Biological and physical retrospective dosimetry

16th EURADOS School: Contribution of dosimetry in the field of nuclear emergency preparedness and radiological accident management, June 2023

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Dose assessment approaches

**PHYSICAL DOSIMETRY**
- Dosimetry badge
  - Fortuitous: Luminescence
  - Electron spin resonance
  - Bioassays

**BIOLOGICAL DOSIMETRY**
- Cytogenetics (Dicentrics, FISH, PCC, MNA)#
  - Biochemical markers
  - Somatic mutations
  - Gene/miRNA expression

**CLINICAL DOSIMETRY**
- Vomiting
- Diarrhoea
- Blood cell count
- Skin reactions...
### Spectrum of damage induced by IR

<table>
<thead>
<tr>
<th>Damage induced by 1 Gy X-rays in a human cell:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical</strong></td>
</tr>
<tr>
<td>• 100,000 ionisations in the cell nucleus</td>
</tr>
<tr>
<td>• 2,000 direct ionisations in the DNA</td>
</tr>
<tr>
<td><strong>Biochemical</strong></td>
</tr>
<tr>
<td>• 1,000 single-strand breaks</td>
</tr>
<tr>
<td>• 1,000 damaged bases</td>
</tr>
<tr>
<td>• 150 DNA protein crosslinks</td>
</tr>
<tr>
<td>• 35 <strong>double-strand breaks</strong></td>
</tr>
<tr>
<td><strong>Cellular</strong></td>
</tr>
<tr>
<td>• 0.1 dicentrics / micronuclei</td>
</tr>
<tr>
<td>• 0.3 lethal events</td>
</tr>
<tr>
<td>• $10^{-5}$ hprt mutations</td>
</tr>
</tbody>
</table>

![Diagram showing the spectrum of damage induced by IR](image)

Biological effects of IR -> Biodosimetry

Chromosome aberrations
Mutations
DNA damage signalling & repair
inflammatory response
necrosis / apoptosis
organ dysfunction
repopulation
ORS, TL
EPR
gene expression,
γ-H2AX, CDKN1A
IR ionisations / excitations
radicals
cancer
OSL, TL
EPR
citulline, Flt3 ligand
CRP, SA, IL-6
WBC, caspases
CVD

Time since exposure
Exposure biomarkers

Pernot et al., Mutat Res 751: 258-286
<table>
<thead>
<tr>
<th>Feature</th>
<th>Feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific to radiation</td>
<td>Persistent effect</td>
</tr>
<tr>
<td>Low background</td>
<td>Ease of sampling</td>
</tr>
<tr>
<td>Low donor variability</td>
<td>Rapid analysis</td>
</tr>
<tr>
<td>Low doubling dose</td>
<td>Low cost</td>
</tr>
<tr>
<td>Dose response calibration</td>
<td>‘Risk meter’</td>
</tr>
</tbody>
</table>
## Characteristics of biodosimetry methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Radiation-responsive tissue</th>
<th>Time since exposure:</th>
<th>Exposure:</th>
<th>Time (h) from sample receipt to dose estimate</th>
<th>Agent specificity</th>
<th>Dose range (Gy) for photon equivalent acute whole body exposure 24 h ago</th>
<th>Triage use</th>
<th>Automated analysis</th>
<th>Standardisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSL/EPR</td>
<td>PED, Teeth / nails, Teeth / bones</td>
<td>d, m, y</td>
<td>P</td>
<td>&lt;1</td>
<td>IR</td>
<td>&gt;0.01</td>
<td>✓</td>
<td>Yes/No</td>
<td>ISO in preparation</td>
</tr>
<tr>
<td>OSL</td>
<td></td>
<td>d</td>
<td>P</td>
<td>&lt;1</td>
<td>IR</td>
<td>&gt;3</td>
<td>✓</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>EPR</td>
<td></td>
<td>d – m – y</td>
<td>P</td>
<td>1-48</td>
<td>IR</td>
<td>&gt;0.05/1</td>
<td>✓</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>γ-H2AX Gene expression</td>
<td>Lymphocytes, Peripheral blood</td>
<td>d</td>
<td>N</td>
<td>3</td>
<td>Genotoxins</td>
<td>&gt;0.2</td>
<td>✓</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>d</td>
<td>N</td>
<td>4 / 36°</td>
<td>Genotoxins</td>
<td>&gt;0.1</td>
<td>✓</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Dicentrics – full</td>
<td>Lymphocytes</td>
<td>d – m</td>
<td>P, N</td>
<td>55</td>
<td>IR</td>
<td>0.1 to 5</td>
<td>✓</td>
<td>Semi</td>
<td>ISO 19238</td>
</tr>
<tr>
<td>Dicentrics - triage</td>
<td>Lymphocytes</td>
<td>d – m</td>
<td>P</td>
<td>52</td>
<td>IR</td>
<td>0.5 to 5</td>
<td>✓</td>
<td>Semi</td>
<td>ISO 21243</td>
</tr>
<tr>
<td>PCC fragments</td>
<td>Lymphocytes</td>
<td>d</td>
<td>N</td>
<td>2°</td>
<td>IR</td>
<td>0.2 to 20</td>
<td>✓</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>PCC rings</td>
<td>Lymphocytes</td>
<td>d – m</td>
<td>P</td>
<td>40°</td>
<td>IR</td>
<td>1 to &gt;20</td>
<td>✓</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Micronuclei</td>
<td>Lymphocytes</td>
<td>d – m</td>
<td>P</td>
<td>75</td>
<td>Genotoxins</td>
<td>0.2 to 4</td>
<td>✓</td>
<td>Yes</td>
<td>ISO in preparation</td>
</tr>
<tr>
<td>MN centromere</td>
<td>Lymphocytes</td>
<td>d – m</td>
<td>P</td>
<td>80</td>
<td>Genotoxins</td>
<td>0.1 to 4</td>
<td>✓</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>FISH</td>
<td>Lymphocytes &amp; their stem cells</td>
<td>d – m – y</td>
<td>P</td>
<td>120</td>
<td>IR</td>
<td>0.25 to 4</td>
<td>✓</td>
<td>No</td>
<td>ISO in preparation</td>
</tr>
<tr>
<td>HPRT</td>
<td>Lymphocytes</td>
<td>d – m</td>
<td>m – y</td>
<td>400</td>
<td>Mutagens</td>
<td>&gt;1</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>GPA</td>
<td>Erythroblasts</td>
<td>d – m</td>
<td>m – y</td>
<td>3</td>
<td>Mutagens</td>
<td>&gt;1</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>TCR</td>
<td>Lymphocytes</td>
<td>d – m</td>
<td>P</td>
<td>180</td>
<td>Mutagens</td>
<td>&gt;0.5</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>Hepatocytes</td>
<td>d</td>
<td>1</td>
<td>Wide range</td>
<td>&gt;1</td>
<td>✓</td>
<td>Yes</td>
<td>Routine diagnostics</td>
<td></td>
</tr>
<tr>
<td>SA</td>
<td>Salivary gland</td>
<td>d</td>
<td>1</td>
<td>Wide range</td>
<td>&gt;1</td>
<td>✓</td>
<td>Yes</td>
<td>Routine diagnostics</td>
<td></td>
</tr>
<tr>
<td>Flt3 ligand</td>
<td>Bone marrow</td>
<td>d</td>
<td>1</td>
<td>Wide range</td>
<td>&gt;1</td>
<td>✓</td>
<td>Yes</td>
<td>Routine diagnostics</td>
<td></td>
</tr>
<tr>
<td>Citrulline</td>
<td>Enterocytes</td>
<td>d</td>
<td>1</td>
<td>Wide range</td>
<td>&gt;1</td>
<td>✓</td>
<td>Yes</td>
<td>Routine diagnostics</td>
<td></td>
</tr>
<tr>
<td>Metabolomics</td>
<td>Range of organs</td>
<td>d</td>
<td>&lt;1</td>
<td>IR</td>
<td></td>
<td>✓</td>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Personal electronic devices
2. PCC fusion method
3. PCC chemically induced
4. PCR / array analysis
5. Longer post-exposure time required for GPA to allow for mutant erythroblasts to mature to erythrocytes

1. **Identify** potentially exposed individuals

2. Take a **sample**
   - A. Blood for biodosimetry
   - B. Personal belongings for OSL/EPR

3. **Measure** radiation-induced signal

4. **Interpret**: Compare the test result with a calibration curve for dose response; estimate uncertainties

5. Inform **clinical treatment** decisions
Dicentric formation

Incorrect repair

Replication
Chromosome dosimetry

- Blood lymphocyte culture

- Blood lymphocyte culture:
  - Phytohemagglutinin
    - culture time = 48h
  - Colcemid
    - culture time = 72h
  - Cytochalasin B
  - harvest slide preparation staining

- Metaphase - Dicentrics/translocations
- Cytokinesis - Micronuclei

Dicentrics - Dose response relationship

Human lymphocytes:

- 0.9 MeV fission neutrons
- 7.6 MeV neutrons
- 250 kVp X-rays
- $^{60}$Co $^3$-rays

Graph showing the dose response relationship for dicentrics in human lymphocytes.
Automatic dicentric hunting

Manual scoring:
~ 50 cells in 1 hr
~ 500 cells in 10 hr

Automatic scoring:
1. Rapid response mode:
   - 150 cells in 30 mins; ~2 min staff effort;
   - +/− 0.5 Gy
2. Full mode:
   3000 cells in 10 h;
   ~ 40 min staff effort;
   +/− 0.15 Gy

The micronucleus assay

Comparison to dicentric assay

- MN arise mainly from acentric fragments
- Scoring easier & faster
- Not specific to radiation
- More background noise (especially for women...)
- Less sensitive (dose limit >0.2 Gy)
- Unstable signal fades like the dicentric
- Cannot easily discriminate between total and partial body exposure


Premature chromosome condensation

1. **Peripheral Blood**
2. **Ficoll Separation**
3. **Fuse in PEG**
4. **Lympocytes**
5. **Chinese Hamster Ovary (CHO) Cells** (grown in BrdU)
6. **Colcemid**
7. **Mitotic Shake off (Metaphase Cells)**
8. **Incubate 1 h** (Medium + PHA + Colcemid)
9. **PCC**
Some limitations with current cytogenetic analysis methods

Ideal scenario: Acute, whole body, homogeneous, external irradiation of a known low LET source, with dose in the region of >0.1 - ~ 5 Gy...

Real life exposures:

- Delayed blood sampling
- Protraction or fractionation, chronic exposures
- Inhomogeneity
- Very high or very low doses
- High LET radiation
- Internally deposited radionuclides
- Mass casualty scenarios
- Inter-scorer, -lab, -assay variation and other confounders

Non-uniform exposure

- Practically all accidents involve inhomogeneous / part-body exposure

- Dicentrics in lymphocytes indicate averaged whole body dose

- Uniform exposure (e.g. in test tube) – Poisson distribution

- Non-uniform – Overdispersion

- Dolphin et al.: ‘Contaminated Poisson’ method

0.3 dicentrics per cell:
Fluorescence in situ hybridisation for stable translocations

DNA Damage - Gamma-H2AX as a marker for DSB

- H2AX is a component of the DNA packaging scaffold (chromatin) in the cell.

- Each radiation-induced DSB induces hundreds of H2AX phosphorylations.

- $^3$H2AX antibody visualises and quantifies individual DSBs at very low doses.

Rothkamm & Horn (2009), Ann Ist Super Sanita 45:265-71; Barnard et al (2013), Genome Integr 4:1
Gene expression, transcriptional responses

Abend et al., 2021: 10.1038/s41598-021-88403-4; Cruz-Garcia et al, 2022; 10.1186/s13014-021-01807-4
**3'-H2AX assay for biological dosimetry**

Blood samples taken after CT scans:

![Image of cellular samples](image)

Rothkamm et al (2007), Radiology 242:244-51
Thermo-/optically- stimulated luminescence

OSL - resistors

(1) Protective coating
(2) Resistive film
(3) Inner electrode
(4) Ni plating
(5) Sn plating
(6) Ceramic substrate

~10-20 mGy detectable but 50% fading in 10 days

EPR dosimetry measures the concentration of radicals with unpaired electrons in a sample.

[called out by measuring how much microwave energy is absorbed due to ‘flipping’ of electron spins in a magnetic field]

Radicals persist sufficiently long only in very few ‘solid’ biological materials:
• Calcified tissues (teeth, bones)
• Finger/toe nails
• Hair
...but also detectable in personal belongings, e.g. glass (watch, glasses, phone...)

\[ \Delta E = E_{1/2} - E_{-1/2} \]

\[ m_s = + 1/2 \]

\[ m_s = - 1/2 \]
EPR in vivo: teeth

Field deployable in vivo EPR tooth dosimeter being developed...
• 17 cm dipole magnet
• SE ~0.9 Gy
• Smaller versions planned

Future work: Improve sensitivity further, develop smaller field-deployable systems suitable for triage

http://www.dartmouth.edu/~eprctr/research/tooth_dosimetry.shtml
Mass casualty radiation accidents

1) Initial clinical triage - vomiting, etc
2) Secondary triage - retrospective dosimetry

Problem
Small very specialised laboratories with limited surge capacity

Solutions
- Score fewer cells at first (e.g. 50 instead of 500) (ISO 21243)
- Automation
- New methods / combination of methods
- Assistance networks
Retrospective dosimetry assistance networks

Regional:
EU: RENEB (Kulka et al, Int J Radiat Biol. 2017 Jan;93(1):2-14); based on recent research projects
Latin America, Japan, Canada, ...

Worldwide:
International Atomic Energy Agency – Response Assistance Network (RANET)
World Health Organization – BioDoseNet
EURADOS WG 10

SATELLITE EVENT
Training Course on "How to Measure and Analyze Luminescence Signals for Potential Applications in Radiation Dosimetry: Theory and Computational Procedures" (WG10)

The training course takes place on Monday, June 12th, 08:00-12:30 and consists of two parts:

PART 1: Steve McKeeve, Emeritus Regents Professor of Physics, Oklahoma State University, USA
"Garbage in Garbage Out" Understanding What You Measure is Critical
The discussion will cover understanding kinetics, what the standard equations mean and don't mean, how to collect the data, essential basic analytical tools, rudimentary and novel peak fitting approaches.

PART 2: Vasilis Fagonis, Emeritus Professor of Physics, McDaniel College, USA and Associate Editor, Radiation Measurements
Luminescence signal analysis with open access software in Python and R
Practical examples will be demonstrated using software codes in Python and R for TL/OSL analysis, using actual experimental data files from dosimetric materials. Codes will be made available at the workshop in the form of Jupiter notebooks which participants will be able to download freely from the web. Using their own laptops, participants can log into their Google Drive account and can run the codes immediately in.

Joint EURADOS WG7 (Internal dosimetry) and WG10 (Retrospective dosimetry) workshop: Biological Dosimetry techniques and EPR applied to accidental intakes of radionuclides - open to all

9th October 2022, 13:30 – 18:00

Aim: To discuss the key open research questions and the potential to address these through collaborative research within and external to EURADOS.

Presentations will include:

* Topic 1: Biological and internal dosimetry for radiation medicine: current state and prospects for the future, Satoshi Tashiro (Hiroshima University)
* Topic 2: Establishment of calibration curves and intercomparison on calibration, Liz Ainsbury (UKHSA)
* Topic 3: Uncertainties in internal dosimetry/biodosimetry, Augusto Giussani (Federal Office for Radiation Protection (FFS) and Paco Barquinero (Universitat Autonoma de Barcelona)

The presentations will be followed by round table discussions on how to move forward with these topics.
Summary

- Retrospective dosimetry quantifies radiation-induced changes to biological or certain physical ‘fortuitous’ materials carried by suspected exposed individuals.

- Development started in the 1960s; today we have a robust set of tools and techniques to estimate dose and in some circumstances say something about circumstances of exposure.

- Development continues!

- For large scale emergency response, networking in development and validation is absolutely key to ensuring we are able to respond with the state of the art for retrospective dose estimation.

- EURADOS WG 10, RENEB and other networks remain essential – this has been and must remain an international effort.
Thank you for listening!

All work/images not referenced are my own or presented on behalf of WG 10 - thank you to all colleagues.

Questions or comments? Interested to join WG 10?

Do get in touch: liz.ainsbury@ukhsa.gov.uk