Biological Dosimetry: Recent developments in RENEB and at the BfS

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October 17th, 2018, ARADOS Meeting

Seoul
Recent developments in RENEB
(Running the European Network of Biological and retrospective Physical dosimetry)
Strategies to handle large scale scenarios

- Automation of processing biological material and analysis

- Scoring strategies
  - Scoring “in triage mode”: rough dose estimation for fast classification
  - Web analysis

- Networking
Networking in retrospective dosimetry

- mutual assistance of laboratories in large scale radiological incidences
- guarantee highest efficiency in processing and scoring of biological and inert samples
- fast classification of individuals based on the received dose:
  - identification of individuals needing medical care
  - reassurance of the „worried well“
- long-term follow up
**Background**

**Chronology of Networking**

- **1986, 1987**
  - "Wake up calls": Chernobyl, Goiania

- **2004**
  - Networking: Russia, Belarus, Ukraine; Help offered internationally

- **2009**
  - Tripartite Network: MoU with IRSN, NRPB/PHE, BfS

- **2010**
  - TENEBS EU project: Pan-European survey

- **2012**
  - RENEB EU project: Realizing the European Network of biological dosimetry and physical retrospective dosimetry

- **2016**
  - MULTIBIODOSE EU project: Focus on large scale emergencies

- **2017**
  - RENEB Association: Running …

**EU project:**
- **Pan-European survey**
- **Realizing …**
- **Running …**
- **Association**
26 RENEB partners (16 European countries) with MoU

- BfS, Germany
- CEA, France
- ENEA, Italy
- ICHTJ, Poland
- INSP, Romania
- ISS, Italy
- IST, Portugal
- IRSN, France
- LAFE, Spain
- NCRRP, Bulgaria
- NCSRD, Greece
- NRPA, Norway
- OKK, Hungary

- PHE, United Kingdom
- SERMAS, Spain
- SU, Sweden
- UAB, Spain
- UGent, Belgium
- UNITUS, Italy
- AMVRC, Italy
- DIT, Ireland
- FZ Jülich
- INFN, Italy
- RPC, Lithuania
- SCK•CEN, Belgium
- US, Spain

Association (Sept. 2018)

Institutions (voting members)
- BfS, Germany
- BIR, Germany
- HGUGM, Spain
- IRSN, France
- NCRRP, Bulgaria
- PHE, United Kingdom
- SU, Sweden
- UAB, Spain
- UGent, Belgium
- INFN, Italy
- IST, Portugal
- NCSRD, Greece
- IRBA, France

Associate members with link to
- HMGU & EURADOS
- NRPA, Norway
Background of the member organizations

- national radiation protection authorities
- research organisations
- universities
- hospitals

→ most with experience in handling radiation accidents
→ some with status as official national biodosimetry laboratory
→ many involved in QA&QM (ISO standards, IAEA Technical Report)
→ others (universities, research organisations) bring in new developments and knowledge
Operational Basis: “Ready-to-use mode” of reliable, proven methods

- Dicentric Assay
- Fluorescence in situ hybridisation (FISH-Assay)
- Micronucleus assay
- Premature Chromosome Condensation (PCC)
- Gamma H2AX Foci
- Electron Paramagnetic Resonance/ Optically Stimulated Luminescence (EPR/OSL) on mobile phone & on tooth, nails

Electronic components and Glass
Operational Basis: “Ready-to-use mode” of reliable, proven methods

Do we need so many different methods?

Yes, because different scenarios demand for different techniques, especially with regard to

- time frame
- access to persons
- sample collection
- shipment, storage
- detection range
- robustness

Choice of best biomarker according to the current needs
# Operational network performance

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sample type</th>
<th>Time frame for sample collection</th>
<th>Classification of individuals: Green / Orange / Red (&lt;1Gy / 1-2Gy / &gt;2Gy)</th>
<th>Detection range (Gy)</th>
<th>Robustness</th>
<th>Implication of individual sensitivity</th>
<th>Stored material: Type and time range for further analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dic</td>
<td>blood</td>
<td>days - months</td>
<td>Time from sample receipt to result</td>
<td>ca 1000</td>
<td>0.1 – 5</td>
<td>high</td>
<td>fixed cells, slides: years</td>
</tr>
<tr>
<td>MN</td>
<td>blood</td>
<td>days - months</td>
<td>RENEb capacity (analysed persons per week)</td>
<td>ca 400</td>
<td>0.2 - 5</td>
<td>medium</td>
<td>slides: years</td>
</tr>
<tr>
<td>FISH</td>
<td>blood</td>
<td>days - years</td>
<td>120 hours</td>
<td>ca 100</td>
<td>0.3 - 4</td>
<td>medium- high</td>
<td>fixed cells: years</td>
</tr>
<tr>
<td>PCC</td>
<td>blood</td>
<td>hours - months</td>
<td>2 - 8 hours</td>
<td>ca 50</td>
<td>0.1 – 20</td>
<td>high</td>
<td>frozen lymphocytes, fixed cells, slides: years</td>
</tr>
<tr>
<td>γH2AX</td>
<td>blood</td>
<td>days</td>
<td>3 hours</td>
<td>ca 1800</td>
<td>0.2 – 5</td>
<td>low</td>
<td>Fixed cells, slides: up to one year</td>
</tr>
<tr>
<td>EPR</td>
<td>PED³</td>
<td>hours- years</td>
<td>&lt;1 hour</td>
<td>ca 770</td>
<td>&gt;1</td>
<td>high</td>
<td>Glass: years</td>
</tr>
<tr>
<td>OSL</td>
<td>PED⁵</td>
<td>hours- months</td>
<td>&lt;1 hour</td>
<td>ca 500</td>
<td>&gt;0.1</td>
<td>high</td>
<td>Resistors: weeks</td>
</tr>
</tbody>
</table>

1. Time between irradiation and sample collection
2. Time from arrival of a sample in the laboratory until the classification of a person, without time for transport/shipment
3. Robustness: high: little influence of disturbing factors; medium: some influence of age, smoking, other agents; low: large influence of other agents and factors;
4. Considering the individual sensitivity of a person
5. Type of the stored material and time frame to perform further analysis;
6. PED: personal electronic device (glass touchscreen);
7. PED: personal electronic device (resistors from circuit board);
Quality Assurance

- mutual assistance of laboratories in large scale radiological incidences
- guarantee the highest efficiency in the processing and in scoring of biological and inert samples

mandatory: comparable results in individual retrospective dose estimation
Quality Assurance in RENEB

- QA Manual
  - for each assay
  - for networking

- Intercomparisons
  - different focus
  - mandatory for partners
  - open to non-partners (also from outside the EU)

- Education and training
  - training exchanges
  - seminars
Intercomparisons within RENEB

- 1st Intercomparison (2013):
  - RENEB partners from the EU
  - The evaluation process was followed by a round of training activities

- 2nd Intercomparison (2014):
  - RENEB candidates and non-EU laboratories

- 3rd Intercomparison (2017):
  - RENEB members and beyond
  - Currently evaluated
Training effect: 1st vs 2nd Intercomparison

Wojcik et al., IJRB 2017
2nd Intercomparison: Material

- 137- Cs gamma source, dose rate 0.478 Gy / min
  (Procedure and Equipment same as 1st exercise 2013)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose (Gy)</th>
<th>Irradiation Time</th>
<th>Donor Gender</th>
<th>Donor Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>RENEB 5 Low dose</td>
<td>0.85</td>
<td>1.8 min</td>
<td>Female</td>
<td>31 years</td>
</tr>
<tr>
<td>RENEB 6 High dose</td>
<td>2.7</td>
<td>5.6 min</td>
<td>Male</td>
<td>22 years</td>
</tr>
</tbody>
</table>

- Irradiation of blood samples for Dic, MN, FISH and PCC assay

*Oestreicher et al., IJRB 2017*
2nd Intercomparison: Shipment worldwide

- **RENEB**
  19 labs, 14 EU countries

- **RENEB-Candidates:**
  4 labs, 3 EU countries

- **Non-RENEB**
  19 labs, 18 countries
  Contact labs in:
  - Japan
  - Canada
  - Uruguay
  - South Africa
  - Serbia (Europe, but not EU)

Total: 42 labs, 35 countries for Dic- & MN assay
2nd Intercomparison: 1st shipment

- TNT-express service: as UN 3373 Biological Substance Category B

Within EU
RENEB partners and candidates – 24 h (28 h)

Non EU
Japan - 63 h
South Africa – 75 h
Canada, Uruguay, Serbia – failure (delivery period > 96 h)

- Temperature range:
  RENEB: 11 – 29 °C
  Japan: 15 – 28 °C
  South Africa: 16 – 26 °C

- Dosemeter: < 1 mSv
2nd Intercomparison: 2nd shipment

- **1 December 2014** (World Courier express service)

  - 3 non EU partners: Canada, Uruguay, Serbia
    - Shifted because of STRIKE

- **8 December 2014** (World Courier express service)
  - Canada: 47 h
  - Uruguay: 44 h
  - Serbia: 46 h

Thermobox: 15 – 25 °C
Intercomparison: Comparison of lab performance

Dic assay: z-score for all estimated doses based on 50 cells

- mean for the lab
- 95% CI of the mean
- boundaries of classification

Oestreicher et al., IJRB 2017
**Intercomparison: Classification performance**

- **Dose: 0.85 Gy**
  - 50 Cells scored

- **Dose: 2.7 Gy**
  - 50 Cells scored

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**Oestreicher et al., IJRB 2017**
Intercomparisons: Other assays

THE FIRST GAMMA-H2AX BIODOSIMETRY INTERCOMPARISON EXERCISE OF THE DEVELOPING EUROPEAN BIODOSIMETRY NETWORK RENEB


Dose assessment intercomparisons within the RENEB network using G0-lymphocyte prematurely condensed chromosomes (PCC assay)

Georgia I. Terzoudi, Gabriel Pantelias, Firouz Darroudi, Katarzyna Barszczewska, Iwona Buraczewska, Julie Depuydt, Dimka Georgieva, Valeria Hadjidjekova, Vasiliki I. Hatzi, Ioanna Karachristou, Maria Károustó, Roberta Meschini, Radhia M’kacher, Alegría Montoro, Fabrizio Palitti, Antonio Pantelis, Gaetano Pepe, Michelle Ricou, Laure Sabatier, Natividad Sebastia, Sylvester Sommer, Anne Vral, Demetrio Zafiropoulos, and Andrzej Wojcik

The second gamma-H2AX assay inter-comparison exercise framework of the European biosimetry network (RENEB)

Jayne Moquet, Stephen Barnard, Albena Staynova, Carita Lindholm, Octávia Monteiro Gil, Vanda Martins, Ute Rößler, Anne Vral, Charlot Vandevoorde, Maria Wojewódzka, and Kai Rothkamm

RENEB intercomparison exercises analyzing micronuclei (CM Assay)


Comparable dose estimates of blinded whole blood samples are obtained independently of culture conditions and analytical approaches. Second RENEB gene expression study

Grainne Manning, Ellina Macaeva, Matthaeus Majewski, Ralf Kriehuber, Kamil Brzóška, Michael Abend, Sven Doucha-Sení, Dominik Oskamp, Sonja Strunz, Roel Quintens, Matthias Port, and Christophe Badie

| Verantwortung für Mensch und Umwelt |
Accident simulation exercise

Brzozowska et al., IJRB 2017
**Accident simulation exercise**

- **Activation of the network**
- **Categorization of an exposure scenario**

**Attention – this is only an exercise**

Dear All,

A radiological emergency has occurred in our country. We are not sure about the number of casualties or worried wells. We are not able to carry out retrospective dosimetry by ourselves and need your assistance.

Please reply ASAP if and how many blood samples/smartphones you can receive during the coming 48 hours to carry out retrospective dosimetry. Please state the biodosimetric tool that you can perform.

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<table>
<thead>
<tr>
<th>Reference lab</th>
<th>Service lab</th>
<th>Please fill in the table by following the instructions given in the sheet &quot;Instructions.&quot;</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Code</th>
<th>Dose in Gy assessed by the assay</th>
<th>Mark triage category by X</th>
<th>Green</th>
<th>Orange</th>
<th>Red</th>
</tr>
</thead>
<tbody>
<tr>
<td>P01</td>
<td>0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>P02</td>
<td>0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>P03</td>
<td>0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>P04</td>
<td>0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>P05</td>
<td>0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>P06</td>
<td>0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>

**Mark the scenario by placing X where it corresponds to the set of doses.**

Only one scenario is possible for one patient.

- VBE < 0.5
- VBE 0.5 - 1.0
- VBE 1.0 - 1.5
- VBE 1.5 - 2.0
- VBE 2.0 - 2.5
- VBE 2.5 - 3.0
- VBE 3.0 - 3.5
- VBE 3.5 - 4.0
- VBE 4.0 - 4.5
- VBE 4.5 - 5.0
- VBE 5.0 - 5.5
- VBE 5.5 - 6.0
- VBE 6.0 - 6.5
- VBE 6.5 - 7.0

Brzozowska et al., IJRB 2017
Developing the Network

- **Open for new techniques**
  - Identification, validation and inclusion of new assays
    - Reporting sheets on the website
    - Literature research, direct contact to scientists
    - Candidates: e.g. gene expression, RAMAN spectroscopy
  - Development of existing assays: e.g. automation, telomere/centromere staining in dic and PCC assay

- **Open for new partners**
  - Identification, qualification and integration of new partners
    - Enhancing the capacity for dose estimation
    - New expertise and competences
Automatic scoring at the Federal Office for Radiation Protection, Germany (BfS)
Dicentric chromosomes (Dic) – Biomarker of exposure

Characteristics
• Highly specific to radiation
• High comparability (in vitro and in vivo)
• Low interindividual variations
• Stability
• Low background
  “Gold standard” for biodosimetry after recent radiation exposure (Hall et al. 2017)

Dose estimation
Routine scoring: 500 – 1000 cells
Minimum resolvable dose: 100 - 200 mGy, 95 % confidence limits

Limiting factors:
Scoring procedure – manual (by eye)
• Well-trained staff
• Labor intensive to analyse large number of cells

Not suitable for research in the low dose range < 100 mGy

Manual scoring
Automatic scoring of dicentric chromosomes

Automated Scoring System
Metafer 4 by MetaSystems

1. Metaphase finding (10 x magnification, MSearch)
2. Capture cells (63 x magnification, AutoCapt)
3. Automated scoring (DCScore)
4. Evaluation of dicentric candidates (by eye)

Enhancement of capacity and speed of the method

False Positive are detected rapidly:
Radiation sensitivity of young children for low doses?

**Blood samples**

- **Newborns**
  - N = 11/male

- **Children (2-5 years)**
  - N = 10/male

- **Adult (20-50 years)**
  - N = 12/male

**In vitro irradiation**

- Toshiba Aquilion/LB Model TSX-201A/1K

1 ml blood / dose / individual

- 0 mGy (sham exposure)
- 41 mGy (120 kVp, 50 mA, 15 sec)
- 978 mGy (120 kVp, 400 mA, 3 x 15 sec)

**Paediatric CT**

- **Head** (girl, 6-10 Jahre)
  - Effective Dose about 2 mSv

**Organ dose (mSv)**

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>Organ dose (mSv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>37</td>
</tr>
<tr>
<td>Thyroid gland</td>
<td>2</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>0.6</td>
</tr>
<tr>
<td>Lung</td>
<td>0.4</td>
</tr>
<tr>
<td>R. Bone marrow</td>
<td>5</td>
</tr>
</tbody>
</table>

E. Nekolla –BFS Seminar 07-2015

Bundesamt für Strahlenschutz

[28]
Results of the pilot study *(Gomolka et al., IJRB 2018)*

**Manual scoring of a low number of metaphase spreads** / “manual low”:
200 cells / individual, i.e. 2000 - 2400 cells / dose point / age group

- **No** significant increase of dicentric chromosomes with dose for single age groups
- **Significant** increase of dicentric chromosomes with dose when all groups are pooled
- **No** significant increase of radiation induced dicentric chromosomes between young and adult donors

Statistical power insufficient
Increased cell number (x10)

- **Automated scoring:** 2000 cells / individual, i.e. 13,000 – 31,000 cells / dose point / age group
- **Manual scoring increased cell number:** 2000 cells / individual, i.e. 26,000 cells / dose point / age group

- Significant increase of dicentric chromosomes with dose for each age group
- Significant increase of radiation induced dicentric chromosomes between young and adult donors

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Oestreicher et al., IJRB 2018, in press

| Verantwortung für Mensch und Umwelt |
Significantly increased risk for young donors to induce dicentric chromosomes at 41 mGy

Oestreicher et al., IJRB 2018
Capacity: Manual vs automatic scoring

<table>
<thead>
<tr>
<th>Number scorers</th>
<th>Cells / day</th>
<th>Cells scored</th>
<th>Time needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual</td>
<td>2</td>
<td>~ 400</td>
<td>104 433</td>
</tr>
<tr>
<td>Automated</td>
<td>1</td>
<td>~ 10 000</td>
<td>180 208</td>
</tr>
</tbody>
</table>

Factor ~ 26 (2x13)

Oestreicher et al., IJRB 2018, in press
Summary

- Increase in cell numbers enables the detection of dose-effect relationships for doses < 100 mGy
- Detection of age-dependent radiosensitivity for low dose level < 100 mGy after in vitro CT exposure
- Young donors showed statistically increased risk for radiation induced dicentric chromosomes at 41 mGy compared to adults.

Capability of automated scoring of dicentric chromosomes

- Reduction of extensive workload
- Decrease of the detection limit
- Opens possibilities for molecular epidemiological studies in radiation protection
Acknowledgement

All members from:

Sarah Baartout, SCK-CEN
Ausrele Kesmiene, IRAC

- Patient contact, information, blood sampling -