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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

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Foreword

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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

the sensitivity for the particular type of material, the available microwave power, and the potential for placing the sample accurately in the most sensitive region of the resonator. It is essential to systematically monitor the sensitivity and the accuracy of the various settings, including the modulation amplitude. While theoretical considerations should be used to decide on the approximate optimal settings, the final tests should be actual measurements with each pertinent parameter empirically confirmed by measurements in which the parameter (e.g. the modulation amplitude) is varied to find the setting that gives the maximum signal-to-noise ratio.

8.4 Measurements of the background signals

EPR signals may be originated by other paramagnetic species in the resonator and also measurement tube for *in vitro* analysis. Therefore, it is essential that measurements of empty resonator and empty measurement tube be made under the same conditions as for samples used for dosimetry. The background signal measured without sample tube may be used to ensure that the background is indeed due to only the resonator.

The following types of the background signal of the resonator and baseline variation may be observed, and if the type is identified, then one can more readily take proper actions to minimize its effect.

If a stable background signal is observed, then the resonator or the sample tube may be contaminated. Carefully cleaning of the resonator or the tube may diminish the effects of this type of background signal.

In the case where the background signal varies with repositioning or reinserting the sample and does not have a consistent nature as expected with a paramagnetic contaminant, this is likely to be due to "microphonics". This can occur with both low frequency (e.g. L-band) and X-band. Dosimetry often requires that the system be pushed to the limits of sensitivity, which makes the system susceptible to microphonic noise. This appears to be due to mechanical effects, especially from modulation fields, but is far from being fully understood. These effects sometimes can be minimized by careful attention to all possible sources of vibrations and rigidity in the physical components and careful electrical grounding of all components.

8.5 Spectrometer stability and monitoring/control of environmental conditions

The spectrometer should be allowed to reach a stable operating temperature in regard to both ambient conditions and the EPR spectrometer's cooling water. For maximum stability under demanding operating conditions such as any combination of high microwave power, high magnetic field modulation amplitude, and variable temperature work, it is important to allow the system to equilibrate under the same conditions as the experiment that is to be performed. One hour is usually adequate to achieve temperature equilibrium.

It is necessary to maintain a controlled environment for the best spectrometer performance. Air flowing through the spectrometer, especially the cavity, may induce temperature fluctuations or microphonics from sample vibration. Large fluctuations in the ambient temperature may degrade performance by reducing the frequency stability of the cavity. Very humid environments may cause water condensation. Condensation inside the cavity may be reduced by maintaining a constant purging stream of dry nitrogen gas. Note that excessive gas flow rates may generate microphonic noise through sample vibration.

Noise pick-up from electromagnetic fields may be encountered in some environments. It may be possible to reduce such noise by shielding or perhaps by turning the noise source off if it is identified to be generated by equipment near the spectrometer.

8.6 Baseline drift

Baseline drift is connected with stability of operation of the spectrometer. Baseline drift should be minimized by optimization of operational conditions. Correction of the baseline drift effects on the spectrum may be performed immediately after measurements with the use of the basic software of the spectrometer, or it may be corrected during subsequent spectra processing of the radiation-induced signal with the use of special software.

Linear baseline drift: The use of very high modulation amplitudes can produce large eddy currents in the sidewalls of the resonator. These currents can interact with the magnetic field to produce a torque on the resonator and create a resonant frequency shift. A linear-field-dependent or modulation-amplitude-dependent baseline is indicative of such an effect. This phenomenon should not be observed if the resonator end plates are properly fitted and torqued.

Slowly and randomly varying baseline: The use of high microwave power or large modulation fields can heat the resonator and the sample. The ensuing thermal drifts in the coupling of the resonator, as well as the frequency of the resonator, can result in a fluctuating offset in the signal. Allow the turned cavity and sample to come to thermal equilibrium before performing the final tuning of the cavity. Once the resonator is equilibrated and properly tuned under equilibrium conditions, one can start acquiring a spectrum. Avoid air drafts around the resonator, as they can randomly change the temperature of the cavity and sample and hence the baseline of the spectrum.

9 Measurements of the samples

9.1 General principles

The goal is to measure the intensity of the radiation-induced signals with uncertainties sufficient to meet desirable dose uncertainties. This requires appropriate conditions for acquisition of the spectra by selection of optimal measurement parameters. In some materials, signals may be present other than those induced by radiation, which requires that the measurement procedure should be optimized to allow for minimizing the potential perturbation from such signals.

9.2 Choice and optimization of the measurement parameters

9.2.1 General

There are three groups of parameters for making measurements with an EPR spectrometer: microwave-related parameters, magnetic field parameters, and signal detection parameters. The aim of this subclause is to provide a basis for proper selection of parameters necessary to record an EPR spectrum of radiation-induced signal from radiation-induced radicals as well as the overlapping signals. Depending on spectrometer type, some of the measurement parameters may not be available.

9.2.2 Microwave-related parameters

9.2.2.1 Microwave frequency. It refers to the resonant frequency of the loaded microwave resonator. It is dependent on the operation band of the spectrometer, type of resonator, and properties of the inserted sample.

9.2.2.2 Microwave power. The EPR signal intensity increases as the square root of the microwave power in the absence of saturation effects. Several microwave power levels should be tried to find the optimal microwave power. Varying the microwave power produces effects on all EPR signals. Influence of microwave power on the baseline drift is described in 8.6. The microwave power should be properly selected in order to achieve the highest ratio of "radiation-induced signal/signals from other sources".

9.2.3 Magnetic field parameters

9.2.3.1 Magnetic field at resonance. The value of magnetic field at resonance is determined by the microwave frequency. The centre of the magnetic field sweep is approximately 350 mT for a frequency of 9,8 GHz and an EPR signal with g-factor near 2,0 (main line of the radiation-induced signal). It is about 40 mT for L-band and 1 200 mT for Q-band.

9.2.3.2 Magnetic field sweep width. Magnetic field sweep width is determined by the type of dosimetric material and sometimes also by the desire to record a standard sample (see 9.5). For example, if a marker sample of MnO is recorded simultaneously with spectrum of the sample at X-band, a typical value of

10 mT is used to record central part of the spectrum near g-factor equal 2,0 together with lines 3 and 4 of the standard.

9.2.3.3 Magnetic field sweep time. EPR signals are recorded with minimal distortion if the sweep time through the signal's peak-to-peak line width is at least 10 times longer than the receiver time constant of the signal channel receiver low-pass filter (principles of selection for this parameter are described below, in 9.2.4.2).

NOTE For undistorted recording of the radiation-induced EPR signal of tooth enamel in X-band (which has approximately 0,4 mT in width), the sweep time should be set up to 125 and 250 times longer than the time constant for a 5 mT and 10 mT sweep width, respectively. Typical sweep times are in the range between 20 s and 80 s.

9.2.4 Signal channel parameters

9.2.4.1 Receiver gain. It is convenient to have sufficient receiver gain in order to readily visualize the signal. With excessive receiver gain, the signal is clipped.

9.2.4.2 Time constant of receiver. The time constant filter reduces random (white) noise. If the scan is too fast for the chosen setting, the signal is distorted and reduced. The use of a time constant, such that the time needed to scan through an EPR signal is about 10 times greater than the length of the time constant, avoids such distortion.

9.2.4.3 Spectrum resolution. Spectrum resolution is determined by the number of channels used by the signal channel analog-to-digital converter (ADC) for spectral acquisition. It should be selected to have enough number of data points to resolve spectral features important for analysis. Typically, to provide a resolution of 0,01 mT and 0,005 mT for sweep width of 10 mT and 5 mT, respectively, 1 024 channels are used.

9.2.4.4 Conversion time. It is the duration of acquisition for each channel. This parameter is chosen in accordance with selected sweep time, which is defined as a product of the number of channels used in spectrum acquisition (spectrum resolution) and the conversion time of each channel.

9.2.4.5 Modulation frequency. The frequency of the magnetic field modulation should be set as high as possible to achieve the best signal-to-noise ratio. Usually, the upper limit is set to prevent broadening of very narrow EPR signals at high modulation frequency. In practice, most commercial EPR spectrometers operate with 50 kHz or 100 kHz modulation frequency. For these values of modulation frequency, EPR lines shall be broader than about 0,002 mT or 0,004 mT, respectively.

9.2.4.6 Modulation amplitude. Field modulation amplitude should not exceed the width of the EPR signal. The use of the higher values is not practical because it leads to broadening of the radiation-induced signal and reduction of signal resolution.

9.2.4.7 Sweep time and number of signal accumulations. In a perfectly stable laboratory environment and spectrometer, signal averaging and acquiring a spectrum with a long sweep (scan) time and a long time constant are equivalent. Unfortunately, such perfect stability is impossible to attain whether in the laboratory or, in the case of *in vivo* tooth dosimetry, in the field. Slow variations in conditions result in baseline drifts. A common cause of such variations is room temperature changes or air drafts around the cavity. For a long sweep time, the variation causes broad features in the spectrum. If the EPR signal is accumulated with a sweep time short compared to the variation time, these baseline features could be averaged out. The baseline drift causes only a direct current offset in each of the scanned spectra. Improvement in baseline stability may be achieved through the use of short sweep times with signal averaging when the laboratory environments are not stable. Alternatively, with the presence of a suitable marker in each sweep (see 9.5), some of the drifts can be corrected by suitable software. Fluctuations in the stability of the magnetic field and microwave frequency are usually compensated by built-in field-frequency lock device in modern spectrometers.

9.2.5 Signal-to-noise ratio enhancement. EPR signal accumulation with averaging produces a signal-to-noise ratio enhancement by square root of the number of accumulations. However, a large number of scans coupled with long sweep times can be a disadvantage because of temporal fluctuations

in spectrometer sensitivity, including the instability of electronic devices, microwave cavity quality factor, and power output.

9.3 Sample positioning and loading

Reproducible positioning of the sample in the resonator is perhaps the most crucial factor for obtaining reproducible and comparable results, and therefore, this aspect needs to be thoroughly documented and the method for achieving it shall be quite robust.

For X-band measurements, the microwave resonator is characterized by a so-called working volume, within which the distribution of microwave field and modulation amplitude are essentially homogeneous. The sample shall be positioned within the working volume, but this does not guarantee a linear dependence between the sample mass and the EPR signal intensity because the sensitivity varies within the working volume. If the sample occupies a significant fraction of the working volume, there is unlikely to be a linear relation between sample mass and the signal intensity and an empirical nonlinear dependence between the EPR intensity and sample mass should be used for normalization. Note that even with the same mass, the sample volume can be out of the linear range if the inner diameters of the sample tubes vary. In this case, it is necessary to have a calibration relationship established for each sample tube. Before using linear mass normalization, it is necessary to determine by measurements the range of masses for the particular type of resonator and for the sample tubes being used, within which the linear dependence of EPR signal intensity versus sample mass is valid. Frequently, the sample tube placement is fixed in the resonator on a special pedestal in order to improve reproducibility of the EPR measurements. In this case, with a smaller sample, a further deviation from linear dependence between EPR intensity and mass occurs because the centre of the sample does not coincide with the centre of the resonator.

The equivalent of sample positioning for *in vivo* tooth dosimetry is the placement of the resonator on the tooth or teeth. This is a crucial parameter to achieve reproducibility because the sensitive volume of the resonators in current use is a cone descending from the loop that comprises the tip of the resonator. It is essential to utilize a sophisticated setup in which the position of the tooth in relationship to the tooth or teeth is established by automatic positioning and support of both the resonator and the head of the subject. The most feasible method to have comparable results between subjects is to have the working volume of the resonator to be less than the volume of irradiated enamel and to have the resonator placed in exactly the same position in regard to the surface of the tooth. If the sensing tip of the resonator has a working volume that is greater than the surface of the tooth, then an empirical relationship shall be established between the dimensions of the tooth and resulting intensity of the signal for the same dose.

9.4 Microwave bridge tuning

The microwave bridge should be tuned each time the sample is positioned, and each time the sample or the internal standard sample is moved, to compensate for any modification of the state of the sample or for the drift of the spectrometer during a set of measurement of a sample. The tuning method used by operators should ensure the best reproducibility of the measurements. Tuning is performed in several stages, at which resonance frequency is adjusted and critical coupling of the resonator is performed. In modern spectrometers, this process may be automated. If parameters and conditions of measurement are not changed, and the same sample is used, it is more likely that reproducible tuning will be achieved, but it is essential to confirm this by the use of an appropriate amplitude monitor (see 9.5).

9.5 Use of standard samples as field markers and amplitude monitors

Fluctuations in the magnetic field/microwave frequency and spectrometer sensitivity can be monitored and corrected for, where appropriate, by the use of appropriate samples. These can be measured at the same time as the investigated samples or at a different time. Depending on whether the sample serves to monitor the magnetic field/microwave frequency or sensitivity stability, it is respectively called a field marker or an amplitude monitor.

Use of the field marker effectively replaces the need for independent measurement of the frequency and magnetic field. The use of a field-frequency lock, which ensures the recording of EPR spectra of any

sample in the same g-factor region (g-factor about 2,0), is extremely useful but, as with any apparatus, its function needs to be verified periodically.

A sample containing Mn^{2+} (MnO in CaO or in MgO) is usually used in the EPR technique as a field marker and an amplitude monitor. This can improve the analysis of spectra so that the position of the EPR signal at the appropriate g-factor is ascertained. The spectral shapes are well known so that, with the positioning established, processing of the data through appropriate software then is fully feasible, including summing of repeated spectral acquisitions or subtraction of the background signal.

9.6 Monitoring reproducibility

It is essential to periodically make repeated measurements of standard samples to ensure that the operating conditions are as expected. The frequency of such monitoring depends on the stability of the system and the types of measurements that are being made, but it is always better to do more than less.

9.7 Procedure to measure anisotropic samples

EPR samples usually have oriented and crystal structure, and they are then usually anisotropic even when the sample is finely ground (e.g. grain size of few hundred microns).

Anisotropy of samples can be averaged by recording several sample spectra at different angles (arbitrary or specially selected) relative to constant magnetic field direction. Subsequently, either the spectra can be summed after their g-factor normalization or the results of radiation-induced signal determination can be averaged.

The sample can be oriented at different angles either manually or with an automatically controlled goniometer.

9.8 Coding of spectra and samples

It is essential that every sample has a unique identifying number. All available information about the sample is linked to this number. Each spectrum file should be linked to the sample number.

10 Determination of the absorbed dose in the samples

10.1 Determination of the radiation-induced signal intensity

The radiation-specific parameter of the EPR spectrum reflecting the dose response should be identified. This radiation-specific parameter, to be defined as intensity, may be any quantity related to the area below the microwave absorption curve, i.e. the area itself or the difference between the maximum positive and maximum negative amplitudes of the first derivative absorption curve known as “peak-to-peak” amplitude.

The laboratory shall implement and establish procedures for the evaluation of the radiation-specific intensity, either manual or computer-assisted.

The purpose of this procedure is twofold:

a) to isolate the radiation-induced signal from other signals originated by other paramagnetic species in the sample, the resonator, and the measurement tube;

b) to determine a relation between the radiation-specific parameter and the absorbed dose in the samples.

The signal isolation can be performed by either

a) subtraction of the unirradiated from the irradiated sample spectrum, or

b) the best fit of the experimental spectrum to a simulated or a measured reference spectrum. For instance, one can use high-dose experimental spectra as reference for the radiation-induced signal.

The same procedure shall be used for all the samples.

10.2 Conversion of the EPR signal into an estimate of absorbed dose

10.2.1 Conversion of the EPR signal into an estimate of absorbed dose for *in vitro* dosimetry

The signal intensity obtained for a sample should be normalized by mass or sensitive volume of the sample (See 9.3). When the signal is accumulated, then the intensity also needs to be normalized by the number of accumulated scans. The intensity may further be normalized by an amplitude monitor measured together with the sample in order to correct the sensitivity of the cavity. Sample absorbing properties may influence the signal intensity. Irradiation of the samples in the course of the additive dose or calibration method shall be performed with a calibrated radiation source such as ^{60}Co or ^{137}Cs.

The signal intensity should be converted to the absorbed dose by the additive dose method or by the calibration method.

The additive dose method: The EPR measurements should be repeated several times for the same sample after each step of irradiation or for several aliquots of subsamples with different given doses. The dose response line or curve is obtained for the sample as a function of given dose. The best-fit line or curve should be extrapolated to the zero ordinate. The distance between the origin and the intersection in the dose axis is the dose to be obtained.

The calibration method: A separate set of samples should be prepared and be given known doses. The best-fit dose response line or curve should be obtained for this set of samples. Assuming that the signal response to dose is the same for this set of calibration samples and for the investigated samples, doses should be estimated as the ones corresponding to the observed signal intensities on this calibration line or curve. Alternatively, a material with a stable EPR signal can be used as the calibration standard after calibrating with irradiated samples.

Caution should be paid:

— to the time between irradiation and reading and to the storage conditions. These should be the same at each step of irradiation and for each calibration sample as for the sample to be investigated. Alternatively, a correction factor should be evaluated and applied. This procedure can be avoided if it is known that the radiation-specific signal intensity is independent of time and of environmental conditions;

— when choosing characteristics of the calibration beam (type, energy) and irradiation conditions (form of the samples, build-up material). In general, it is better to choose characteristics of calibration beams and irradiation conditions that are as close as possible to the conditions in which the sample was irradiated. Usually in irradiation facilities, the delivered dose is calibrated in a media (air, water, tissue) different from the matter of the samples. Therefore, it is usually necessary to evaluate and apply dose conversion factors to convert the delivered dose to absorbed dose in the sample medium.

10.2.2 Conversion of the EPR signal into an estimate of absorbed dose for *in vivo* tooth dosimetry

This requires that procedures are established to achieve reproducible geometric relationship between the sensitive volume of the resonator and the tooth *in vivo*. To accomplish this, the resonator needs to be designed specifically for the anatomical site and an apparatus to ensure accurate placement needs to be developed for the specific purpose.

Because of the inherent difficulty in matching the geometry, it is essential that an intrinsic standard be a rigid part of the apparatus. This is then utilized as an intensity standard for calculation of the dose.

11 Measurement uncertainty

To be meaningful, a measurement of absorbed dose shall be accompanied by an estimate of uncertainty.

Components of uncertainty shall be identified as belonging to one of two categories:

— Type A: those evaluated by statistical methods; or

— Type B: those evaluated by other means.

The identification of Type A and Type B uncertainties is based on the ISO Guide to the Expression of Uncertainty in Measurement (GUM). The purpose of using this type of characterization is to promote an understanding of how uncertainty statements are developed and to provide a basis for the international comparison of measurement results.

All identified sources of uncertainties should be declared and the uncertainty budget be reported.

12 Investigation of dose that has been questioned

Dose investigations should be conducted in case of unexpected dose reading or upon request of customer. Dose investigation may consist of (but is not limited to) the following steps:

— repetition of the sample measurement;

— evaluation of the possible deviation in the dose measurement procedure by comparison with other samples measured in the same time period;

— control exposure of the sample with known dose;

— measurements of the control samples with the set of known doses;

— request of additional information on the sample prehistory.

Generally, dose investigation can be done in three steps: analysis of sample conditions, evaluation of the EPR spectra, and applied dose calibration. Below are some recommendations on the process of the dose investigations.

Incorrect dose report can be caused by sample alteration due to uncontrolled chemical contamination, physical treatment, or wrong sample weighting. If the sample with unexpected dose reading is available, it is recommended to visually inspect it on the presence of any deviation from normal appearance, e.g. abnormal colour, presence of foreign inclusions, etc. For example, it is known that sometimes, carious black inclusions in tooth enamel sample may have an EPR signal which can look similar to a radiation-induced one and can be mistakenly used for dose measurements. If this is the case, the inclusions can be manually taken out of sample, and EPR measurements can be repeated. The questioned sample also can be split into parts and their EPR spectra can be compared. Another possible reason for abnormal (high) dose reading is a presence in EPR spectrum of the sample of some non-radiation signals which can be responsible for dose over-estimation. Most often, those signals are induced by mechanical stress (mechanically induced signal), sample overheat during preparation, and/or by solar or UV. Mechanically induced radicals can be stable over time and interfere with the radiation-induced signal. Consequently, the mechanical treatment of a sample should be minimized and performed as gently as possible. The signals induced by mechanical stress and UV exposure are likely to be induced on the sample surface, and therefore, chemical etching may counteract this effect, if the sample can tolerate such treatment.

Incorrect dose measurement can be also caused by EPR spectrometer malfunction or presence of some foreign radicals in the resonator or sample tube with EPR signal(s) positioned nearby radiation-induced signal. Therefore, it is recommended to measure EPR spectrum of empty resonator and empty sample tube used for questioned dose measurement. Low radiation-induced signal intensity may be caused by the presence of water in the resonator during measurements or wrong sample tube positioning in the resonator. In this case, repeated EPR measurement can provide more accurate dose assessment.

Application of wrong dose calibration factor can be another reason for incorrect dose measurement. The following steps are recommended to evaluate correctness of this: verification of radiation source calibration, check of energy dependence of dose response in the specific sample, abnormally high or low radiation sensitivity of questioned sample. Repeated control exposure with known dose from a properly calibrated source can verify this part of the dose measurement procedure.

13 Quality assurance (QA) and quality control (QC)

In-house quality assurance and quality control practices should be established.

In the case of EPR dosimetry, having in place quality assurance means that:

a) there is a written manual which describes all elements of the EPR dose measurement procedure including, but not limited to, sample collection, sample preparation, EPR measurements, dose determination, and uncertainty analysis;

b) the personnel have the necessary knowledge, experience, and qualification to perform EPR dosimetry work;

c) all equipment used for EPR dose measurements, including equipment that supports the measuring process (e.g. for environmental conditions) having a significant effect on the accuracy or validity of the result of the test, calibration, or sampling shall be calibrated before being put into service;

d) the laboratory should have an established program and procedure for QA.

Generally speaking, quality control (QC) emphasizes testing of procedures, materials, and equipment involved in dose measurements to uncover failures which can cause wrong or inaccurate dose measurements. Typically, it is based on established quality control procedures for monitoring the validity of tests and calibrations undertaken. In the case of EPR dosimetry, QC procedures may include, but are not limited to, the following elements:

— measurements of the same EPR standard samples (e.g. "weak pitch" or Mn^{2+} in some crystals like CaO) at the same recording and environmental conditions;

— assessment of the spectrometer sensitivity and stability;

— measurements of the empty sample tube used for the dose measurements at the same recording parameters as used for measurements of investigated samples;

— estimation of the noise level in the EPR spectrometer;

— multiple measurements of some control samples, e.g. known to be unirradiated (zero-dose) sample and high-dose sample; constant monitoring and recording of environmental conditions (temperature and humidity) in the lab;

— calibration of the microbalance(s) which is(are) used for sample weighting;

— verification of the sample radiation sensitivity if universal calibration curve using for dose measurements;

— participation in interlaboratory comparisons and proficiency testing;

— establishment and periodic (annual or semi-annual) verification of traceability of the reported doses to national or international standard. The laboratory establishes traceability of its own measurement and measuring instruments to the SI by means of an unbroken chain of calibrations or comparisons linking them to relevant primary standards of the SI units of measurement. The link to SI units may be achieved by reference to national measurement standards. National measurement standards may be primary standards, which are primary realizations of the SI units or agreed representations of SI units based on fundamental physical constants, or they may be secondary standards which are standards calibrated by another national metrology institute. When using external calibration services, traceability of measurement shall be ensured by the use of calibration services from laboratories that can demonstrate competence, measurement capability, and traceability. The calibration certificates issued by these laboratories shall contain the measurement results, including the measurement uncertainty;

— maintenance of reviews' records, including any significant changes in the procedures;

— in case if QC data are found to be outside pre-defined criteria, planned action should be taken to correct the problem and to prevent incorrect results from being reported. The laboratory should have a policy and a procedure for implementing corrective action when nonconforming work or departures from the policies and procedures in the management system or technical operations have been identified. The procedure for corrective action shall start with an investigation to determine the root cause(s) of the problem.

14 Minimum documentation requirements

The laboratory shall establish and maintain procedures for identification, collection, indexing, access, filing, storage, maintenance, and disposal of quality and technical records. All records shall be legible and shall be stored and retained in such a way that they are readily retrievable in facilities that provide a suitable environment to prevent damage or deterioration and to prevent loss. Retention times of records shall be established. Records may be in any media, such as hard copy or electronic media. All records shall be held secure and in confidence.

The laboratory shall retain records of original observations, derived data, and sufficient information to establish an audit trail, calibration records, staff records, and a copy of each test report for a defined period typically not to exceed five years. The records for each test or calibration shall contain sufficient information to facilitate, if possible, identification of factors affecting the uncertainty and to enable the test or calibration to be repeated under conditions as close as possible to the original. The records shall include the identity of personnel responsible for the sampling, performance of each test, and/or calibration and checking of results.

In the case of EPR dosimetry, minimum documentation shall include the following (additional records may be required depending on the measurements that are being made):

All information related to the application of procedure from sample collection to final dose estimation.

— all parameters related to the EPR spectra, sample data (mass), and protocol for the dose calculation;

— all available description of the samples that were used for dose measurements, e.g. where, when, and from whom they were collected;

— in the case of biological samples, available information on donor, e.g. age, gender, place of birth. Available information about irradiation in the past etc. Special code system which prevents immediate direct identification of the donor and sample should be developed and implemented;

— results of the appropriate periodic sensitivity and stability tests.

