Update on AFRRI’s Cytogenetic Biodosimetry Activities

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AFRRI

Uniformed Services University
The opinions, conclusions, and recommendations expressed or implied do not necessarily reflect the views of the Department of Defense or any other department or agency of the federal government.

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Outline

• Cytogenetic Biodosimetry – Background
• AFRRI’s Cytogenetic Biodosimetry Laboratory
• Dicentric Chromosome Aberration (DCA) Assay
  - Manual scoring
  - Automated scoring
• Premature Chromosome Condensation (PCC) Assay
  - Centromeric painting to score dicentrics
  - Multiple endpoints
    (excess fragments, rings, length ratio, and dicentrics)
• DoD Biodosimetry Network
Cytogenetic Biodosimetry
# Table 1. Comparison of Cytogenetic Aberration Assays Used for Dose Assessment

<table>
<thead>
<tr>
<th>Cytogenetic Aberration Assays</th>
<th>Premature chromosome condensation (PCC)</th>
<th>Dicentric (and ring) (DCA)</th>
<th>Fluorescent in situ hybridization (FISH)</th>
<th>Cytokinesis-block micronucleus (CBMN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical aberrations scored for biological dosimetry applications</td>
<td>excess chromosome fragments; dicentrics(^b) and rings</td>
<td>dicentrics(^b) (and rings(^a))</td>
<td>dicentrics(^b) (and rings)</td>
<td>micronuclei</td>
</tr>
<tr>
<td>Typical radiation scenario applications</td>
<td>translocations(^b)</td>
<td>translocations(^b)</td>
<td></td>
<td>nucleoplasmic bridges</td>
</tr>
<tr>
<td>Photon equivalent, acute dose range (Gy) for whole-body dose assessment</td>
<td>acute</td>
<td>acute</td>
<td></td>
<td>acute</td>
</tr>
<tr>
<td>Useful for partial-body exposure applications</td>
<td>recent exposure</td>
<td>protracted recent exposure</td>
<td></td>
<td>protracted recent exposure</td>
</tr>
<tr>
<td>For higher doses</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>Status of assay standardization</td>
<td>Yes</td>
<td>Yes</td>
<td>NA(^c)</td>
<td>NA</td>
</tr>
<tr>
<td>Status of assay standardization</td>
<td>ISO standards ([3, 4])</td>
<td></td>
<td>NA</td>
<td>ISO standard — pending, and ([5])</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.
Experimental Design
- Human peripheral blood ex vivo irradiation model

Radiation Dose
0,1,3,5 Gy

- 0.6 Gy/min
  \(^{137}\text{Cs}\) gamma rays

- Treatment with 100 nM calyculin A
  \(\text{30 min}\)
  before harvesting

PCC Assay
Culture at 37°C for 48 h
+ PHA

DCA Assay
Culture at 37°C for 24 h
+ PHA

Analysis of chromosome morphology
(fragments and rings)

Analysis of chromosome morphology
(dicentrics and rings)

Treatment with 0.1 µg/ml colcemid
\(\text{24 h}\)
before harvesting
AFRRI Resources – Cytogenetic Activities

AFRRI
Multiple Radiation Sources and
Dosimetry Support

www.metasystems.org/

Hanabi Metaphase Chromosome Harvesters

Metafer

Hanabi Metaphase Chromosome Harvesters

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Hanabi Metaphase Chromosome Harvesters

Metafer

Hanabi Metaphase Chromosome Harvesters

AFRRI
Uniformed Services University
MetaSystems Inc.
Ikaros Karyotyping Software

[Image of chromosome analysis software interface]
Dicentric Chromosome Aberration (DCA) Studies

- Performance exercises and inter-comparisons
- Quality control studies:
  - SOPs
  - Equipment checks & maintenance records
  - Validation reports
- Enhancement of processing and throughput

2013-Nov-25 Experiment
# Exercises/Inter-laboratory Comparisons

## Recent AFRRI cytogenetic biodosimetry exercises and inter-comparison studies

<table>
<thead>
<tr>
<th>Report Date</th>
<th>P.O.C.</th>
<th>Blood shipping &amp; culture</th>
<th>Quick scan scoring (20 spreads per scored sample)</th>
<th>Triage scoring (50 or 500 spreads per samples)</th>
<th>Comment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 2014</td>
<td>Special Forces Exercise (North Carolina)</td>
<td>+ (n = 1)</td>
<td></td>
<td>+ (n=1, duplicate)</td>
<td>Task completed &lt;60 h for result report from receipt of blood samples</td>
</tr>
<tr>
<td>May 30, 2014</td>
<td>Wilkins (Health Canada)</td>
<td>+ (n=10)</td>
<td>+ (n=10)</td>
<td>+ (n=10)</td>
<td>&lt;60 h (Quick Scan) for result report to be sent from receipt of blood</td>
</tr>
<tr>
<td>Nov 2014</td>
<td>Wilkins (RENEB-1a)</td>
<td>-</td>
<td>-</td>
<td>+ (n=2, duplicates)</td>
<td>Task completed and report submitted</td>
</tr>
<tr>
<td>Jan 2015</td>
<td>Wilkins (RENEB-1b)</td>
<td>-</td>
<td>-</td>
<td>+ (n=2, duplicates)</td>
<td>Task completed and report submitted</td>
</tr>
<tr>
<td>Nov 2015</td>
<td>Wilkins (Health Canada)</td>
<td>+(n=10)</td>
<td>+</td>
<td></td>
<td>Task completed</td>
</tr>
<tr>
<td>Oct 2016</td>
<td>Wilkins (Health Canada)</td>
<td>+(n=11)</td>
<td>-</td>
<td>+(n=1)</td>
<td>Task completed</td>
</tr>
<tr>
<td>Jan 2017</td>
<td>Wilkins (RENEB-II)</td>
<td>-</td>
<td>-</td>
<td></td>
<td>500 spreads (n=3)</td>
</tr>
<tr>
<td>Mar 2018</td>
<td>Wilkins (Health Canada)</td>
<td>+(n=10)</td>
<td>-</td>
<td>+(n=10)</td>
<td>On-going</td>
</tr>
</tbody>
</table>
Automatic Scoring of Dicentric Chromosomes as a Tool in Large Scale Radiation Accidents

- Semi-automated dicentric scoring is less efficient than manual scoring of dicentrics (Left panel).
- Calibration curves produced by 6 labs using semi-automated scoring, each with their own selected classifiers, were not statistically different from each other (right panel).
- Blind test was performed by the 6 labs using semi-automated dicentric scoring and they were able to distinguish doses within ±0.5 Gy.

Hypothesis/Methods

Ho: Can the use of automated dicentrics scoring enhance the throughput of analysis supporting dose assessment by cytogenetics?

Conduct DCA assay (outlined in previous slide)

Create spreads using HANABI spreader

Stain and coverslip slides

Perform an “MSearch” at 10x

AutoCapture images at 63x

Run DC Score to detect dicentrics

Data analysis

Hanabi Metaphase Chromosome Harvesters

Hanabi Metaphase Chromosome Spreader

MetaSystems Inc, Metafer
www.metasystems.org/
MSearch -> AutoCapture -> DC Score
10X  63X  63X
Dose Response Using the Automated Scoring of the Dicentric Chromosome Aberration Assay: Total-Body Irradiation (TBI) vs Partial-Body Irradiation (PBI)

\[ y = 0.0411 \pm 0.0200 + 0.0480 \pm 0.0237x + 0.0175 \pm 0.0048x^2 \]

\[ R^2 = 0.9887 \]

\[ y = 1.0176 \pm 0.0429 + 0.0486 \pm 0.0507x + 0.0080 \pm 0.0103x^2 \]

\[ R^2 = 0.8916 \]
Hypothesis

Use of centromeric PNA-FISH in the existing PCC assay can more accurately identify dicentrics for dose assessment.

Jason Hsiao
Fluorescent in-situ hybridization

Centromeres stained with fluorescent red probe

Dicentrics (or more) can be easily visualized

Score 50 dicentrics or 100 spreads per dose across dose-response range

Construct calibration curve with linear quadratic fit
Fitted Calibration Curve

\[ y = -0.5899 \pm 0.28 + 0.6903 \pm 0.0645x - 0.0095 \pm 0.0025x^2 \]

\[ R^2 = 0.9931 \]
Various endpoints are currently being considered for use in dose assessment using the PCC assay, however, there is no consensus as to the optimum endpoints to use. Studies performed here were focused on evaluating four PCC endpoints (i.e., PCC fragments, rings, LR, and dicentrics) for dose assessment following TBI and PBI.
Dose responses for the four PCC parameters in all G2/M-PCC were determined and fitted with various models. These radiation calibration curves would be used in the case of a TBI.
The fraction of damaged cells, knowing the dose, can then be determined from the calibration curves of the dose response of fraction of damaged cells containing ≥48 fragments per cell (Figure A), ≥1 ring per cell (Figure B), ≥10 LR per cell (Figure C), and ≥1 dicentric per cell (Figure 3D) here shown for 100% irradiated cells. These calibration curves would be proportional adjusted for the fraction of the body exposed.
\[ \text{Dose}_{\text{PBI}} = \text{Dose}_{\text{TBI}} \]

If the PBI dose was equal to the TBI dose, then we would use the TBI radiation curves for dose assessment.

\[ \text{Dose}_{\text{PBI}} > \text{Dose}_{\text{TBI}} \]

If the PBI dose was significantly greater than the TBI dose, then we would suspect a partial-body exposure and use the appropriate PBI calibration curves.
Dose responses for the four PCC parameters in damaged $G_2/M$-PCC were determined and fitted with various models (Figures A-D). These radiation calibration curves would be used in the case of PBI and represent the first introduction of the use of the four novel endpoints (i.e., $Q_{PCC}$, $Q_R$, $Q_{LR}$, and $Q_D$) for application in dose assessment for PBI using the PCC assay.
DOD Biodosimetry Network: Initial Design Involving AFRRI & NDC

Abstract for platform presentation at the 2018 CIRMS 26th Annual Meeting, NIST, Gaithersburg, MD, 16-18 April 2018; submit to Ms. Renata Freindorf (renata@cirms.org).

Update on AFRRI’s Cytogenetic Biodosimetry Activities

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Scientific Research Department, Armed Forces Radiobiology Research Institute, Uniformed Services University of the Health Sciences, Bethesda, MD 20889, USA;

ABSTRACT

Cytogenetic biodosimetry using the IAEA manual and relevant ISO standards is the generally accepted method for radiation dose assessment in cases of suspected radiation over-exposures. The Armed Forces Radiobiology Research Institute (AFRRI) Biodosimetry Center provides biodosimetry capability based on the use of the dicentric chromosome aberration (DCA) and premature chromosome condensation (PCC) cytogenetic assays. In the last year the number of donors contributing to AFRRI’s baseline for use of the dicentric chromosome aberration (DCA) assay has doubled to 20, which improves our ability to assess potentially low-dose exposures. We have recently obtained a commercial software application to permit routine karyotyping of metaphase spreads in cases where radiation-induced chromosome aberrations are detected in order to evaluate for potential clonal aberrations. Our laboratory replaced its automated metaphase finder and applied the use of the automated scoring software to develop a dose-response calibration curve that permits rapid scoring of dicentric aberrations in cases of suspected radiation accidents. In the last few years we have participated in multiple exercises/inter-comparisons and successfully demonstrated blood collection and shipping in a military deployment activity as well as the ability to use both the conventional- and QuickScan-DCA analysis methods for dose assessment. In addition, efforts to establish the premature chromosome condensation (PCC) assay are underway to provide the laboratory with a second cytogenetic biodosimetry assay with robust capability for assessment of partial-body and higher doses (>5 Gy). Blood was exposed to $^{137}$Cs gamma ray doses 0 – 26 Gy at 0.59 Gy/min. Cultures were incubated for 2 hr at 37°C following with 48 hrs in the presence of PHA with the final 0.5 hr. with 100 nM calyculin A. Dicecntrics in PCC spreads were measured using the centromeric protein nucleic acid (PNA) probe using fluorescence in situ hybridization. Results from the analysis of excess PCC fragments, rings, and dicentrics will be reported including the use of the analysis methods for partial-body and high-dose exposure cases.

[The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy or position of DoD, AFRRI, USUHS, nor the U.S. Government. Funding support provided by AFRRI RBB4431317 and RBB4352317.]
Dear Bill,

The time we have allocated for each speaker is 30 min. Please plan for a 25 min presentation followed by a 5 min Question and Answer period.

Thank you!

Ronnie Minniti

*Co-Chair of the CIRMS Medical Applications Subcommittee*

______________________________
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Hi Bill,

Each speaker has a 25-min slot, with a common agenda of a 20-minute presentation, with around 5 minutes of questions. Does this answer your question?

Best,

Regina

Regina Fulkerson <rmkenned@gmail.com>

Session Chairs:
Regina Fulkerson <rmkenned@gmail.com>;
Wesley Culberson <wsculberson@wisc.edu>;
Minniti, Ronaldo (Fed) <ronnie.minniti@nist.gov>