube axis[1]. In rectangular resonators, the EPR sensitivity along the vertical axis decreases proportionally to the square of the cosine. It is approximately 75 % of the maximal sensitivity at the borders of the working volume and 25 % at 10 mm from the centre. The extension of the working volume inside of dielectric high-Q resonators can be smaller[14].

6.2.13 Use of standard samples

Standard samples are used as a magnetic field marker and monitor of the EPR spectrometer sensitivity in order to correct magnetic field position and intensity of measured EPR signals.

Samples containing Mn^{2+}:MnO in CaO or MgO, or Cr^{3+}:Al_2O_3, or pitch samples can be used as field markers and sensitivity monitors.

The use of a field marker sample replaces the need for independent, more precise measurement of the microwave frequency and magnetic field position with external devices. The magnetic field position of a marker signal can be used in spectrum processing as reference for the field positions of RIS and BGS.

A sensitivity monitor sample is used to correct for day-to-day sensitivity variations of the spectrometer. The frequency of measuring a monitor sample depends on the actual stability of the spectrometer and

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environmental conditions. The sensitivity monitor sample can be measured simultaneously with each enamel sample, or prior to and after each sample. The sensitivity monitor shall be measured at least twice per day, i.e., prior to and after the series of measurements for the day. Sensitivity corrections (normalization by monitor intensity) for measurements are recommended if observed variations in the standard sample intensity are larger than repeatability of its measurements.

NOTE If standard samples are placed within the cavity and are measured simultaneously with the investigated samples, they are called internal standard samples. External standard samples are measured prior to/after the investigated samples at the same position inside of the resonator.

6.2.14 Number of measurement repetitions

The uncertainty of the RIS intensity of an enamel powder sample due to the anisotropic EPR response of the individual grains shall be reduced by averaging repeated measurements performed after shaking of the sample in the sample tube or turning the sample tube in the resonator.

Recommended are at least three repeated measurements, with shaking of the sample twice or turning the sample tube twice by 120°. In case of large grains (approaching 1 mm) more repeated measurements may be necessary to average the signal anisotropy.

NOTE Tooth enamel is crystalline, and EPR signal position and intensity depend on the orientation of the external magnetic field relative to the crystal axis. This anisotropy is widely averaged out by measuring powder samples containing numerous randomly oriented small crystals. Remaining anisotropy of the powder samples can be further averaged by repeated measurements after rearrangement of the powder grains in the sample tube or turning the sample tube in the resonator. Subsequently, either the spectra can be summed after their g-factor normalization and baseline correction or the results of RIS intensity from each measurement can be averaged.

7 Assessment of the RIS intensity

7.1 General

The RIS intensity of tooth enamel is evaluated as multiple, I, to a RIS reference spectrum or the peak-to-peak amplitude, A, of the $g_{\perp}$-component of the RIS.

The peak-to-peak amplitude A might be measured directly at the measured EPR spectrum from an enamel sample when absorbed dose was larger than approximately 500 mGy[1][8].

In general, evaluation of RIS intensity or amplitude requires mathematical processing of the spectra in order to consider interferences with the BGS[1].

For spectrum processing, the magnetic field positions of all evolved EPR spectra shall be aligned on basis of measurements of field marker samples, or microwave frequency and magnetic field.

Spectra processing protocols for assessment of the RIS intensity may include either:

— **EPR spectrum fitting**. Assessment of the RIS and BGS contributions in the EPR spectrum under analysis by fitting with reference spectra for RIS and BGS, which were reconstructed from analytical or numerical functions, or measured spectra. The intensity of the RIS from the analysed EPR spectrum results from the fitting routine as multiple of the RIS reference spectrum[10][15] to[20].

or

— **EPR spectrum subtraction**. Subtraction of a reference BGS signal reconstructed from measurements of unexposed enamel samples from the EPR spectrum under analysis. The intensity of the RIS is evaluated as amplitude A of the RIS from the difference spectrum[1].

The exact protocol for assessment of the RIS intensity shall be established by each laboratory considering the following critical aspects of spectrum fitting and spectrum subtraction as listed below:

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Critical aspects of spectrum fitting:

a) Fitting can be performed by minimizing the squared difference between experimental and simulated spectra using the linear or non-linear least-square method.

b) With application of the linear least-square method, the amplitude parameter of components (reference spectra) is varied for fitting.

c) With application of the non-linear least-square method, the amplitude parameter of the components, as well the parameters magnetic field shift and EPR line width, can be set as variable for fitting. In this case, temporary magnetic field fluctuations, which may have occurred during spectrum recording, can be considered.

d) The simulated spectrum is composed of at least two components describing the RIS and BGS. Further components might be included for improved description of background signals.

e) In the fitting routine, spectra components are used in numerical form, which can be realized from component presentations in analytical or numerical form.

f) Spectra of components of the BGS and RIS in numerical form can be obtained from measured spectra of unexposed enamel samples and highly exposed (>10 Gy) samples, respectively.

g) Spectra of components of the BGS and RIS in numerical form can be obtained from simulation software for EPR powder spectra using theoretical or empirically evaluated parameters for g-values, EPR line width, and shape of the RIS and BGS.

h) Descriptions of RIS and BGS in analytical form are the sum of 1st derivatives of Gaussian and/or Lorentzian functions with theoretical or empirically evaluated parameters for g-values, EPR line width and shape of the RIS and BGS.

Critical aspects of spectrum subtraction:

— The BGS signal used for subtraction shall be of average intensity, line width and shape for the samples under investigation.

— An average RIS can be obtained from measurement of mixed enamel powder prepared from at least three teeth or as average spectrum from individual measurements of samples from at least three teeth.

— With spectrum subtraction, the intensity of the average BGS might be manually adjusted in order to achieve most flat baseline beside the RIS.

7.2 Intrinsic EPR signals from microwave resonator and sample tube

The EPR spectrum of the empty sample tube may contain stable signals due to paramagnetic centres from impurities in the resonator or the material of the sample tube and variable pulses due to microphonic noise.

The stable component of the empty tube EPR spectrum can be controlled and/or assessed as the average spectrum from several measurements during a day or even within several days.

If stable EPR signals exist at the position of the RIS, the empty tube spectrum shall be considered in the spectrum processing protocol.

NOTE Microphonic noise can result from mechanical vibrations, sonic waves or ventilation at the location of the microwave resonator.

8 Irradiation of tooth enamel calibration samples for low linear energy transfer (LET) exposure

This applies to the calibration of the RIS dose response for the exposure of teeth with photons.

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The samples shall be irradiated as whole teeth or enamel powder with grain size larger than 0,2 mm.

The irradiation may be performed with a calibrated radiation source with ^{60}Co or ^{137}Cs isotopes. The use of X-ray sources with acceleration voltage below 300 kV is not recommended. High MV X-rays can be used for calibration.

The applied calibration dose values shall be in units of absorbed dose in enamel (hydroxyapatite).

The calibration dose in other units delivered by the calibration source shall be converted to the unit of absorbed dose in enamel. The dose conversion coefficients are dependent on photon energy (ISO/ASTM 51261)[21].

At irradiation, secondary electrons equilibrium should be assured. To insure electronic equilibrium for irradiation in air with gamma-rays from ^{60}Co or ^{137}Cs isotope sources, it is recommended to place samples in PMMA or Aluminum containers with wall thickness of 5 mm or 2,5 mm, respectively[1][22].

With use of ^{60}Co or ^{137}Cs isotopes, the calibration dose in units of air kerma delivered by the calibration source shall be converted to the unit of absorbed dose in enamel with the dose conversion factor 0,993[1][7][22].

NOTE When grain size of enamel powder was larger than 0,2 mm, no significant difference was observed between the RIS response from irradiated powder and powder prepared from irradiated whole teeth[9][22][23].

9 Conversion of the RIS intensity into an estimate of absorbed dose

A linear relation, see Formula (4), between the RIS intensity, I_{RIS}, from tooth enamel and the absorbed dose D in enamel is assumed for the estimation of absorbed dose, with the dose calibration factor c and an EPR signal bias b. The assumption of linear EPR signal to dose relation is valid up to 100 Gy with deviation of less than 0,5 %[24]. The EPR signal bias b can result from unknown exposures from natural radiation and medical applications and/or can be caused by the spectrum processing procedure due to incomplete elimination of the BGS contribution. Values of the signal bias are typically equivalent to dose values from approximately 20 mGy to 300 mGy[25].

$$I_{RIS} = b + c \cdot D \tag{4}$$

For evaluation of absorbed dose in enamel of a sample, the signal intensity, I_{RIS}, shall be normalized by sample mass (see 6.2.12) and an amplitude monitor (see 6.2.13).

The absorbed dose D_i of an individual sample may be evaluated from the individual signal intensity, $I_{RIS,i}$, using Formula (5) with individual dose calibration factor, c_i, and an EPR signal bias, b_i.

$$D_i = (I_{RIS,i} - b_i)/c_i \tag{5}$$

The individual dose calibration factor c_i may be estimated or approximated with the

a) additive dose method: this method is most time and labour consuming and destroys the sample with irradiation to high dose, but is effective when sensitivity variation is of concerned, or

b) one point calibration: fastest but least accurate method, which has limited use e.g. for high absorbed dose above 1 Gy, or

c) dose calibration curve: most frequently used method, which does not destroy dose information in the sample.

The individual EPR signal bias b_i cannot be evaluated with any of the methods but only approximated as average value from a dose calibration curve.

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The exact protocol for RIS intensity-to-absorbed dose conversion shall be established by each laboratory considering the following critical aspects of the different procedures as listed below:

— **Additive dose method**. Provides an estimate of the individual dose calibration factor, c_i. The sample is exposed additionally to several doses from a calibration source. The absorbed dose in the sample is estimated as intercept point of the applied dose axis and the linear regression line of the signal-to-dose relation with slope c_i. The estimated dose D for the sample is the individual dose, D_i, increased by a dose value equivalent to b_i/c_i.

— **One point calibration**. Provides an estimate of the individual dose calibration factor, c_i. The sample is exposed additionally with one large calibration dose, D_c, from a calibration source. The individual dose calibration factor is estimated from the original RIS intensity, $I_{RIS,i}$, and the intensity after calibration irradiation, $I_{RIS,I}(D_c)$, as $c_i = (I_{RIS,i}(D_c) - I_{RIS,i})/D_c$. The absorbed dose in the sample is estimated as $D = I_{RIS,i}/c_i$. The estimated dose, D, for the sample is the individual dose, D_i, increased by a dose value equivalent to b_i/c_i.

— **Dose calibration curve**. Provides an estimate of an average dose calibration factor, c, and average EPR signal bias, b. Sets of various teeth or mixed enamel powder prepared from various teeth are exposed to several doses from a calibration source in order to establish a RIS signal intensity-to-absorbed dose calibration curve. Dose calibration factor c and EPR signal bias, b, are evaluated from linear regression analysis of the calibration curve. The individual absorbed dose, D_i, in a sample is evaluated with Formula (5) using the average dose calibration factor, c, and average EPR signal bias, b. The variation of the individual dose calibration factors, c_i, relative to the average, c, is given by the individual radiation sensitivity of enamel samples, which varied by 10 %[26].

NOTE Typical annual dose contribution from environmental background radiation is approximately 1 mGy.

10 Calculation of uncertainty on dose estimate

In line with ISO/IEC 17025, the resulting estimated dose represents the best estimate possible given the associated dispersion, which arises from the experimental and intrinsic uncertainties. It is thus necessary to estimate measurement uncertainty using appropriate methods.

The general procedure for assessing uncertainty relies on formal combination of all the sources of experimental uncertainty (ISO/IEC Guide 98-3)[27]. In brief, the relationship between the dose and the input quantities should first be clearly defined, the sources of uncertainty relevant to the particular case should be identified and quantified, then the combined uncertainty should be calculated (ISO 5725-1)[28].

The recommended methodology to calculate uncertainty on absorbed dose in the context of the EPR dosimetry with tooth enamel is defined below.

In practice, the usual method used to estimate the uncertainties on the dose is to combine the confidence limits on the frequency with the uncertainties on the calibration coefficients (ignoring the covariance components). However, the uncertainty associated with an assessment varies widely depending on a large number of factors as listed below. As such, it is recommended that the uncertainty is assessed according to the above procedure on a case by case basis.

— Sample preparation.

— Sample mass normalization at measurements.

— EPR measurements.

— Spectrometer noise.

— Sample positioning.

— Spectrometer stability.

— Spectrum processing.

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— EPR signal to dose conversion.

— Calibration of EPR dose response

— Calibration gamma source.

— Calibration curve - uncertainty may be determined through the propagation of uncertainties in its intercept and slope.

— Dose bias - natural radiation background, life-time medical exposures, solar UV exposure.

The laboratory shall define the methods used to determine confidence limits. The laboratory shall report the method used to determine the standard uncertainty (the standard deviation) and the expanded uncertainty (which gives the 95 % confidence interval). The laboratory should also retain records of the uncertainty budget (a list of the uncertainty components and how they were evaluated) together with details of any systematic errors accounted for and all other corrections and constants employed.

It should also be noted that it is recommended in ISO 11929-1[29] and others that the standardized uncertainty is multiplied by coverage factor, k, such that the coverage probability of the confidence intervals corresponds to approximately 95 %.

11 Minimum detectable dose

To assess the performance of the method and to compare the performance between laboratories, the parameters decision threshold and detection limit should be calculated.

The definition of decision threshold is the critical amplitude following from the hypothesis test for 95 % probability of an un-irradiated sample, and hence allowing for a false positive error rate α of 5 % indicating exposure of the sample by EPR measurement. That is, within the distribution of measured EPR signal amplitudes from unexposed samples, there is probability α of 5 % that the amplitude is larger than the critical amplitude. The critical amplitude is the decision threshold at which it may be decided whether a sample was exposed or not. The dose value that is obtained with the EPR signal-to-dose response curve from the critical amplitude is called critical dose.

The definition of the detection limit follows from the hypothesis test for 95 % probability that the sample was exposed, and hence allowing for a false negative error rate β of 5 % to indicate exposure of the sample by EPR measurement so that if in reality the true value is equal to or exceeds the detection limit. The probability of wrongly not rejecting the hypothesis (error of the second kind) shall be at most equal to a given value β. A graphical illustration of the definitions of critical amplitude and detection limit is shown in Figure 2.

The critical amplitude, I_{CL}, see Formula (6),and the limit of detection, I_{DL}, see Formula (7), of EPR signal intensity are calculated from the mean of measurements of unexposed samples (b_0) and the estimated standard deviation of n EPR measurements of unexposed samples, σ_0, and samples exposed to a dose, D_{DL}. σ_{DL}, respectively.

$$I_{CL} = b_0 + t_{(1-\alpha,n-2)}\sigma_0 \tag{6}$$

$$I_{DL} = I_{CL} + t_{(1-\beta,n-2)}\sigma_{DL} \tag{7}$$

The estimated standard deviation shall be multiplied by the Student's critical value $t_{(1-[\alpha \text{ or } \beta],n-2)}$, the (1-[$\alpha$ or β]) percentage point of Student's t distribution with the single-sided confidence interval chosen according to the desired confidence level (1-[α or β]) and number of samples n.

The standard deviations may be evaluated in a calibration design from the 90 % prediction bands of an unweighted linear least-squares fit of the EPR signal-to-dose response curves in the case of constant uncertainty. Alternatively, in the case of dose dependent uncertainty, the values of the standard

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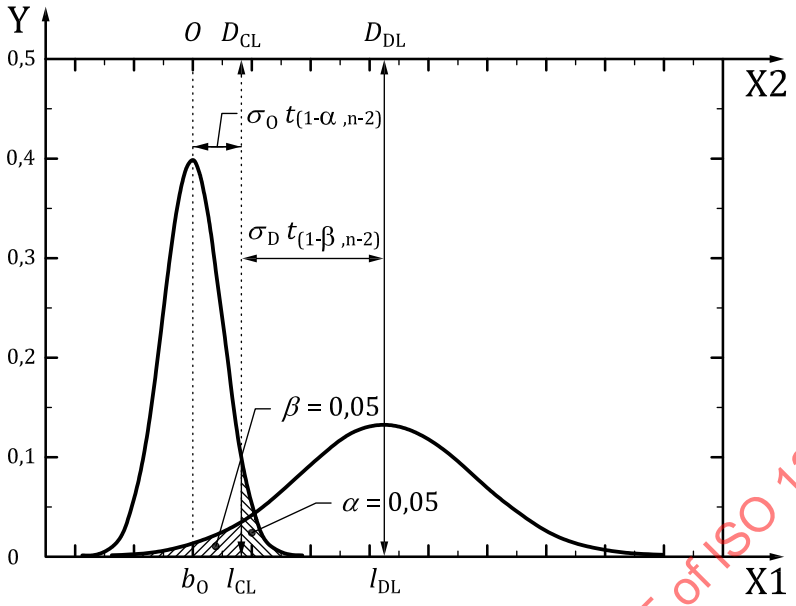
deviations may be predicted from a model function proposed for the analytical formulation of the variance of EPR measurements on the absorbed dose.



Key

X1 EPR signal amplitude, I

X2 absorbed dose, D

Y relative frequency

Figure 2 — Graphical illustration of the definitions of decision threshold (critical amplitude, I_{CL}) and critical dose, D_{CL}, detection limit of signal amplitude, I_{DL}, and of absorbed dose, D_{DL}

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12 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.

13 Laboratory safety requirements

13.1 General

The use of this document can involve hazardous materials, operations, and equipment. It does not purport to address all of the safety or environmental problems associated with its use. It is the responsibility of users of this document to take appropriate measures to ensure the safety and health of personnel and the environment prior to application of this document, and fulfil statutory and regulatory requirements for this purpose.

13.2 Magnetic field safety requirements

With commercial EPR spectrometers, the magnetic field is restricted to the region between the pole caps of the magnets, and therefore, there is no associated health risk.

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There is no potential to project the 0,5 mT line defined in ISO/IEC 17043[30] beyond the confines of the spectrometer[30]. The establishment of the 0,5 mT limit is based on concerns about potential effects on pacemakers, which are the only significant source of risk to health from the magnetic fields that are employed with EPR.

13.3 Electromagnetic frequency requirements

EPR spectrometers using X-band microwaves have no hazard for exposure of operators, as the spectrometers fully constrain the microwave to the sample with no significant amount distributed outside of the resonator.

13.4 Chemical safety requirements

Certain chemicals and pharmaceuticals are used routinely in the procedures covered in this document. When present in sample preparation and sterilization procedures, they are mostly used in small volumes and in dilutions that generally present no health hazard. They are, however, prepared and stored in concentrated stock solutions. Laboratories shall adhere to their institutional, regional or national requirements for chemical safety and hazard communication, e.g., the globally harmonized system (GHS).

13.5 Health risks from tooth samples

Extracted teeth should be considered infectious because they contain blood and have the potential to cause harm. The safe handling of extracted teeth requires methods for antimicrobial and antiviral control[31]. In dental clinics, the widespread method for sterilization of extracted teeth is the use of (1 to 5) % sodium hypochlorite for 24 h[32].

13.6 Optical safety requirements

When black light lamps are used in checks for the sample preparation quality procedure, shielding and working procedures shall be in place to avoid direct irradiation of the skin or eyes of laboratory staff.

14 Responsibility of the customer

This clause concerns items that are not controlled by the laboratory. Prior to preparation and measurement of tooth enamel samples, coordination between the customer and the measuring laboratory should occur. Essential requirements should be explained to the customer and this may be by a standardized instruction sheet.

15 Responsibility of the service laboratory

The laboratory shall establish and maintain a QA program, which covers all aspects of EPR dosimetry with tooth enamel. The laboratory's QA program shall include periodic internal checks of equipment operations, reagent suitability, and various performance checks (i.e. inter-laboratory comparison, operator qualifications, sample protocol, measurement, dose estimations, report generation, etc.).

16 Quality assurance and quality control (QA and QC)

16.1 General

Quality management shall assure continued improvement of operations. This document describes specific quality assurance and quality control procedures for laboratories performing EPR dosimetry with tooth enamel, which shall be in accordance with ISO/IEC 17025.

EPR dosimetry deals with small sample masses and low intensity signals. Proper choices of apparatus and measurement conditions are critical in order to obtain high sensitivity of measurements. It is also

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important to monitor stability of the apparatus in order to achieve high reliability of the measurement results.

The individuals carrying out EPR dosimetry shall be appropriately trained. All individuals should participate in intra- and inter-laboratory comparisons. A set of calibration samples (at least five) should be used routinely to verify that the accuracy of results is well within the expected range.

16.2 Performance checks

16.2.1 General

Performance checks with suitably qualified EPR dosimetry laboratories and networks (e.g. EURADOS, RENEB) shall be established through periodic inter-laboratory comparisons.

ISO 5725 is dedicated to statistical analysis to test the reliability and the precision of a technique[28]. The tests proposed can be applied only if many samples (at least five) are analysed.

16.2.2 Performance checks by inter-laboratory comparisons

Proficiency tests are essential tools for the quality assurance of the laboratories, as they constitute an objective evaluation of their performance, from both a human and technical point of view.

The presence of individual laboratories or data that appear to be inconsistent with all other laboratories may change the estimates. To discard or correct inconsistent data, two approaches can be used (ISO 5725-2 and ISO 5725-5).

a) numerical outlier tests (Cochran and Grubbs tests): To discard data that give rise to a test statistic that exceeds the critical value of the test at the 5 % significance level;

b) robust methods for data analysis: To yield robust values of the average and standard deviation of the data.

The procedure is as follows:

— the outlier test for laboratory inter-comparison performance requires a minimum of five laboratories for statistical robustness (ISO 5725-1);

— estimation of the overall, inter-laboratory mean value and standard deviation, once outliers are discarded or corrected. The preferred method is the calculation of the robust parameters;

— determination of the laboratory's performance: Calculate the z-score parameter, reference value and the estimated deviation from the laboratory results. Determine the u-score parameter (this evaluation includes both participant measurements and reference value uncertainties).

16.2.3 Performance checks of sample preparation

Inspection with black light (UV-A, wavelength >315 nm) of enamel powder may be used to identify dentine remains in the samples. Such check may be routinely included in the sample preparation protocol to ensure minimal dentine remains. At the first use of a black light source it should be ensured, through checks with test enamel samples that selected wavelength, power and exposure time do not induce EPR signals in enamel.

The presence of impurities or mechanically and thermally induced EPR signals in prepared samples shall be checked and documented.

Data to be registered for sample preparation shall include: operator name, preparation date, grain size of enamel powder, and if applicable the existence of impurity signals.

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