Dose assessment by cytogenetic analysis

Biodosimetry Research Team, NIRS
Yumiko Suto
Content

1) Biodosimetry
2) Dicentric chromosome assay (DCA)
3) NIRS Biodosimetry System
4) Case reports
   - JCO criticality accident
   - TEPCO Fukushima Daiichi NPS accident
1) Biodosimetry

2) Dicentric chromosome assay (DCA)

3) NIRS Biodosimetry System

4) Case reports
   - JCO criticality accident
   - TEPCO Fukushima Daiichi NPS accident
Acute Radiation Syndrome (ARS)

Less than 1 - 2 Gy / More than 4 - 5 Gy?

<table>
<thead>
<tr>
<th>Dose Range (Gy)</th>
<th>Prodromal Effects</th>
<th>Manifest Symptoms</th>
<th>Survival Expectancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 - 1.0</td>
<td>None to Mild (3 h to up to 48 h)</td>
<td>None to slight decrease in blood count</td>
<td>Almost Certain</td>
</tr>
<tr>
<td>1.0 - 3.5</td>
<td>Mild to moderate (1 h up to 48 h)</td>
<td>Mild to Severe Bone Marrow Damage</td>
<td>0 to 10% Death</td>
</tr>
<tr>
<td>3.5 - 7.5</td>
<td>Severe (1 h up to 48 h)</td>
<td>Pancytopenia, Mild to Moderate GI Damage</td>
<td>10 to 100% Death (within 2 to 6 weeks)</td>
</tr>
<tr>
<td>7.5 - 10.0</td>
<td>Severe (&lt;1 h up to 48 h)</td>
<td>Combined BM and GI Damage</td>
<td>90 to 100% Death (within 1 to 3 weeks)</td>
</tr>
<tr>
<td>&gt;10.0</td>
<td>Severe (minutes to &lt;48 h)</td>
<td>GI, Neurological and Cardiovascular Damage</td>
<td>100% Death (within 2 - 12 weeks)</td>
</tr>
</tbody>
</table>

(Modified from AFRRI 2003, Prasanna et al. 2004)
What is dose assessment?

In order to assess an effective dose, it is necessary to evaluate the external exposure caused by gamma ray or neutron, and the internal exposure caused by ingestion from contaminated food or soil and inhalation from radioactive fallout.

→ Useful information for the medical triage and management of radiological casualties with suspected ARS
**External exposure**

Individual monitoring for exposure is done by:

- Clinical dosimetry
  * Medical symptoms, blood cell counts...
- Physical dosimetry
  * Personal dosimeter
  * Dose reconstruction
- Biodosimetry
  * Chromosome Analysis
  * ESR, new technologies...
Chromosome aberrations induced by radiation

- Fragment
- Deletion
- Translocation
- Dicentric chromosome and fragment
- Ring chromosome and fragment
- Metaphase
Chromosome analysis of PBMCs with in vitro neutron irradiation.

(Suto et al., unpublished data)

Mathematical relation between dose and aberration frequency

Low LET • • • aberrations/cell = aD^2 + bD + c
High LET • • • aberrations/cell = aD + c

(D: dose)

(Russell J., et al., 1980)
<table>
<thead>
<tr>
<th>Typical aberrations scored for biological dosimetry applications</th>
<th>Premature chromosome condensation (PCC)</th>
<th>Dicentric (and ring) (DCA)</th>
<th>Fluorescent in situ hybridization (FISH)</th>
<th>Cytokinesis-block micronucleus (CBMKN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>excess chromosome fragments; dicentrics and rings</td>
<td>dicentrics and rings</td>
<td>dicentrics (and rings)</td>
<td>micronuclei</td>
<td>translocations</td>
</tr>
<tr>
<td>translocations</td>
<td></td>
<td></td>
<td>nucleoplasmic bridges</td>
<td></td>
</tr>
<tr>
<td>Typical radiation scenario applications</td>
<td>acute</td>
<td>acute</td>
<td>acute</td>
<td>acute</td>
</tr>
<tr>
<td>recent exposure</td>
<td>recent exposure</td>
<td>protracted recent exposure</td>
<td>protracted old exposure</td>
<td>protracted recent exposure</td>
</tr>
<tr>
<td>Photon equivalent, acute dose range (Gy) for whole-body dose assessment</td>
<td>0.2 to 20</td>
<td>0.1 to 5</td>
<td>0.25 to 4</td>
<td>0.3 to 4</td>
</tr>
<tr>
<td>Useful for partial-body exposure applications</td>
<td>Yes</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Useful for triage dose assessment</td>
<td>Yes</td>
<td>Yes</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>Status of assay standardization</td>
<td>NA</td>
<td>ISO standards</td>
<td>NA</td>
<td>ISO standard — pending, and [5]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>For higher doses</th>
<th>Karyotyping Stable aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower background Less variable Gold Standard</td>
<td></td>
</tr>
<tr>
<td>Higher background Effect of other events</td>
<td></td>
</tr>
</tbody>
</table>

a Table modified from TMT Handbook [6].

b Specific chromosome aberrations typically detected by use of centromeric and whole-chromosome specific DNA hybridization probes.

c NA: not applicable/not available.

(IAEA, 2011, modified)
**FLOWCHART OF CYTOGENETIC DOSIMETRY**

**Triage**

- Suspected Acute-radiation Overexposure
  - Criticality Accident Radioterrorism

**Dose Estimation**

**Complementary Techniques**

- G0/G1 - PCC Assay
  - 5 h
- G2 - PCC Assay
  - 48 h

**Main Stream**

**Dicentric Assay**

**Micronuclei Assay**

- PNA - FISH (centromere / telomere)
- M - FISH / Chromosome painting

PCC: Premature Chromosome Condensation
PNA: Peptide Nucleic Acid
M-FISH: Multiplex in situ Hybridization
Standardization of protocols

IAEA

Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies

WHO BioDose Net, IABERD, etc.

ISO

ISO 17099 (draft): CBMN assay
ISO 17136 (draft): calibration curve
ISO 19238: Dicentric Chromosome Assay (DCA)
ISO 21243: DCA for mass casualty
The principle of biodosimetry

1) Collect information and prepare for dose assessment
   * the population at risk, radionuclides, etc.

2) Take biological samples

3) Measure biological indicator(s)
   for radiation-induced damages

4) Compare the test result with a calibration curve
   → triage mode / full scoring mode
   → inform clinical treatment decisions
   * total vs. partial body exposure?
   * time since exposure?
   * acute, fractionated, or chronic exposure?
1) Biodosimetry

2) Dicentric chromosome assay (DCA)

3) NIRS Biodosimetry System

4) Case reports
   - JCO criticality accident
   - TEPCO Fukushima Daiichi NPS accident
Data analysis

* A standard dose-response calibration curve is established experimentally in advance.

* For each patient, the frequency of dicentric chromosomes/cell will be applied to the standard curve and the dose can be estimated.

Dose Estimate, CABAS:
Calibration curve fitting
Dose estimations and statistics
G function (fractionated / protracted exposure)
Distribution analysis
Criticality analysis, etc.

Marta Szłuińska et al. (2007): CABAS 2.0
Automation of chromosome image analysis - metaphase detection (and dicentric scoring) -
1) Biodosimetry

2) Dicentric chromosome assay (DCA)

3) NIRS Biodosimetry System

4) Case reports
   - JCO criticality accident
   - TEPCO Fukushima Daiichi NPS accident
NIRS Biodosimetry System

**Radiation Accident (Emergency Call)**

- **Day 0**
  - Blood collection, isolation of PBMCs, cell culture (48h, 1\textsuperscript{st} division)
  - Harvesting, fixation, chromosome preparation, staining

- **Day 1**
  - 3-10 mL

- **Day 3**
  - *Automatic* metaphase-finding $\rightarrow$ chromosome-image analysis
  - medical triage scoring $\rightarrow$ report to the doctors

- **Day 4**
  - Biodosimetry scoring
  - $\rightarrow$ Upload the result to their records
  - Explain to the patient at their next consult

- **FISH analysis**
- **Genomic analysis**

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**NIRS DCA System**

- Based on IAEA Manual, ISO 19238, ISO 21243
- Questionnaire & Informed consent

**Explanation**

- **Dose estimation**
  - **Calibration curve**
  - **Dose (Gy)**

- **Age, sex, alcohol, smoking, medicine, histories of medical and occupational exposures, etc.**
NIRS Biodosimetry System

~ Proceeding to Molecular Cytogenetics ~

1) Dicentric analysis with PNA-FISH (especially for high-dose exposure)
2) M-FISH for translocation analysis
3) Three-color painting (FISH) for translocation analysis
4) G0/G1 PCC assay with PNA-FISH / M-FISH
Chromosome Network, NIRS, Japan

* Asahikawa Medical College
* Hirosaki University
* NIRS
* Tokyo Medical and Dental University
* Human Service Center
* Osaka Prefecture University
* Nagasaki University
* Radiation Effects Research Foundation
* Hiroshima University
* Nagasaki University
Realising the European Network of Biodosimetry (RENEB)

16 EU countries (23 laboratories)
* dicentric assay, FISH assay, micronucleus assay, PCC assay, Gamma-H2AX assay and EPR/OSL assay, ...
NIRS-IAEA Workshop on Cytogenetic Biodosimetry for Asia 2011
and NIRS-ISTC Workshop on Cytogenetic Biodosimetry
in cooperation with WHO
26-27 January 2011

Organized by National Institute of Radiological Sciences (NIRS), International Science and Technology Center (ISTC) and International Atomic Energy Agency (IAEA) in cooperation with World Health Organization (WHO)

Asia Inter-comparison Study in 2008

NIRS activity of international networking for Biodosimetry

$^{60}$Co-gamma ray (0.6 Gy/min)
Donor 1: 4.5 Gy (A) and 0.45 Gy (B), Donor 2: 4.5 Gy (C)
Clinical cytogeneticists in Japan

220 experts certified by the Japan Society of Human Genetics and retired experts

Pooling the expert lists to support for the radiation accidents (with combined disaster)

Information and experience → to each lab

~ expansion of pool of cytogeneticists ~

Educational and training courses on cytogenetics of radiation exposure and periodic exercises

* Our NIRS lab has been certified as training lab by the Japan Society of Human Genetics since November 2010.
1) Biodosimetry

2) Dicentric chromosome assay (DCA)

3) NIRS Biodosimetry System

4) Case reports
   - JCO criticality accident
   - TEPCO Fukushima Daiichi NPS accident
Cytogenetic Dose Estimates

JCO criticality accident (1999)
Chromosome aberration analysis in persons exposed to low-level radiation from the JCO criticality accident in Tokai-mura.

Masao Sasaki 1, Isamu Hayata 2, Nanao Kamada 3, Yoshiaki Kodama 4, Seiji Kodama 5

(1 Kyoto Univ.; 2 NIRS; 3 Hiroshima Univ.; 4 RERF; 5 Nagasaki Univ.)

Cytogenetical dose estimation for 3 severely exposed patients in the JCO criticality accident in Tokai-mura.

Isamu Hayata 1, Reiko Kanda 1, Masako Minamihisamatsu 1, Akira Furukawa 1, Masao Sasaki 2

(1 NIRS; 2 Kyoto Univ.)

The criticality accident in a uranium conversion test plant in Tokai-mura

On September 30, 1999, at around 10:35 a.m., the criticality accident occurred in a uranium conversion test plant of the JCO Ltd., in Tokai-mura, Ibaraki Prefecture, Japan. As two workers (A and B) were pouring uranyl nitrate into a precipitation tank, the solution emitted a flash of blue light and the alarms went off to warn against gamma rays.

Another worker (C) who was in the corridor next to the room immediately recognized that a criticality accident had taken place and ordered the two workers to evacuate.
Cytogenetic Analysis

46 persons in total were analyzed:

* 3 JCO patients (A, B and C)
* 7 other workers of JCO whose cell counts were abnormal
* 26 other JCO workers
* 7 workers who worked at other places near JCO
* 3 firemen

→ Chromosomal aberrations were detected
  only in the three patients.
Prematurely condensed chromosomes having PCC-rings (arrows) in a lymphocyte of Patient A.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Marker</th>
<th>Frequency of chromosomal aberrations/cell</th>
<th>Dose estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>PCC-R</td>
<td>150/100, 9h, - 23h, - 48h, 150/100</td>
<td>&gt;20 GyEq*</td>
</tr>
<tr>
<td></td>
<td>Dic</td>
<td>445/50, 197/20, 73/8, 715/78</td>
<td>22.6 (15.0→30) GyE**</td>
</tr>
<tr>
<td></td>
<td>Dic+Rc</td>
<td>563/50, 250/20, 90/8, 903/78</td>
<td>24.5 (16.0→30) GyE**</td>
</tr>
<tr>
<td>B</td>
<td>PCC-R</td>
<td>77/100, 9h, - 23h, - 48h, 77/100</td>
<td>7.4 (6.5→8.2) GyE*</td>
</tr>
<tr>
<td></td>
<td>Dic</td>
<td>199/75, 127/50, 153/50, 479/175</td>
<td>8.3 (6.9→9.8) GyE**</td>
</tr>
<tr>
<td></td>
<td>Dic+Rc</td>
<td>224/75, 147/50, 166/50, 537/175</td>
<td>8.3 (6.9→10.0) GyE**</td>
</tr>
<tr>
<td>C</td>
<td>PCC-R</td>
<td>24/100, 9h, - 23h, - 48h, 24/100</td>
<td>2.3 (1.8→2.8) GyE*</td>
</tr>
<tr>
<td></td>
<td>Dic+Rc</td>
<td>63/100, 64/100, 64/100, 191/300</td>
<td>3.0 (2.8→3.2) GyE***</td>
</tr>
</tbody>
</table>

NIRS-R-47 (2002), modified
Cytogenetic Dose Estimates

TEPCO Fukushima Daiichi NPS accident (2011)
The earthquake affected the Fukushima Daiichi Nuclear Power Plant (NPP) and caused serious damages to NPP, resulting in a large amount of radioactive materials being released into the environment.

- I-131: $1.6 \times 10^{17}$ Bq
- Cs-134: $1.8 \times 10^{16}$ Bq
- Cs-137: $1.5 \times 10^{16}$ Bq

As of June 6, 2011
(by Nuclear and Industrial Safety)
Sending Experts : REMAT

Radiation Emergency Medical Assistance Team

National Institute of Radiological Sciences (NIRS)
The transportation system was paralyzed.  
(It took 10 – 14 days for sending blood samples.)
11 March  Checked all facilities and stocks in NIRS

→ 1,000 patients could be received
   200 patients at one time could be handled

12 ～ Information from REMAT
via the NIRS Information Sharing System

1) I-131, I-133, Cs-134, Cs-137 → External exposure (gamma-rays)
2) Lower dose exposure
   (Higher dose exposure = mainly because of unexpected access to radioactive wastes that were scattered around the 1F site after the hydrogen explosions)

→ possibly one or a few workers per one time would be received as patients (none from residents)
→ could be conveyed by JSDF

Priority: workers at 1F site with suspect overexposure (more than 1 Gy) or uncertain exposure

→ Notice to the Government, TEPCO, Fukushima Pref. and hospitals
(1 April, 2011, all the site-workers got APDs.)
NIRS DCA System

**Radiation Accident (24-h Emergency Call)**

- Blood collection, isolation of PBMCs, cell culture (48h, 1st division)
- Harvesting, fixation, chromosome preparation, staining

**Automatic** metaphase-finding → chromosome-image analysis

medical triage scoring → report to the doctors

- Biodosimetry scoring
- Upload the result to medical report

- Explain to the patient at their next consult

- **FISH analysis**
- Genomic analysis

**Day 0**

- Blood collection, isolation of PBMCs, cell culture (48h, 1st division)
- Harvesting, fixation, chromosome preparation, staining

**Day 1**

- Biodosimetry scoring
- Upload the result to medical report

**Day 3**

- Biodosimetry scoring
- Upload the result to medical report

**Day 4**

- Biodosimetry scoring
- Upload the result to medical report

NIRS DCA System based on IAEA Manual 2011, ISO 19238, ISO 21243

**Questionnaire & Informed consent**

- Age, sex, alcohol, smoking, medicine, histories of medical and occupational exposures, etc.

**Automatic** metaphase-finding → chromosome-image analysis

Dose estimation

- DIC/cell
- Calibration curve
- Dose (Gy)
Biodosimetry of restoration workers for Tokyo Electric Power Company (TEPCO) Fukushima Daiichi Nuclear Power Station Accident

Yumiko Suto 1, Momoki Hirai 1, Miho Akiyama 1, Gen Kobashi 1, Masanari Itokawa 2, Makoto Akashi 1, Nobuyuki Sugiura 1,3

(1 NIRS, JAPAN; 2 Tokyo Metropolitan Institute of Medical Science, JAPAN; 3 NSRA, JAPAN)

(Suto et al., 2013b)
NIRS received 1F site-workers with suspected overexposure for medical care and physical and biological dosimetry

For biodosimetry, 12 individuals were received from 21 March to 1 July 2011.

→ Patients were back every week for internal dose assessment.

Re-examination of DCA: after 3 months and 1 year

(Suto et al., 2013b)
• No individuals showed values exceeding the lower limit level of medical triage for ARS (1 Gy).

• Six individuals took an annual health examination (29 July - 6 August 2012): every individual showed either a decreasing tendency or equal values to the results obtained from the former examination.

(Suto et al., 2013b)
Exposed dose to emergency workers at 1F NPP

External exposure

<table>
<thead>
<tr>
<th>Dose (mSv)</th>
<th>Mar</th>
<th>Apr</th>
</tr>
</thead>
<tbody>
<tr>
<td>250&lt;</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>100~150</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>50~100</td>
<td>163</td>
<td>25</td>
</tr>
<tr>
<td>≤50</td>
<td>3545</td>
<td>5727</td>
</tr>
<tr>
<td><strong>total (person)</strong></td>
<td><strong>3745</strong></td>
<td><strong>5752</strong></td>
</tr>
<tr>
<td><strong>max (mSv)</strong></td>
<td><strong>199</strong></td>
<td><strong>85</strong></td>
</tr>
<tr>
<td><strong>ave (mSv)</strong></td>
<td><strong>14</strong></td>
<td><strong>1.1</strong></td>
</tr>
</tbody>
</table>

External+internal exposure

<table>
<thead>
<tr>
<th>Dose (mSv)</th>
<th>Mar~Nov</th>
</tr>
</thead>
<tbody>
<tr>
<td>250&lt;</td>
<td>6</td>
</tr>
<tr>
<td>100~150</td>
<td>139</td>
</tr>
<tr>
<td>50~100</td>
<td>686</td>
</tr>
<tr>
<td>≤50</td>
<td>17989</td>
</tr>
<tr>
<td><strong>total (person)</strong></td>
<td><strong>18846</strong></td>
</tr>
<tr>
<td><strong>max (mSv)</strong></td>
<td><strong>679</strong></td>
</tr>
<tr>
<td><strong>ave (mSv)</strong></td>
<td><strong>12</strong></td>
</tr>
</tbody>
</table>

No Acute Radiation Syndrome

(from TEPCO press release 2012.12.27)
Summary

◆ Biodosimetry based on cytogenetic assays has been used to estimate the absorbed dose in the exposed individual.

→ to get useful information for the medical triage and management of radiological casualties with suspected acute radiation syndrome (ARS) when a radiation accident or unplanned radiation exposure occurs.

◆ Dicentric Chromosome Assay (DCA) is the ‘Gold Standard’ for biodosimetry, which has been used in the past radiation accidents.

→ also applied to the JCO criticality accident (1999, Japan) and the Fukushima Daiichi NPS accident (2011, Japan).

◆ Further efforts are being made to develop more rapid methods and regional / international networking in consideration of mass-casualty radiation accidents.