Update on AFRRI's Cytogenetic Biodosimetry Activities

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AFRRI

Uniformed Services University

Disclaimer

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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

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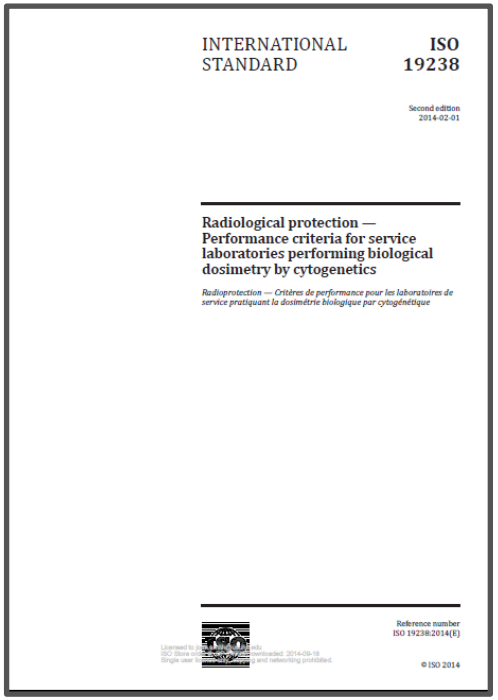
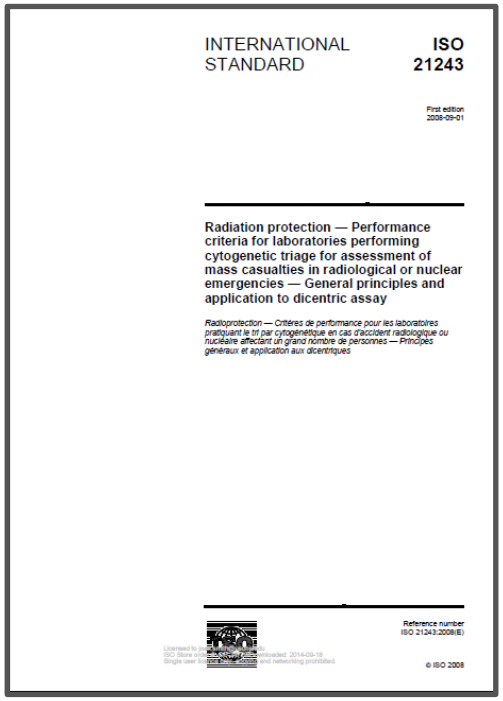
Cytogenetic Biodosimetry

DCA - Reference



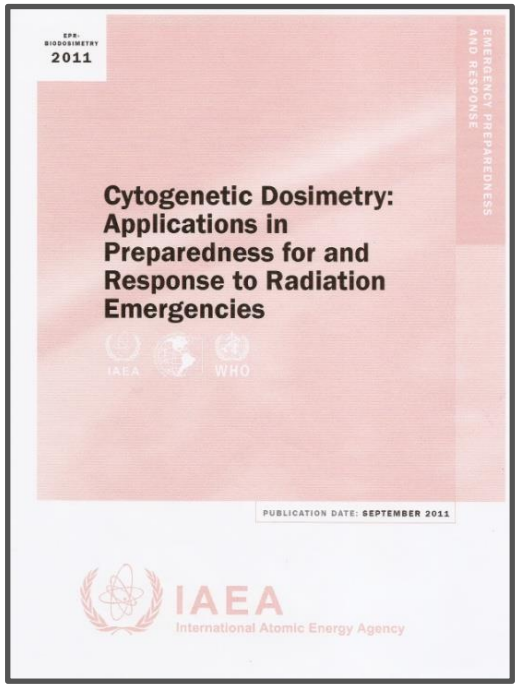
2014

DCA - Triage



2008

Manual



2011

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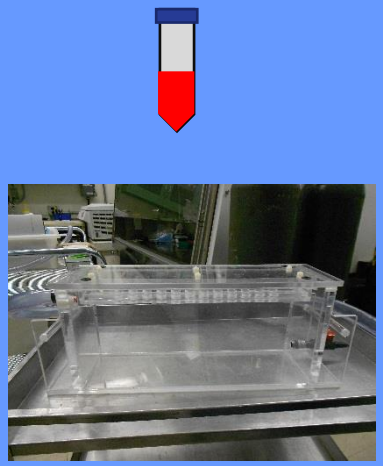
TABLE 1. COMPARISON OF CYTOGENETIC ABERRATION ASSAYS USED FOR DOSE ASSESSMENT[a]

	Cytogenetic Aberration Assays			
	Premature chromosome condensation (PCC)	Dicentric (and ring) (DCA)	Fluorescent *in situ* hybridization (FISH)	Cytokinesis-block micronucleus (CBMN)
Typical aberrations scored for biological dosimetry applications	excess chromosome fragments; dicentrics[b] and rings translocations[b]	dicentrics[b] (and rings) Lower background Less variable Gold Standard	dicentrics[b] (and rings) translocations[b] Higher background Effect of other events	micronuclei nucleoplasmic bridges
Typical radiation scenario applications	acute recent exposure	acute protracted recent exposure	acute protracted old exposure	acute protracted recent exposure
Photon equivalent, acute dose range (Gy) for whole-body dose assessment	0.2 to 20 For higher doses	0.1 to 5	0.25 to 4 Karyotyping Stable aberrations	0.3 to 4
Useful for partial-body exposure applications	Yes	Yes	NA[c]	NA
Useful for triage dose assessment	Yes	Yes	NA	Yes
Status of assay standardization	NA	ISO standards [3, 4]	NA	ISO standard — pending, and [5]

[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)

Experimental Design
- Human peripheral blood *ex vivo* irradiation model



Roberston Chamber (37°C)

0.6 Gy /min ^{137}Cs gamma rays

Radiation Dose
0,1,3,5 Gy

PCC Assay

Culture at 37 ° C for 48 h + **PHA**

DCA Assay

Culture at 37 ° C for 24 h + **PHA**

Treatment with 100 nM calyculin A **30 min** before harvesting

Treatment with 0.1 µg/ml colcemid **24 h** before harvesting

Analysis of chromosome morphology (fragments and rings)

Analysis of chromosome morphology (dicentrics and rings)

AFRRI Resources – Cytogenetic Activities

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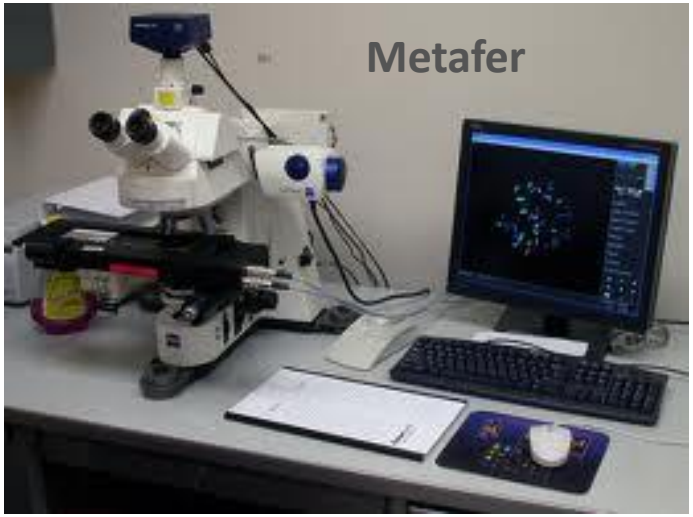
Multiple Radiation

Sources and

Dosimetry Support

www.metasystems.org/

Hanabi
Metaphase
Chromosome
Harvesters



Hanabi
Metaphase
Chromosome
Harvesters

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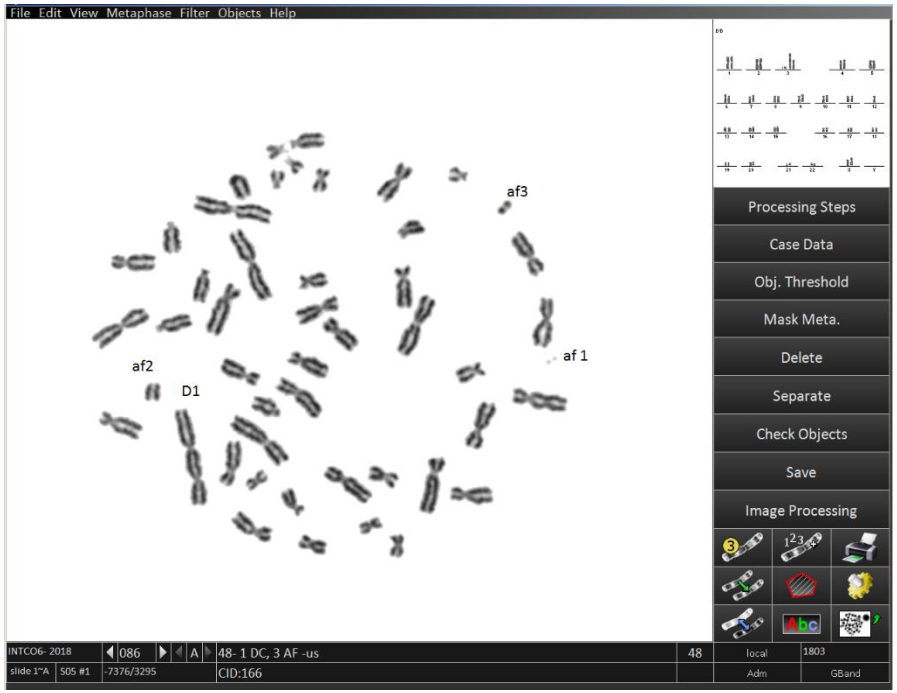
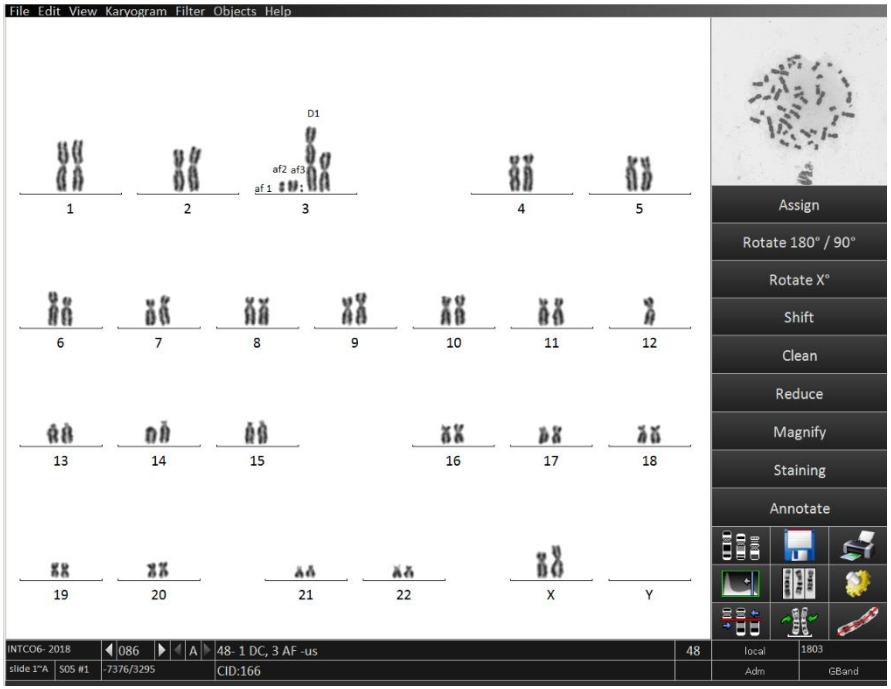
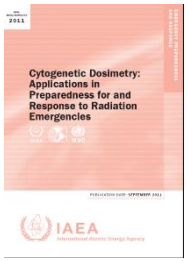
MetaSystems Inc.
Ikaros Karyotyping Software





MetaSystems



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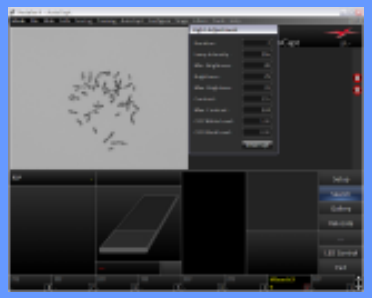
Metasystems Inc. - Metafer

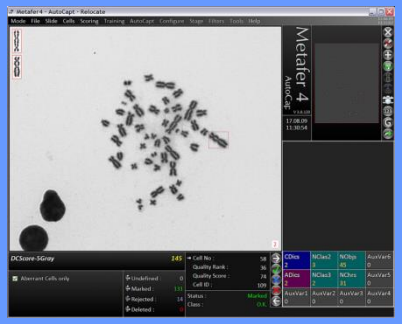
MSearch

Auto Capture



Metafer

DC Score



Share Cloud

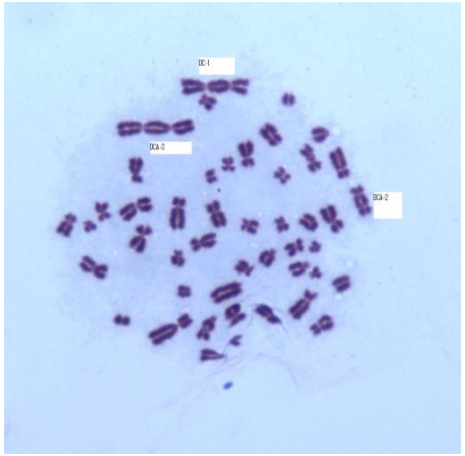
www.metasystems.org/

Sugarman SL, Livingston GK, et al. The Internet's Role in a Radiation Mass Casualty, Health Phys 106(5 Suppl 2): S65-70, 2014.

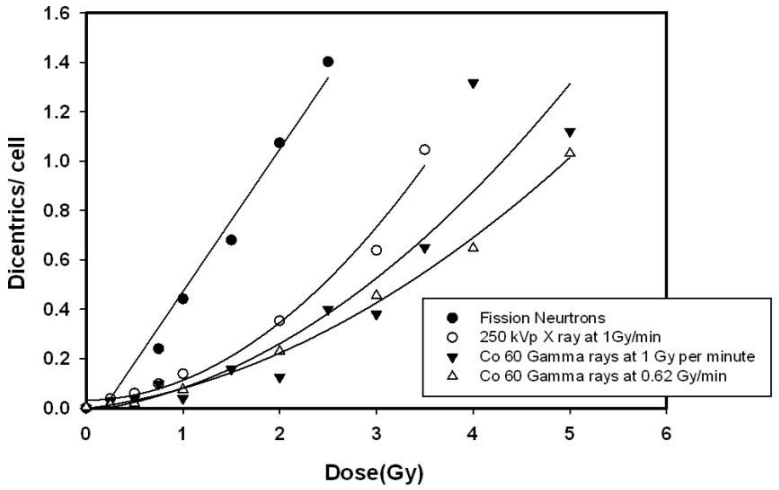
Romm H et al. Automatic scoring of dicentric chromosomes as a tool in large scale radiation accidents. Mutat Res. 756(1-2):174-83, 2013

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

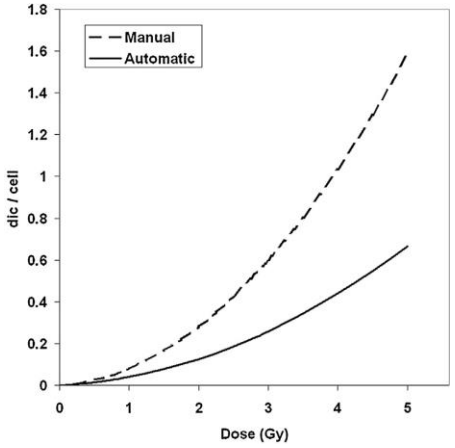
Uniformed Services University of the Health Sciences
Armed Forces Radiobiology Research Institute

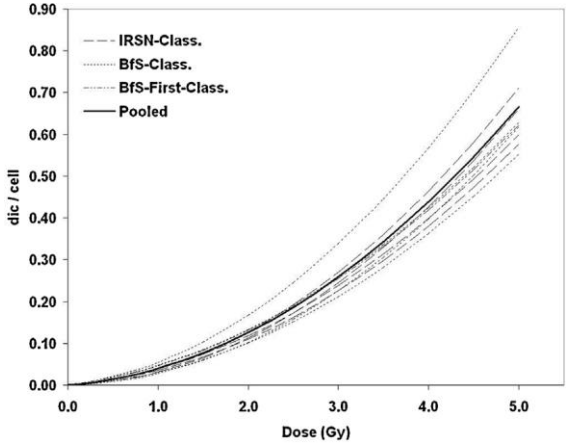
Recent AFRRI cytogenetic biodosimetry exercises and inter-comparison studies

Report Date	P.O.C.	Blood shipping & culture	Quick scan scoring (20 spreads per scored sample)	Triage scoring (50 or 500 spreads per samples)	Comment(s)
May 2014	Special Forces Exercise (North Carolina)	+ (n = 1)		+ (n=1, duplicate)	Task completed <60 h for result report from receipt of blood samples
May 30, 2014	Wilkins (Health Canada)	+ (n=10)	+ (n=10)	+ (n=10)	<60 h (Quick Scan) for result report to be sent from receipt of blood
Nov 2014	Wilkins (RENEB-1a)	-	-	+ (n=2, duplicates)	Task completed and report submitted
Jan 2015	Wilkins (RENEB-1b)	-	-	+ (n=2, duplicates)	Task completed and report submitted
Nov 2015	Wilkins (Health Canada)	+(n=10)	+	+	Task completed
Oct 2016	Wilkins (Health Canada)	+(n=11)	-	+(n=1)	Task completed
Jan 2017	Wilkins (RENEB-II)	-	-	500 spreads (n=3)	Task completed
Mar 2018	Wilkins (Health Canada)	+(n=10)	-	+(n=10)	On-going

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.





Romm H et al. Mutat Res 756:174-183, 2013.
Romm, H et al. Health Phy 106(6): 764-771, 2014

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Hypothesis/Methods

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

Brett O'Brien

Conduct DCA assay (outlined in previous slide)

Create spreads using HANABI spreader

Stain and coverslip slides

Perform an "MSearch" at 10x

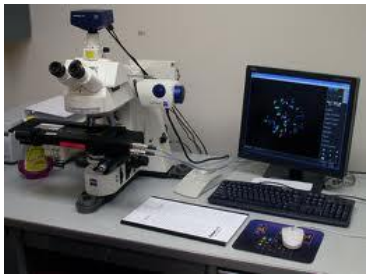
AutoCapture images at 63x

Run DC Score to detect dicentrics

Data analysis

Hanabi Metaphase Chromosome Harvesters

Hanabi Metaphase Chromosome Spreader



MetaSystems Inc, Metafer
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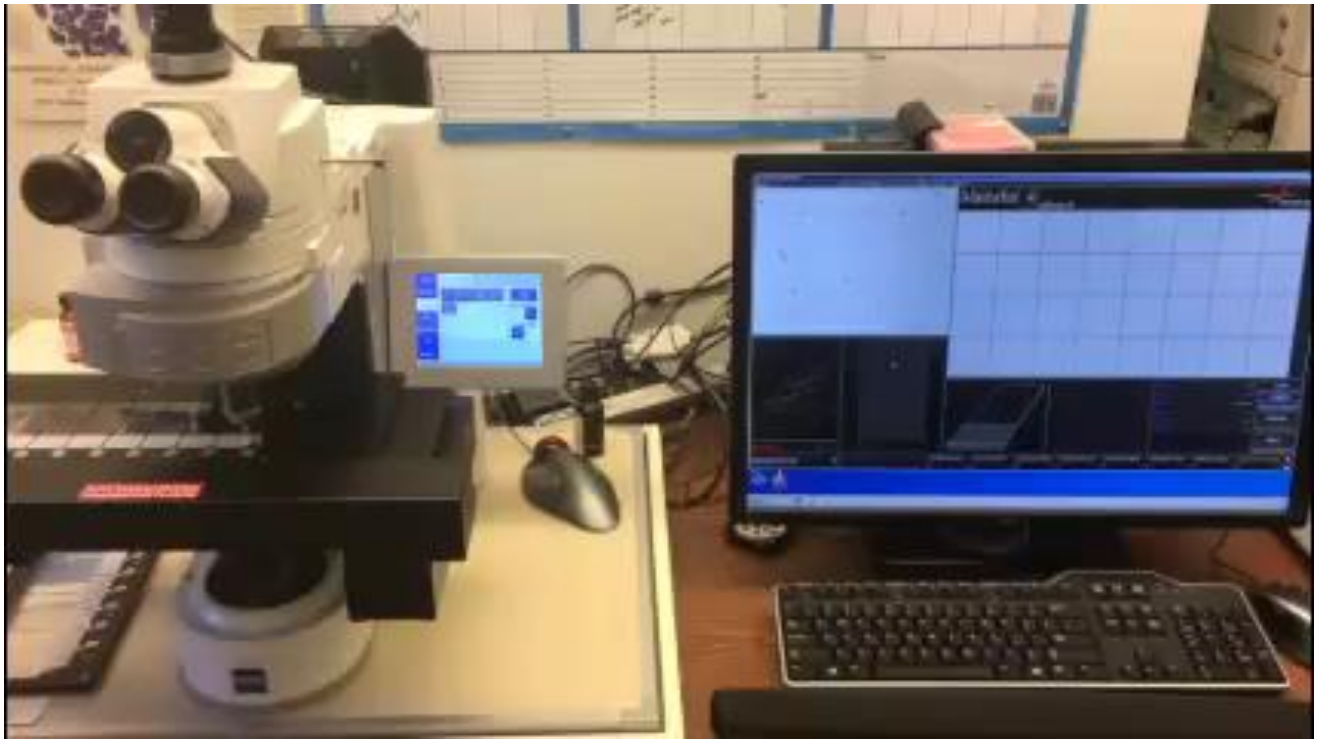
MSearch -> AutoCapture -> DC Score

10X 63X 63X



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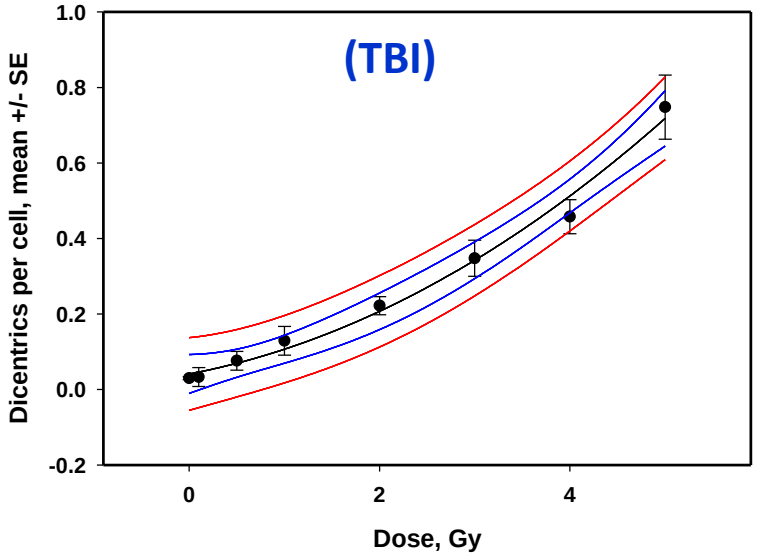
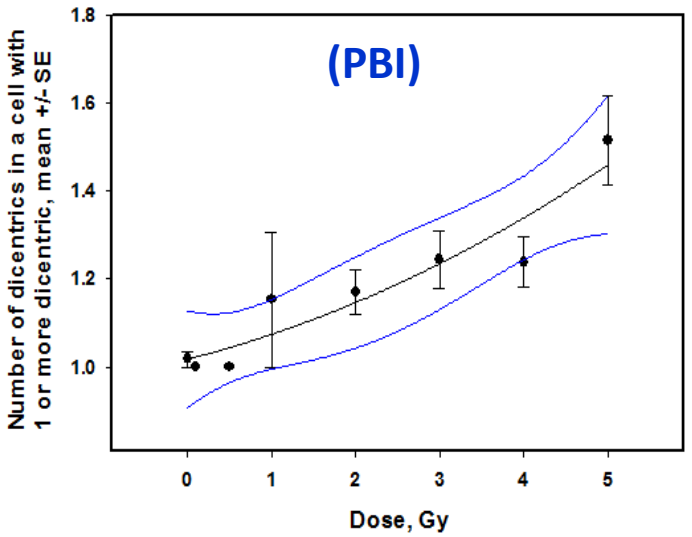
Dose Response Using the Automated Scoring of the Dicentric Chromosome Aberration Assay: Total-Body Irradiation (TBI) vs Partial-Body Irradiation (PBI)

$y = 0.0411 (+/- 0.0200) + 0.0480 (+/- 0.0237)x + 0.0175 (+/- 0.0048) x2$
$R^2 = 0.9887$



$y = 1.0176 (+/- 0.0429) + 0.0486 (+/- 0.0507)x + 0.0080 (+/- 0.0103) x2$
$R^2 = 0.8916$



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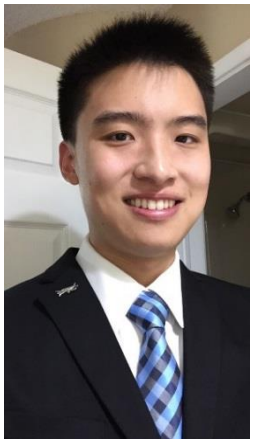
Hypothesis



Jason Hsiao

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

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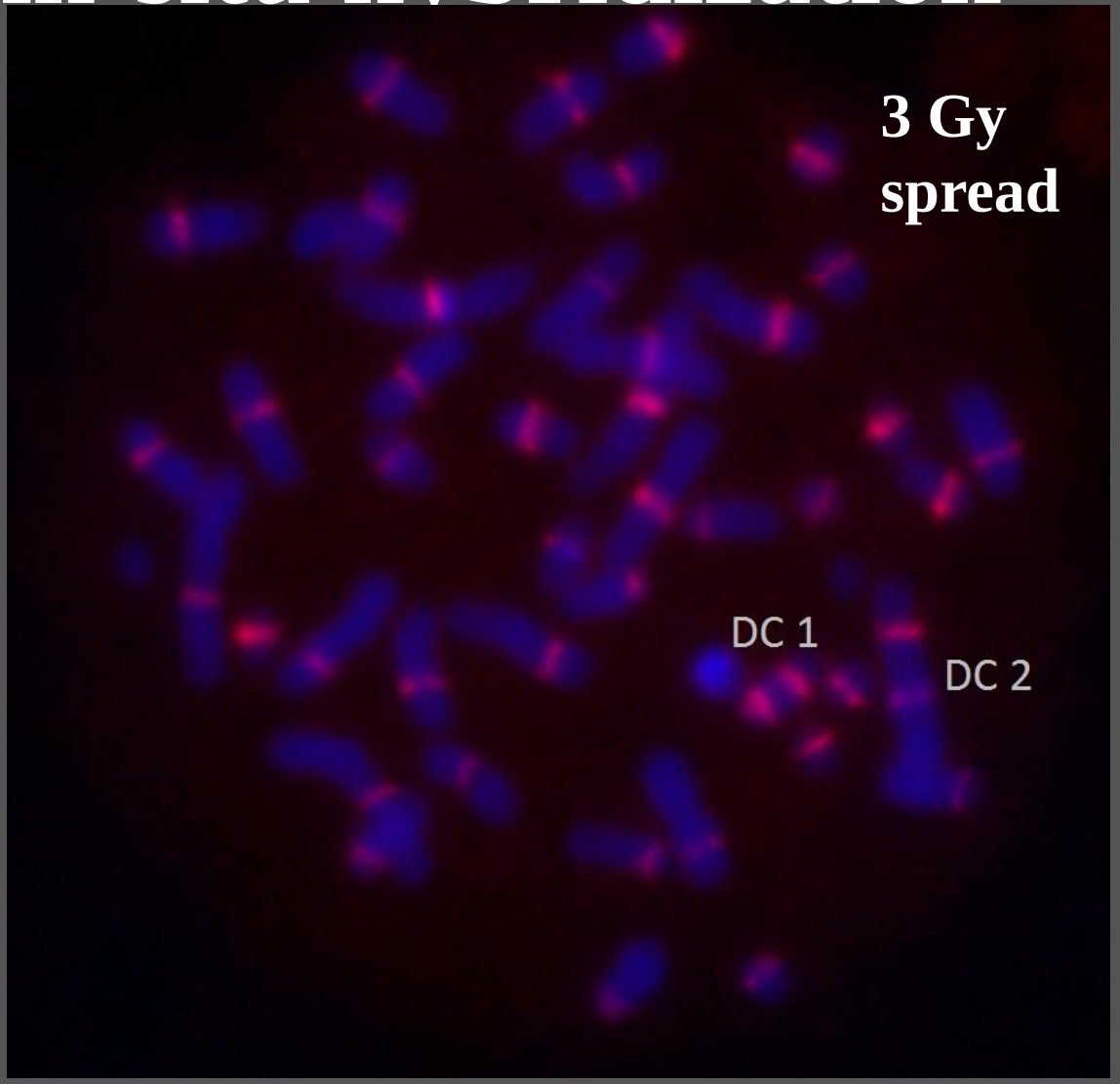
Fluorescent in-situ hybridization

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

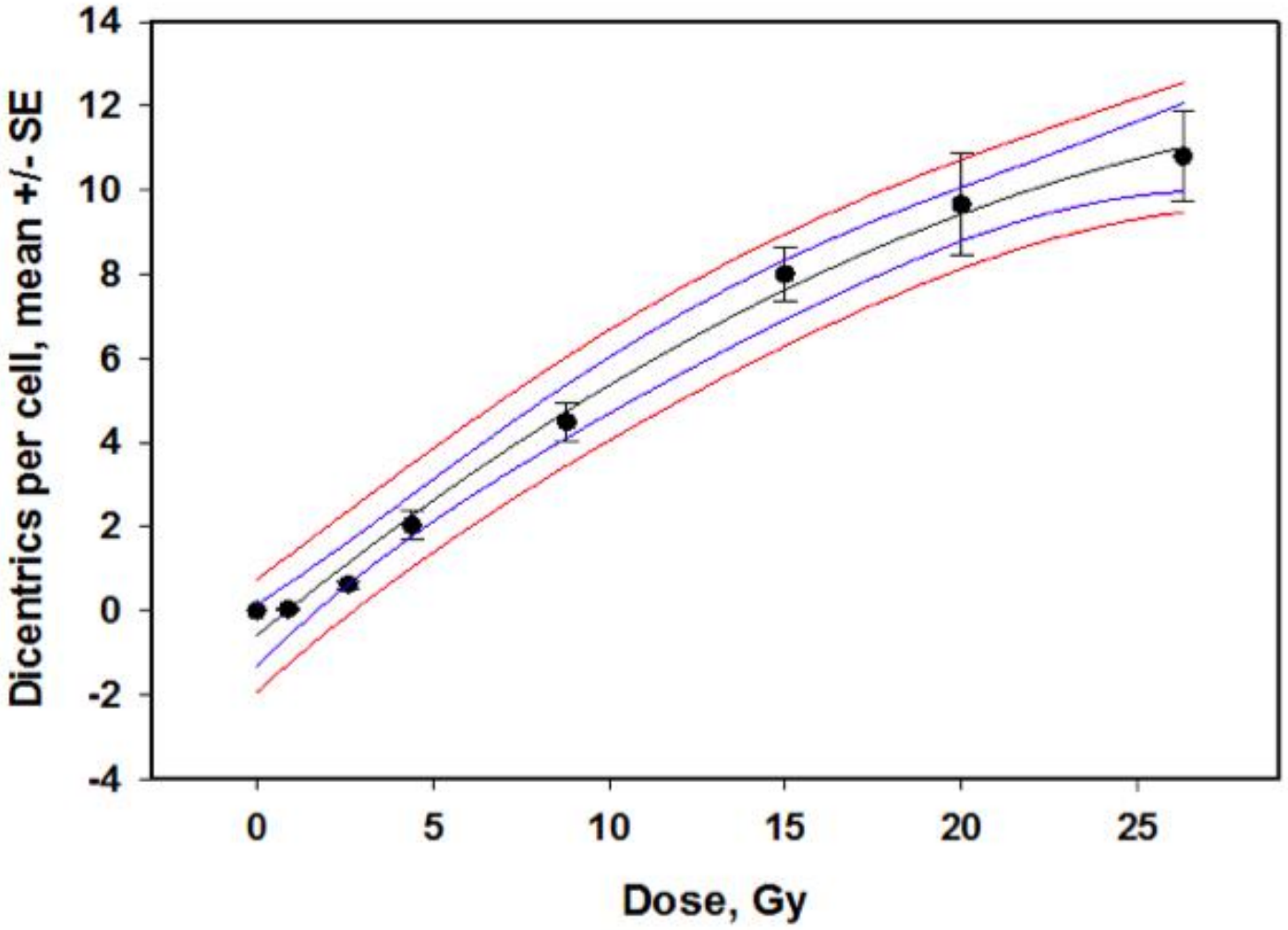
Construct calibration curve with linear quadratic fit



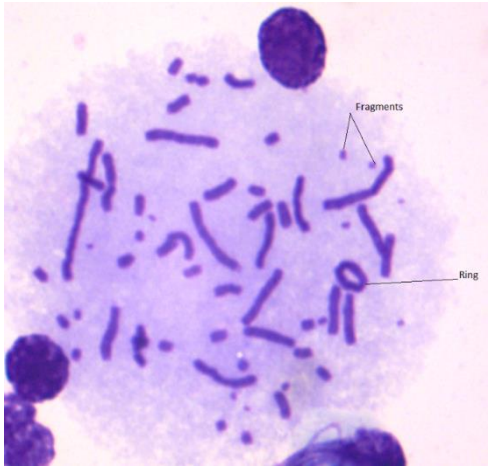
Fitted Calibration Curve

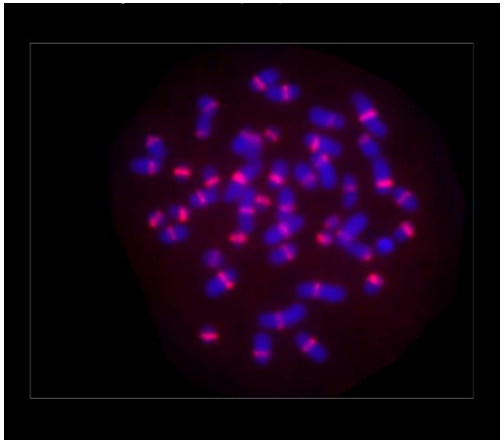
$y = -0.5899\ (+/-\ 0.28) + 0.6903\ (+/-\ 0.0645)x + -0.0095\ (+/-\ 0.0025)\ x^2$

$R^2 = 0.9931$



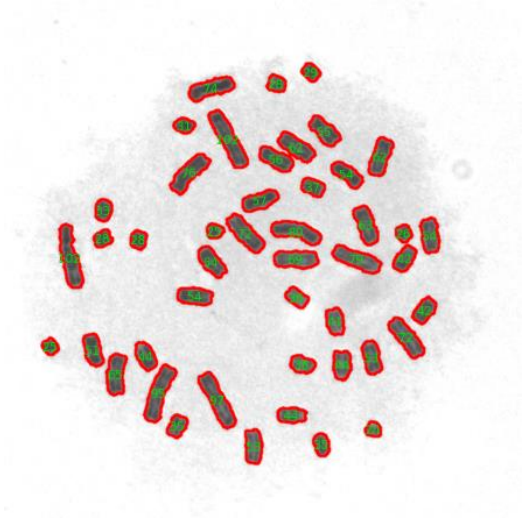
PCC fragments, rings

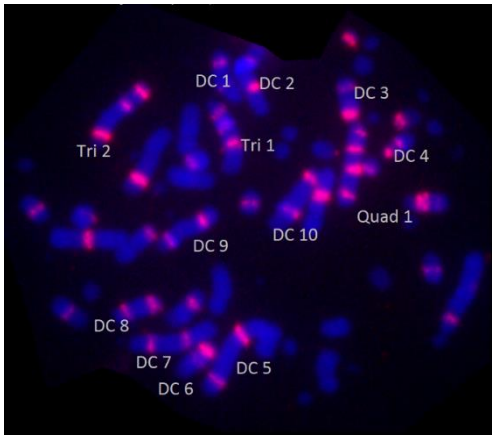




dicentrics

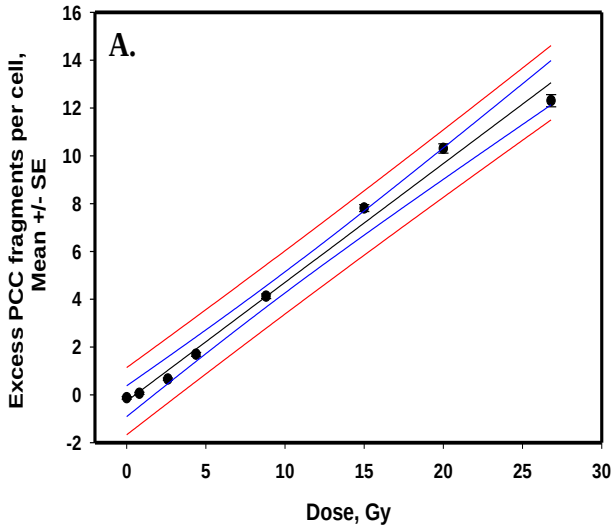
Length Ratios



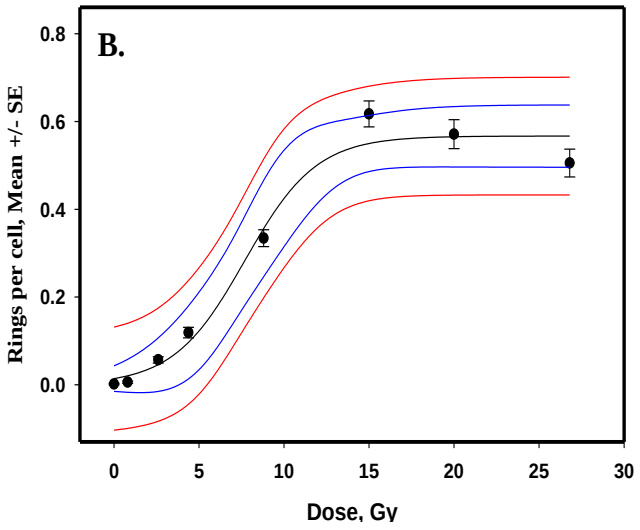


Various endpoints are currently being considered for use in dose assessment using the PCC assay, however, there is no consensus as to the optimum endpoints to use. Studies performed here were focused on evaluating four PCC endpoints (i.e., PCC fragments, rings, LR, and dicentrics) for dose assessment following TBI and PBI.

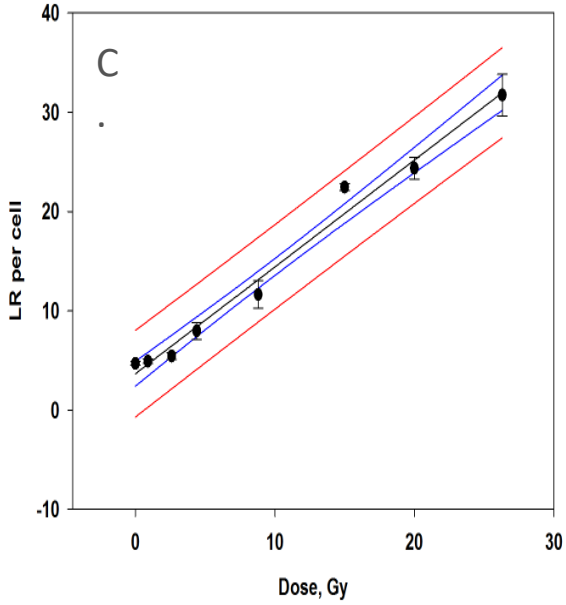
PCC fragments



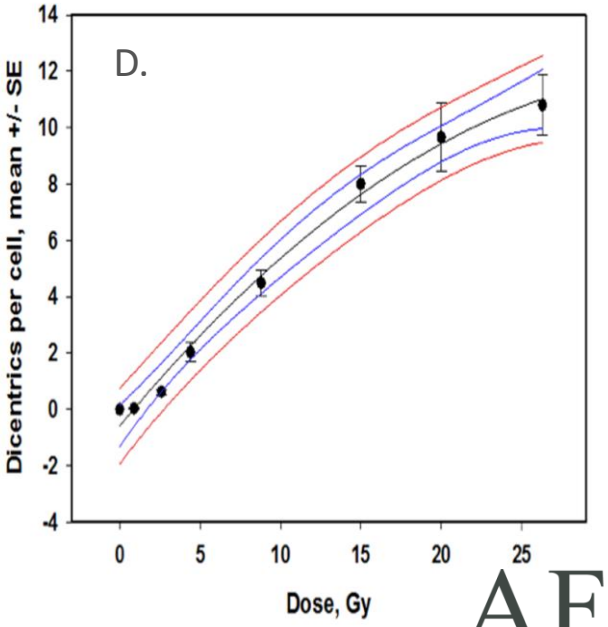
PCC rings



Length ratios



Dicentrics



Dose responses for the four PCC parameters in all G_2/M-PCC were determined and fitted with various models. These radiation calibration curves would be used in the case of a **TBI**.

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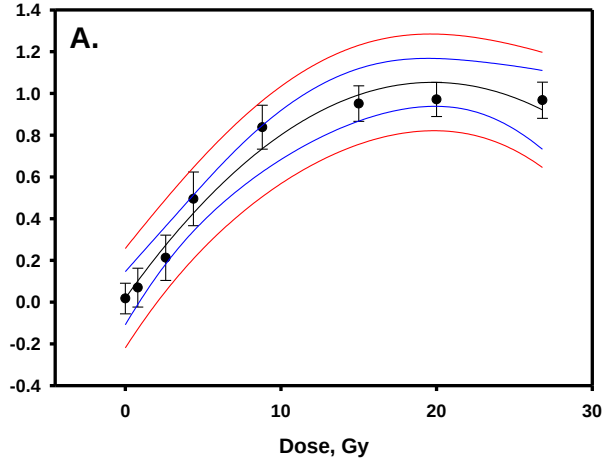
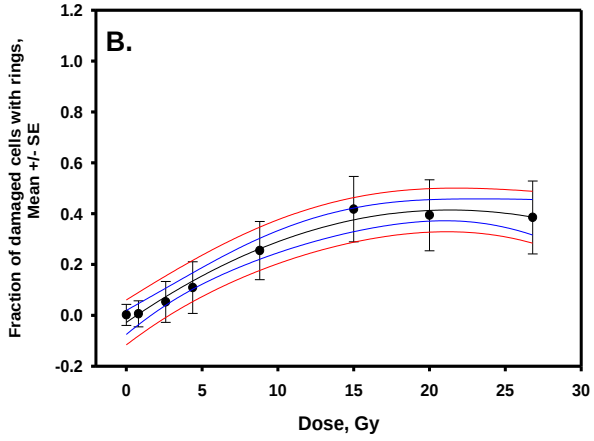
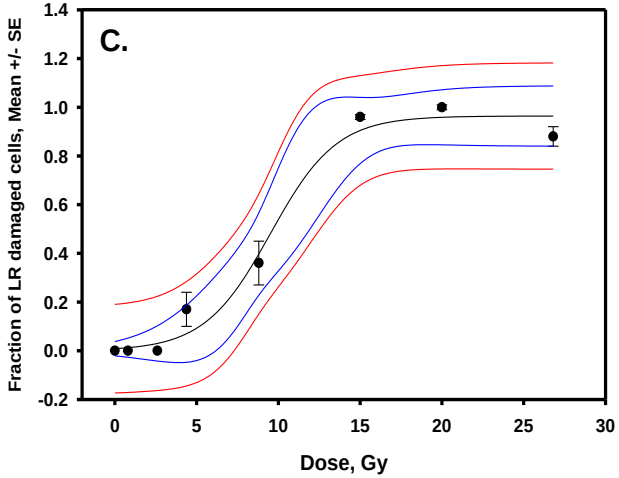
PCC fragments



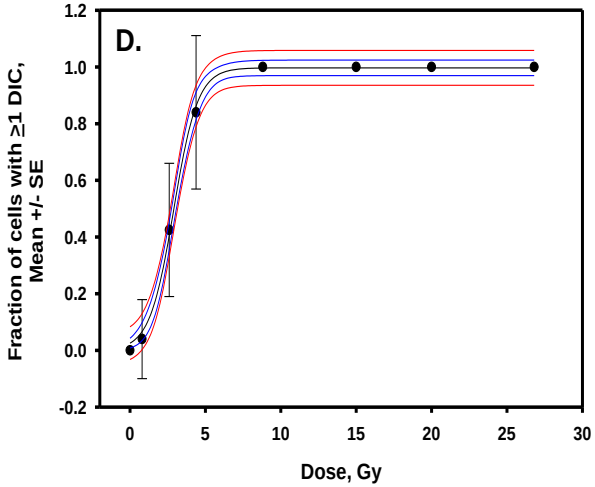
PCC rings



Length ratios



Dicentrics



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.

$$\mathbf{Dose_{PBI} = Dose_{TBI}}$$

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

$$\mathbf{Dose_{PBI} > Dose_{TBI}}$$

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.

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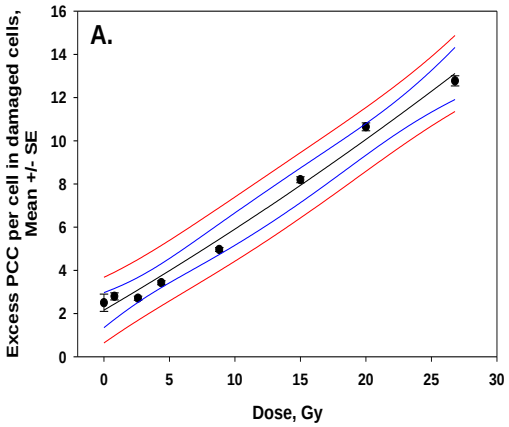
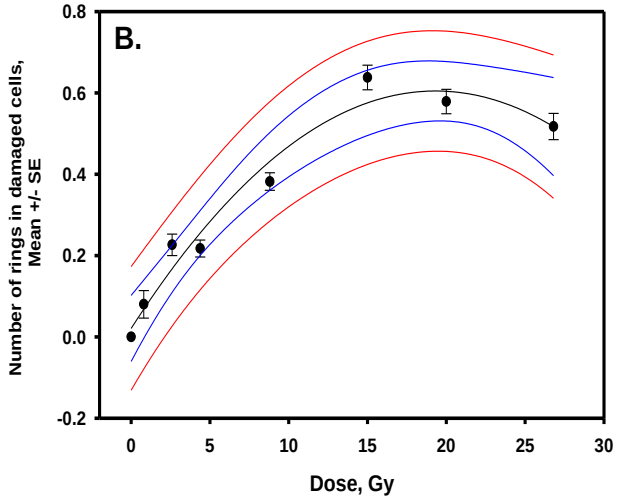
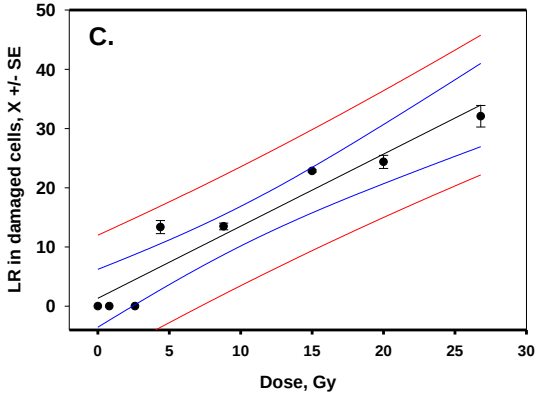
PCC fragments



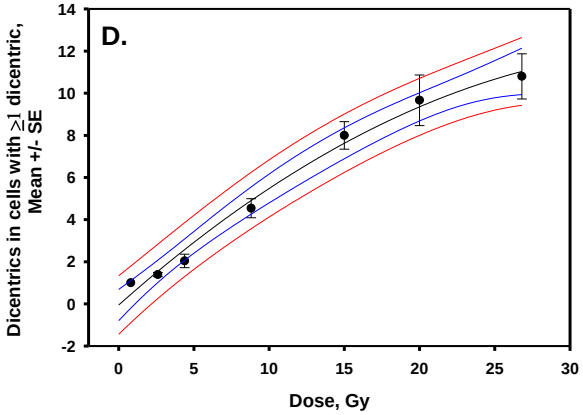
PCC rings



Length ratios

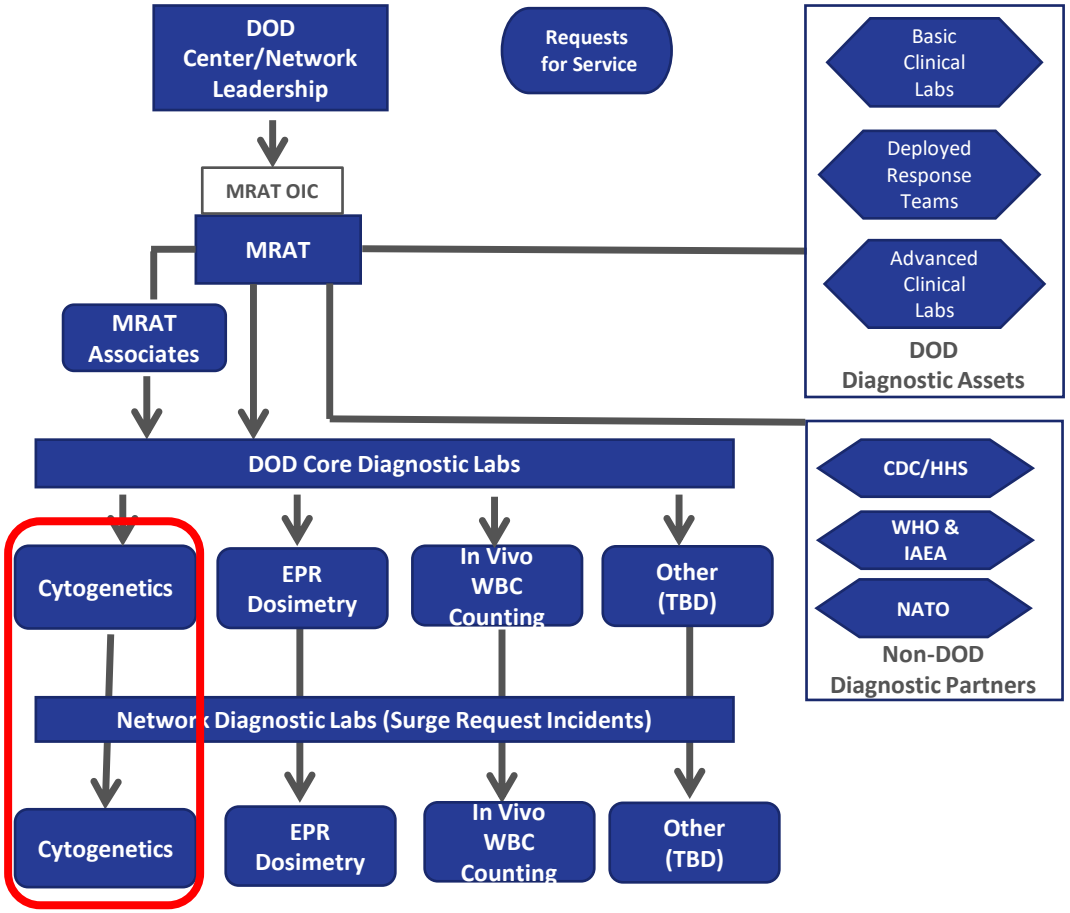


Dicentrics



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



Blakely WF, Romanyukha A, Hayes SM, Reyes RA, Stewart HM Jr, Hoefer MH, Williams A, Sharp T, Huff LA. U.S. Department of Defense Multiple-Parameter Biodosimetry Network. Radiat Prot Dosimetry. 172(1-3): 58-71, 2016; doi: 10.1093/rpd/ncw295. Epub 2016 Nov 24

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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

William F. Blakely, Uma Subramanian, Kuang-Heng Hsiao, Brett O'Brien, Lyudmila Romanyukha, and David L. Bolduc

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. Dicentrics 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.]

Uniformed Services University

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>