The Scientific Medical Effects of Ionizing Radiation Course, conducted once a year, focuses on the latest research about the medical effects of ionizing radiation to help clinicians, health physicists, and medical planners preserve troop health in the face of radiological/nuclear terrorism or warfare.

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Disclosures

• All equipment suppliers are commercial vendors.
• No expressed or implied partnerships with these companies.
• Views expressed are those of the author; no endorsement by DoD, AFRRI/USU or NIAID/NIH is implied.
Objective

Develop current “gold standard” chromosome aberration radiation dose assessment method for mass casualty applications
Outline

Section I
  Background

Section II
  Dicentric assay for clinical triage

Section III
  Laboratory automation for sample processing and Laboratory Information Management System (LIMS)

Section IV
  Inter-laboratory comparison study
Section I

Background
Diagnostic Biodosimetry

Premise

• Dose to the irradiated person is the primary determinant of the nature, onset, severity, and duration of acute radiation syndrome.

• Early diagnostic information on the absorbed dose is essential for effective clinical management.

• Medical personnel rely heavily on clinical signs and biological-based assessments of radiation dose.

• With appropriate medical and intensive care, the likelihood of survival can be increased significantly.
# Radiation Dose, Clinical Status, and Outcome

<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)
Qualities of an Ideal Biodosimeter

• Dose-effect relationship
• Specific to ionizing radiation
• Low inter-individual variation
• Early availability of results
• Partial-body exposures
• Persistency
• Fractionated and chronic exposures
• Radiation quality effects
• Noninvasive or semi-invasive sampling
• Automation
The Dicentric Assay – Effective and Validated Biodosimetry Method

- Validated cytogenetic “gold standard” biodosimetry assay
- Dicentric chromosomes seen in human peripheral blood lymphocytes, are specific to ionizing radiation exposure
- Dose-effect relationship
- Strong correlation between *in vitro* and *in vivo* yield
- Technical manual (IAEA) and international standards (ISO) are available for performing dicentric assay for radiation dose assessment.
- Performed in “reference cytogenetic reach-back biodosimetry laboratory”
- Needs cytogenetic expertise and labor intensive
- Applications in radiation mass casualties will require automation.
Dicentric Chromosome: A Specific Radiation Biomarker

Mechanism of dicentric induction

Metaphase spread with a dicentric chromosome
AFFRI’s Dose-Response Calibration Curves

Prasanna et al. (2002)
Dicentric Assay: Current Status

IAEA revised manual

- ISO standard for accrediting cytogenetic biodosimetry laboratories (ISO TC85/SC 2, 19238, 2003) is available.
- “Radiation Protection — Performance criteria for rapid cytogenetic assessment of individual exposures in radiological or nuclear mass casualties - I. General principles and application to the dicentric assay, (ISO TC85/SC 2, WG-18, 21243) soon be ready for ratification.
Conclusions: Section I

• Dicentric assay is currently the “gold-standard” dose assessment method.

• Technical manual and standards are available for performing dicentric assay for radiation dose assessment.

• Applications in radiation mass casualties will require further assay development.
Section II
Dicentric Assay for Confirmation of Clinical Triage
Guidelines for Confirmation of Clinical Triage by Biodosimetry

- Time of emesis is less than 2 hours after exposure

- An approximate 50% decline in peripheral blood lymphocyte counts over 12 hours

- Geographical location-based physical dosimetry indicate above 3-Gy dose

- Multi-parameter clinical symptoms indicate imminent acute radiation syndrome
Dose Assessment: Cytogenetic Biodosimetry Strategy

A. Physical Dosimetry

B. Prodromal Syndromes

C. Lymphocyte Depletion Kinetics

D. Lymphocyte Cytogenetics

Prasanna et al. 2004
Emergency Triage Analysis for Mass Casualties

- Triage for management of the Acute Radiation Syndrome (ARS) will require estimation of whole-body dose in the ranges of >4 Gy, 2-4 Gy and <2Gy

- Scoring 20-50 metaphases per patient allows stratification of patients into dose categories

- Current activities of ISO focus on developing performance criteria for assessment of individual exposures in radiological or nuclear mass casualties
Triage Dose Prediction Model: Confirm “Significant” Exposure (20 cells)
Dose Prediction by Analyzing 50 Cells

![Graph showing Dic/cell vs Dose (Gy) with LCL and UCL curves.](image)

- Predicted dose (Gy)
- Dic/cell vs Dose (Gy)
- LCL
- UCL
Cytogenetic Dose Assessment in Mass Casualties: Challenges and Solutions

• Labor-intensive and time-consuming
  • Automate
    • Sample processing
    • Chromosome-aberration analysis
• Needs expertise
  • Establish inter-laboratory networks
  • Train individuals
  • Perform tele-analysis via Virtual Private Network (VPN)
  • Automate analysis
Conclusions: Section II

- Triage for management of the Acute Radiation Syndrome (ARS) will require estimation of whole-body dose in the ranges of >4 Gy, 2-4 Gy and <2 Gy.

- Scoring 20-50 metaphases per patient allows stratification of patients into dose categories.

- Current activities of ISO focus on developing performance criteria for assessment of individual exposures in radiological or nuclear mass casualties.

- Dicentric assay can be used for confirming clinical triage in radiation mass casualties
Section III

Laboratory Automation and Laboratory Information Management System
Sample Processing: Assay Overview and Industrial Analysis

Prelogin Dispatch Blood Collection Kit
- Capacity: 20 samples/kit
- Vol: 96
- Rate: 12 min/kit
- Person: 1

Sample Check In
- Capacity: 20 samples/kit
- Vol: 96
- Total Time: 1 h
- Person: 1

Blood Cell Counter
- Capacity: 20 samples/holder
- Vol: 96
- Rate: 20 min/run
- Person: 1

Liquid Handler
- Capacity: 96 tubes/holder
- Vol: 96
- Time: 1 h
- Person: 1

Stainer
- Capacity: 150 slides
- Vol: 192
- Rate: 30 min/run
- Person: 1

Metaphase Spreader
- Capacity: 5 slides/run
- Vol: 192
- Rate: 5 min/run
- Person: 1

Metaphase Harvester
- Capacity: 96 tubes/run
- Vol: 96
- Rate: 3 h/run
- Person: 1

Incubator
- Capacity: 500 tubes/chamber
- Vol: 96
- Total Time: 48 h
- Person: 1

CoverSlipper
- Capacity: 150 slides
- Vol: 192
- Rate: 30 min/run
- Person: 1

Metaphase Finder
- Capacity: 150 slides
- Vol: 192
- Rate: 8-12 h/run
- Person: 1

Microscope Satellites
- Capacity: 12 samples/person
- Vol: 96
- (20 spreads/sample)
- Time: 8 h
- Persons: 8

Confirmation
- Time: 8 h
- Persons: 1-2

Report
- Time: 8 h
- Persons: 1-2

1 sample = 1 patient = 20 spreads/patient

Martin et al. 2007
Automation Challenges

- System Integration
  - Liquid handling
- Protocol Optimization
  - Metaphase harvester
- Equipment Customization
  - Metaphase finder
- Data management and Sample Tracking
  - LIMS (Sample Master®) → CytoTrak™
System Integration for “Walk-Away” Automated Blood Processing
Protocol Optimization: Metaphase Harvesting

• Equipment is originally designed to harvest metaphase spreads from cell cultures.
• Manual protocols are automated.
• Standard Operating Procedures (SOP) are developed for harvesting metaphase spreads from peripheral blood and isolated lymphocytes.

Metaphase harvester
Equipment Customization: Bar-code Integration in Metaphase Finder and Satellite-Scoring Stations

- Customized to scan barcodes on slides as it automatically locates chromosome spreads.
- Software customizations allow barcode information to be associated with data and image files.
- Allows case file data storage on a centralized server.
- Customized network of satellite chromosome-aberration analysis workstations provides direct access to case files on the centralized server, eliminating data transcription errors.
LIMS (SampleMaster Pro®)

- Quality Control and Quality Assurance
- Sample tracking
- Data entry
- Sample scheduling and stability
- Electronic data transfer
- Chemical inventory
- Resource management
- Sample conditions
- Test set up
- Security
- Auditing
- Configuration
- Reporting
- Instrument integration
The CytoTrack™ System for Sample Tracking and Data Management: Overview

- LIMS Server and SQL relational database represent the heart.
- Individual equipments are integrated via customized interfaces and data translators.
- Data translation and processing modules running on centralized server communicati between equipments by importing, recording, and exploring data automatically.
- The server also monitors the status of each equipment, reagent inventories, staff training, and accreditation and environmental conditions.

CytoTrack component and data flow
High-Throughput Batch Processing of Samples

Sample Prelogin (30 min)

Batch process 88 samples (liquid handler (30 min))

Incubate (24 h)

Add colcemid

Incubate (24 h)

Harvest metaphase spreads (2.5 h)

Spreading (1 h)

Staining (30 min)

Batch I processing (~53 hours)

Analysis

Sample Prelogin (30 min)

Batch process 88 samples (liquid handler (30 min))

Incubate (24 h)

Add colcemid

Incubate (24 h)

Harvest metaphase spreads (2.5 h)

Spreading (1 h)

Staining (30 min)

Batch II processing (~53 hours)

Analysis

MEIR, July 08
Analysis of Samples

Data Collect

Remote Analyzing Stations

Samples

Blood Handler

Incubator

Harvester

Spreader

Metaphase finder

Auto-Stainer

LIMS Server

Metaphase spread images

Encrypted traffic in VPN tunnel

Internet

Remote Analyzing Stations

Analyzed Info retrieval & Backup on LIMS

Samples

Slides

LIMS

Prasanna 32
Conclusions: Section III

- Sample throughput for the current gold standard method can be increased significantly by laboratory automation.

- Transitioned manual sample preparation methods to automated walk away platforms for cytogenetic dose assessment.

- Processing of 500 to 1000 samples per week is possible.

- Quality control and quality assurance can be improved by implementing LIMS.

- Automated cytogenetic laboratory can support physical transfer of slides or images for rapid dicentric analysis for dose assessment.
Section IV

Quality Control Essentials

Inter-Laboratory Comparison Study
Collaborators

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• T.C. Pellmar (AFRRI)
• H. Romm (BFS, Germany)
• R. Wilkins (Health Canada)
• M. Yoshida (NIRS, Japan)
Background and Objective

• Variations in sample preparation methodology

• Analysis of dicentrics by different scorers

• Differences in dose-effect calibration curves

  Will influence dose assessment.

• **Objective:** Perform first inter-laboratory comparison after establishment of ISO guidelines
Inter-Laboratory Comparison Study: Laboratories and Roles

- **AFRRI**
  - Dosimetry
  - Radiation Exposure
  - Preparation of samples
  - Analysis
  - Report

- **REAC/TS, US**
  - Preparation of samples
  - Analysis

- **Budesamt fur Strahlenschutz, Germany**
  - Preparation of samples
  - Analysis

- **Health Canada**
  - Preparation of samples
  - Analysis

- **NIRS, Japan**
  - Preparation of samples
  - Analysis
Phase I Studies

• Construct dose-response calibration curves (Dose range 0.25 to 5.0 Gy)

• Compare calibration curves and determine variability
Calibration Curves

Phase II Studies

• Dose blinded study

• Irradiate samples with unknown doses

• Estimate radiation dose and determine accuracy
Distribution of Estimated Biological Doses for Laboratories A - E.

## Estimated Biological Doses in All Labs

<table>
<thead>
<tr>
<th>Physical Dose (Gy)</th>
<th>Estimated Biological Dose (Gy) and (SD) in various laboratories</th>
<th>Mean (SD)</th>
<th>Percent Error in Estimated Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>0.75</td>
<td>0.81</td>
<td>0.72</td>
<td>0.79</td>
</tr>
<tr>
<td>1.5</td>
<td>1.45</td>
<td>1.30</td>
<td>1.66</td>
</tr>
<tr>
<td>3.0</td>
<td>2.76</td>
<td>3.21</td>
<td>2.80</td>
</tr>
<tr>
<td>4.5</td>
<td>4.35</td>
<td>4.47</td>
<td>4.30</td>
</tr>
</tbody>
</table>
Conclusions: Section IV

• Dose-effect relationships for calibration curves between laboratories are similar but coefficients of the calibration curves differ.

• More importantly, estimated radiation doses from dose blinded samples by a comparison with each laboratory’s calibration curve were quite accurate in all laboratories at all doses.

• Developing a mathematical dose prediction algorithm for confirmation of clinical triage.
Summary

• Further developed and validated the “gold-standard” biodosimetry method for radiation mass casualty applications.

• Automation, system integration, protocol optimization, equipment customization, and data management solutions are critical for dicentric assay’s application in mass casualties.

• The laboratory is ready for advanced “stress” testing of automated systems and CytoTrack (LIMS) using real samples from a simulated mass casualty event.

• Degree of variability on dose effect calibration curves and assessed dose for the dose blinded samples between laboratories was determined.

• Mathematical algorithms for triage dose assessment is developed using inter-laboratory comparison study data, but needs testing.

• High throughput cytogenetics can be valuable for triage dose prediction in mass casualties.
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