EPRBioDose2026

Biodosimetry into the Future

ABSTRACT BOOK

August 30th – September 3rd, 2026

The Westin Ottawa

Ottawa, Ontario, Canada

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Acknowledgements

The Organizing Committee sincerely thanks the Local Organizing Committee, IABERD Executive Committee and Scientific Council, session chairs, keynote speakers, presenters, and authors. We also gratefully acknowledge our host institution, sponsors, and The Westin Ottawa staff for their support of EPRBioDose2026, with special thanks to Annick Laporte for logo design.

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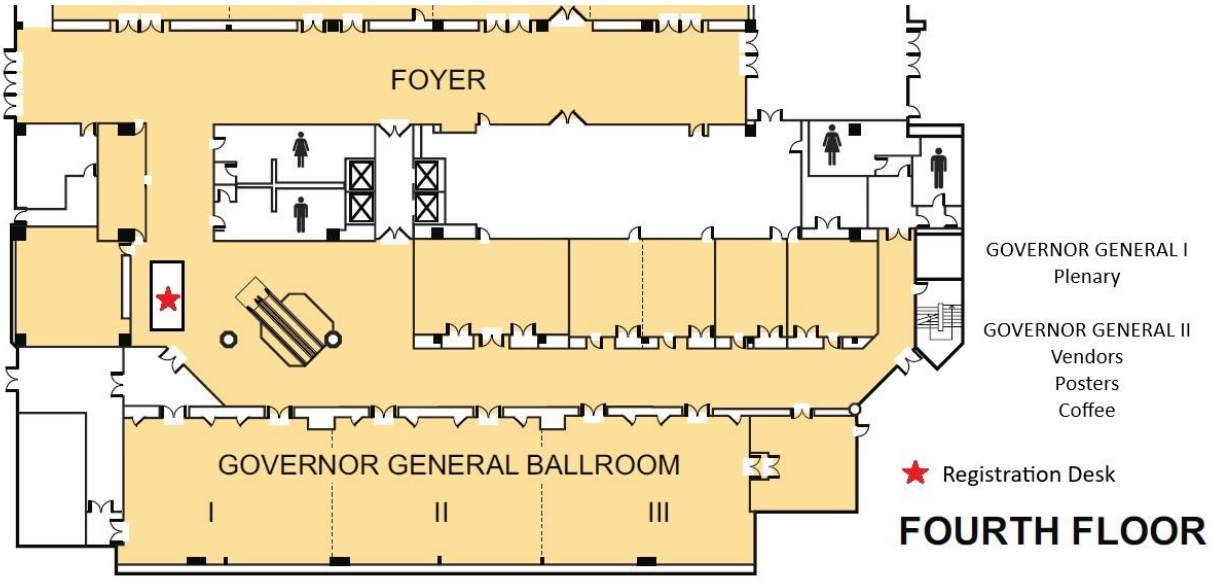
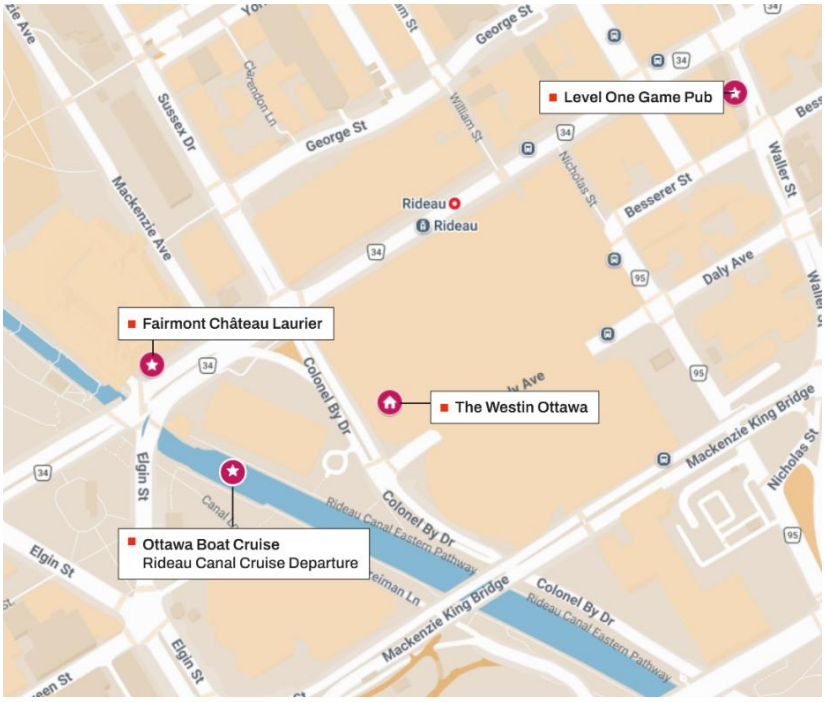
Venue & Key Locations

The Westin Ottawa - 11 Colonel By Drive, Ottawa, Ontario K1N 9H4, Canada

Venue Floor Plan (4th floor)



Key Locations



Interactive Map:
https://bit.ly/4wJ7BWX

Timetable

TIME	DAY −1 Sat · Aug 29	DAY 0 Sun · Aug 30	DAY 1 Mon · Aug 31	DAY 2 Tue · Sep 1	DAY 3 Wed · Sep 2	DAY 4 Thu · Sep 3	DAY 5 Fri · Sep 4
8:00			Registration 8:00–9:00				AECL | CNL Tour 7:00–18:30 Bus pick-up at the Westin
8:30	Training Courses 8:30–17:00 Location: 775 Brookfield Road Health Canada			Keynote 8:30–9:15	Keynote 8:30–9:15		
9:00			Welcome · 9:00–9:20			Session 7 9:00–10:15	
9:30			Keynote 9:20–10:05	Session 2 9:15–10:30	Session 5 9:15–10:30		
10:00			Coffee · 10:05–10:30			Coffee · 10:15–10:45	
10:30			Session 1 10:30–12:30	Coffee · 10:30–11:00	Coffee · 10:30–11:00	Session 8 10:45–12:00	
11:00				Session 3 11:00–12:30	Session 6 11:00–12:30		
11:30							
12:00						Closing · 12:00	
12:30		WHO BioDoseNet 10:00–17:00 Governor General I	Lunch 12:30–13:30	Lunch 12:30–13:30	Photo · 12:30–12:45		
13:00					Lunch & GA 12:45–14:00		
13:30			Posters 13:30–15:00	Posters 13:30–15:00			
14:00							
14:30					Boat Cruise 14:30–16:00		
15:00			Coffee · 15:00–15:30	Coffee · 15:00–15:30			
15:30			Round Table 15:30–17:30	Session 4 15:30–17:00			
16:00							
16:30							
17:00							
17:30							
18:00		Ice Breaker 18:00–20:00 Daly's (3rd floor)		Student | ECS Night Out 18:00–23:00	Banquet 18:00–22:00 TwentyTwo (22nd floor)		
...							

EPRBioDose2026 Program

Ottawa · Canada
The Westin Hotel · 11 Colonel By Dr.
4th Floor, Governor General I

DAY 1 | Monday, August 31

TIME	PROGRAM
8:00 – 9:00	**Registration & Coffee** · The Westin Hotel · 4th Floor Lobby
9:00 – 9:20	OPENING **Opening Remarks & Land Acknowledgement** **Tim Singer** Director General, Environmental & Radiation Health Sciences Directorate, Health Canada **Matthias Port** President, IABERD **Zhanat Kenbayeva** WHO Representative
9:20 – 10:05	KEYNOTE LECTURE 1 *Chair · Ruth Wilkins (Health Canada, Canada)*
KL1	**Perspectives in Multiple-Parameter Biological, Biophysical, and Physical Dosimetry** **William Blakely** Armed Forces Radiobiology Research Institute, United States
10:05 – 10:30	**Coffee Break** · Poster Room · Governor General II
10:30 – 12:30	SESSION 1 **New Biomarker Discovery** *Chairs · Sangeeta Murugkar (Carleton University, Canada), Lobna Alkebsi (Quantum and Radiological Science and Technology, Japan)*
S1-1	**Biomarkers and Bioresponse Following Internal Radiation Exposure** **Gayle Woloschak** Northwestern University, United States
S1-2	**New Functionalities in Multiharmonic Analysis (Sponsor presentation)** **Mikolaj Baranowski** Novilet, Poland

DAY 1 | Monday, August 31

TIME	PROGRAM
S1-3	**Biomarker Discovery in Minipigs Reveals Sex- and Survival-Associated Proteomic Signatures** **Ales Tichy** University of Defence, Czech Republic
S1-4	**Application of the Adverse Outcome Pathway Framework to Identify Multi-endpoint Biomarkers of Radiation Exposure** **Ngoc Vuong** Health Canada, Canada
S1-5	**Molecular Echoes of High-Dose Radiation: Persistent Blood Transcriptome Remodelling in Rhesus Macaques** **Sabrina Fischer** Bundeswehr Institute of Radiobiology, Germany
S1-6	**Transcriptional Signatures Along the Linear Energy Transfer Profile of Proton and Carbon-Ion Tracks in Skin and Blood** **Yannick Comoglio** United Kingdom Health Security Agency, United Kingdom
S1-7	**Unmasking Radiation Signatures: Integrating Automated RNA Isolation and Globin-Depleted Nanopore Sequencing for Point-of-Care Biodosimetry** **Samantha Stewart** Bundeswehr Institute of Radiobiology, Germany
S1-8	**CLOCK: meChanisms of the Transcriptional Control of the Circadian Clock** **Milagrosa Lopez Riego** United Kingdom Health Security Agency, United Kingdom
S1-9	**Identifying Urinary Exosomes and Their Content as Biomarkers of Radiation Exposure** **Antonella Bertucci** Canadian Nuclear Laboratories, Canada
12:30 – 13:30	**Lunch** · Governor General I
13:30 – 15:00	**Poster Session 1** · Odd-Numbered · Governor General II
15:00 – 15:30	**Coffee Break** · Poster Room · Governor General II

DAY 1 | Monday, August 31

TIME	PROGRAM
15:30 – 17:30	ROUND TABLE SESSION **Artificial Intelligence in Retrospective Biological and Physical Dosimetry: From Emergency Triage to Integrated Dose Assessment** **Moderators** **Maurizio Marrale** (University of Palermo, Italy) **Adayabalam Balajee** (Oak Ridge Institute for Science & Education, United States) **Panelists** **Matthias Port** (Bundeswehr Medical Academy, Germany) **Zhanat Kenbayeva** (World Health Organization, Switzerland) **Yohei Fujishima** (Hirosaki University, Japan) **Nadica Maltar-Strmečki** (Ruđer Bošković Institute, Croatia) **Kim Hyoungtaek** (Korea Atomic Energy Research Institute, Republic of Korea) **Prarthana Pasricha** (Carleton University, Canada) **Mohamed Amine Benadjaoud** (Autorité de sûreté nucléaire et de radioprotection, France)

DAY 2 | Tuesday, September 1

TIME	PROGRAM
8:00 – 8:30	**Coffee Break** · Poster Room · Governor General II
8:30 – 9:15	**KEYNOTE LECTURE 2** *Chair · Matthias Port (Bundeswehr Medical Academy, Germany)*
KL2	**Cutaneous Radiation Injury: Clinical Analysis and EPR Biodosimetry for Local Exposure Assessment** **Minsu Cho** Korea Institute of Radiological & Medical Sciences, Republic of Korea
9:15 – 10:30	**SESSION 2** **Recent Developments in EPR and Biological Dosimetry** *Chairs · Antonella Bertucci (Canadian Nuclear Laboratories, Canada), Samantha Stewart (Bundeswehr Institute of Radiobiology, Germany)*
S2-1	**Current Challenges and Future Perspectives in Cytogenetic Biodosimetry** **Tomisato Miura** Hirosaki University, Japan
S2-2	**Forensic Electron Paramagnetic Resonance Dosimetry for Former US Nuclear Workers** **Alexander Romanyukha** Uniformed Services University for the Health Sciences / Armed Forces Radiobiology Research Institute, United States
S2-3	**(of Cytogenetic Staining Methods Incorporating a New Modified C-Banding Technique** **Donovan Anderson** Hirosaki University, Japan
S2-4	**Development of an Automated Calyculin-A Induced Premature Chromosome Condensation (PCC) Assay for Biodosimetry** **Hailey Adams** Carleton University | Health Canada, Canada
S2-5	**Development of Radiation Injury Triage and Assessment System (RITAS): A High-Throughput Biodosimetry Platform for Radiation Emergency Response** **Helen Turner** Columbia University, United States
10:30 – 11:00	**Coffee Break** · Poster Room · Governor General II

DAY 2 | Tuesday, September 1

TIME	PROGRAM
11:00 – 12:30	**SESSION 3** **Retrospective and Accidental Dose Assessment — EPR** *Chairs · Stephen Swarts (University of Florida, United States), Hanna Przybyla (Ontario Tech University, Canada)*
S3-1	**Electron Paramagnetic Resonance for Retrospective and Accidental Dose Assessment: Biological and Fortuitous Materials** **Hasan Tuner** Balikesir University, Türkiye
S3-2	**Recent Achievements and Current Challenges in EPR Dosimetry Using Fingernails** **Samayeh Azariasl** Hiroshima University, Japan
S3-3	**Investigating the Use of Alanine-Based Chip Cards for Emergency Personal Dosimetry Following Ionizing Radiation Exposures** **Joel Beazley** University of Oxford, United Kingdom
S3-4	**Improvement of Sugar-EPR Dosimetry for Emergency Use** **Hiroshi Yasuda** Hiroshima University, Japan
	EPR Dosimetry of Sprague-Dawley Rat Teeth **Amna Hassan** Canadian Nuclear Laboratories, Canada
S3-5	**In Vivo EPR Tooth Dosimetry for a Healthcare Worker Detects the Signal Composed of Medical and Occupational Radiation Exposure** **Ichiro Yamaguchi** National Institute of Public Health, Japan
12:30 – 13:30	**Lunch** · Governor General I
13:30 – 15:00	**Poster Session 2** · Even-Numbered · Governor General II
15:00 – 15:30	**Coffee Break** · Poster Room · Governor General II

DAY 2 | Tuesday, September 1

TIME	PROGRAM
15:30 – 17:00	**SESSION 4** **Retrospective and Accidental Dose Assessment — Biodosimetry** *Chairs · Christina Beinke (Bundeswehr Institute of Radiobiology, Germany), Yohei Fujishima (Hirosaki University, Japan)*
S4-1	**Emerging Technologies in Radiation Biodosimetry : Harnessing Novel Approaches for a Radiological/Nuclear Public Health Emergency Toolkit** **Merriline Satyamitra** National Institute of Allergy and Infectious Diseases (NIH/NIAID), United States
S4-2	**A Simple, Rapid, Portable and Cost-Effective PCR Method for Radiation Biodosimetry** **Ashveny Ashok** United Kingdom Health Security Agency, United Kingdom
S4-3	**Recent Developments in the Field of High Dose Biodosimetry in India** **Usha Yadav** Bhabha Atomic Research Centre, India
S4-4	**The Impact of Inter- and Intra-individual Variability on the DNA Damage Response to Ionising Radiation** **Hannah Mancey** United Kingdom Health Security Agency, United Kingdom
18:00 – 23:00	**Night Out for Students & Early Career Scientists** · Château Laurier, Level One

DAY 3 | Wednesday, September 2

TIME	PROGRAM
8:00 – 8:30	**Coffee Break** · Poster Room · Governor General II
8:30 – 9:15	**KEYNOTE LECTURE 3** *Chair · Maurizio Marrale (Università degli Studi di Palermo, Italy)*
KL3	**The Maturation of EPR Biodosimetry for Mass Casualty Response** **Jason Sidabras** Medical College of Wisconsin, United States
9:15 – 10:30	**SESSION 5** **Space Radiation Dosimetry** *Chairs · Hiroshi Yasuda (Hiroshima University, Japan), Valerie Goh Swee Ting (Singapore Nuclear Research and Safety Institute, Singapore)*
S5-1	**Space Radiation Environment Challenges for Biodosimetry** **Marcelo Vazquez** Canadian Nuclear Laboratories, Canada
S5-2	**Astronaut Biodosimetry in Canada** **Ruth Wilkins** Health Canada, Canada
S5-3	**Machine Learning and Sample Size Calculations in Astronaut Biodosimetry** **Benjamin (** Carleton University / Health Canada, Canada
S5-4	**Simulating a Lymphocyte Cell in Geant4-DNA to Model Radiation Response in Space** **Dinindu Gunasekara** Carleton University / Health Canada, Canada
10:30 – 11:00	**Coffee Break** · Poster Room · Governor General II
11:00 – 12:30	**SESSION 6** **Biodosimetry for Low Dose and Medical Exposures** *Chairs · Alegria Montoro (La Fe University and Polytechnic Hospital, Spain), Prabodha Kumar Meher (Stockholm University, Sweden)*
S6-1	**Biological Dosimetry for Low Dose and Medical Exposures – Where Are We and Where Are We Heading?** **Andrzej Wojcik** Stockholm University, Sweden
S6-2	**Microdosimetric Considerations for Uncertainty Quantification in Cytogenetic Dosimetry** **Finn Dodgson** Carleton University, Canada

DAY 3 | Wednesday, September 2

TIME	PROGRAM
S6-3	**EPR Study of Dose-Rate Dependent Radiolytic Radical Formation During Ultra-High Dose-Rate Irradiation In Vitro and In Vivo** **Johanna Pehlivan** Karlsruhe Institute of Technology, Germany
S6-4	**Investigating the γ-H2AX Response for Low Dose and Low Energy-Range X-ray Exposures** **Lindsay Beaton-Green** Health Canada, Canada
S6-5	**Establishment of CBMN Dose-Response Curves with Age- and Sex-Adjusted Background Correction for Biodosimetry** **Lobna Alkebsi** Quantum and Radiological Science and Technology, Japan
S6-6	**Multimodal Nonlinear Optical Microscopy Detection of In Vivo Radiation Exposure in Murine Skin** **Parminder Riarh** Carleton University, Canada
12:30 – 12:45	**Group Photo** · Governor General I
12:45 – 14:00	**Lunch & GA Meeting** · Governor General I
14:30 – 16:00	**Boat Cruise of the Rideau Canal** · Free afternoon
18:00 – 22:00	**Banquet Dinner** · The Westin · Venue TwentyTwo

DAY 4 | Thursday, September 3

TIME	PROGRAM
8:00 – 9:00	**Coffee Break** · Poster Room · Governor General II
9:00 – 10:15	**SESSION 7** **Reference Dosimetry / Alanine Dosimetry** *Chairs · Oswaldo Baffa (University of São Paulo, Brazil), Patricia Nicolucci (University of São Paulo, Brazil)*
S7-1	**Dosimetry for Biological Studies: State of the Art, Recent Advances and Challenges** **Morgane Dos Santos** Autorité de sûreté nucléaire et de radioprotection, France
S7-2	**Alanine Spectrum Decomposition for Simultaneous Dosimetry and LET Estimations** **Erwan Martinet-Gerphagnon** Autorité de sûreté nucléaire et de radioprotection, France
S7-3	**Validating Reference Dosimetry in Magnetic Fields Using Alanine Dosimeters – a Pan-Canadian Study** **Malcolm McEwen** National Research Council, Canada
S7-4	**X-ray Microdosimetry in Aluminum Oxide** **Connor McNairn** Carleton University, Canada
10:15 – 10:45	**Coffee Break** · Poster Room · Governor General II
10:45 – 12:00	**SESSION 8** **Networking, Exercises, Emergency Response** *Chairs · Lindsay Beaton (Health Canada, Canada), Sabrina Fischer (Bundeswehr Institute of Radiobiology, Germany)*
S8-1	**Networking, Exercises, and Emergency response** **Stephen Barnard** United Kingdom Health Security Agency, United Kingdom
S8-2	**Development of a National Biological Dosimetry Protocol in Spain: Interlaboratory Comparisons and Future Network Implementation** **Alegria Montoro** La Fe University and Polytechnic Hospital, Spain
S8-3	**Enhanced Operational Capacity for a Laboratory Using Triage-Mode Based Biodosimetry: Implementation and Training** **Juan Martinez** Autorité de sûreté nucléaire et de radioprotection, France

DAY 4 | Thursday, September 3

TIME	PROGRAM
S8-4	**UKHSA RCCE Emergency Response Exercise 2025 — Biological and Physical Dosimetry: Comparison of Techniques for Full and Triage Dose Estimations in the Event of a Mass Casualty Nuclear Incident** **Lauren Ashworth-Donn** United Kingdom Health Security Agency, United Kingdom
12:00	CLOSING **Closing Remarks**

Poster Session

The poster program is divided into two sessions. Odd-numbered posters will be presented during Poster Session 1 on Monday, August 31st, from 13:30–15:00 in Governor General II. Even-numbered posters will be presented during Poster Session 2 on Tuesday, September 1st, from 13:30–15:00 in Governor General II.

**Posters are listed in numerical order by assigned poster ID. Posters with an oral presentation counterpart are cross-referenced by oral session number; their abstracts are published in the Oral Presentations section.*

Poster ID	Title & Presenter
P01	**In silico Identification of Circulating miRNA for Radiation Exposure Assessment** *Hae Young Ko (Korea Institute of Radiological & Medical Sciences, Republic of Korea)*
P02 (S1-3)	**Biomarker Discovery in Minipigs Reveals Sex- and Survival-Associated Proteomic Signatures** *Aleš Tichý (University of Defence, Military Faculty of Medicine, Czech Republic)*
P05	**A Saliva-Based Microfluidic Sensor for Point-of-Care Diagnosis of Acute Radiation Syndrome** *Sabrina Fischer (Bundeswehr Institute of Radiobiology, Germany)*
P06 (S1-5)	**Molecular Echoes of High-Dose Radiation: Persistent Blood Transcriptome Remodelling in Rhesus Macaques** *Sabrina Fischer (Bundeswehr Institute of Radiobiology, Germany)*
P07 (S1-7)	**Unmasking Radiation Signatures: Integrating Automated RNA Isolation and Globin-Depleted Nanopore Sequencing for Point-of-Care Biodosimetry** *Samantha Stewart (Bundeswehr Institute of Radiobiology, Germany)*
P09	**Application of the TGx-DDI Biomarker to Detect Radiation-Induced DNA Damage Responses in Human Lymphocytes** *Saadia Khilji (Health Canada, Canada)*
P10 (S2-3)	**Comparison of Cytogenetic Staining Methods Incorporating a New Modified C-Banding Technique** *Donovan Anderson (Hirosaki University, Japan)*
P11 (S2-4)	**Development of an Automated Calyculin-A Induced Premature Chromosome Condensation (PCC) Assay for Biodosimetry** *Hailey Adams (Carleton University / Health Canada, Canada)*
P13	**A Highly Efficient and Sensitive Approach for Detecting Chromosome Aberrations in Cytogenetic Biodosimetry Using Checkpoint Bypass** *Ayaka Okimoto (Hirosaki University, Japan)*
P14	**Use of the p21 inhibitor UC2288 as a strategy to circumvent cell cycle arrest during proliferation for dicentric chromosome analysis** *Christina Beinke (Bundeswehr Institute of Radiobiology, Germany)*

Poster ID	Title & Presenter
P15	**Quantification of Golgi morphology using imaging flow cytometry for radiation biodosimetry** *Younghyun Lee (Soonchunhyang University, Republic of Korea)*
P16	**Serum-free culture supports reliable chromosomal aberration analysis for clinical cytogenetics and biodosimetry** *Tomisato Miura (Hirosaki University, Japan)*
P17	**EPR dosimetry in bone samples of former United States nuclear workers** *Alexander Romanyukha (Uniformed Services University of the Health Sciences, United States)*
P18	**Assessing various processing methods to optimize the detection of the stable radiation induced signal (RIS) in nail clippings** *Steven Swarts (University of Florida, United States)*
P19	**Feasibility Study of ESR Dosimetry in Turtle Shells** *Amber Harshman (Oak Ridge National Laboratory, United States)*
P20	**EPR spectral classification of smartphone screen glass: From variability to dosimetric insight** *Hasan Tuner (Balikesir University, Türkiye)*
P21	**Development of Preliminary STG-EPR Dosimetry Protocol for Radiological Emergency** *Jae Seok Kim (Korea Institute of Radiological and Medical Sciences, Republic of Korea)*
P23	**Absorption dose ratio for fingernails and fingers by incident direction and energy of mono-energy photons** *Ichiro Yamaguchi (National Institute of Public Health, Japan)*
P27	**Using fluorescence in situ hybridization (FISH) to evaluate multiple biomarkers of recent and past exposure to ionizing radiation** *Vita Lai (Canadian Nuclear Laboratories, Canada)*
P29	**Implementation of the γH2AX Assay as a Biodosimetry Tool for Radiation Emergencies** *Jakub Vavra (National Radiation Protection Institute, Czech Republic)*
P30	**γH2AX as a biodosimetric marker of ionizing radiation: the use of Metafer systems and flow cytometry** *Lenka Andrejsová (University of Defence, Czech Republic)*
P31	**Cases with suspected exposure to ionizing radiation referred to the Biological Dosimetry Laboratory of the Hospital General Universitario Gregorio Marañón, Madrid (Spain)** *Maria Jesus Prieto Rodriguez (Hospital General Universitario Gregorio Marañón, Spain)*
P32	**Translocation Frequencies in Peripheral Blood Mononuclear Cells of Cancer Patients Undergoing External Beam Radiotherapy** *Prabodha Kumar Meher (Stockholm University, Sweden)*

Poster ID	Title & Presenter
P33	**Validating an imaging flow cytometry-based γ-H2AX assay for triage screening in case of a radiological event** *Steve Pecoskie (Canadian Nuclear Laboratories, Canada)*
P34	**Accelerated Triage of Suspected Irradiated Individuals Based on Dicentric Chromosome Analysis of Human Peripheral Blood** *Zuzana Šinkorová (University of Defence, Czech Republic)*
P35 (S4-2)	**A Simple, Rapid, Portable and Cost-Effective PCR Method for Radiation Biodosimetry** *Ashveny Ashok (United Kingdom Health Security Agency, United Kingdom)*
P37	**Accurate estimation of clinically significant radiation doses up to 5 Gy using qRT-PCR–based gene expression analysis** *Ivonne Romero (University of La Frontera, Chile)*
P40 (S5-3)	**Machine Learning and Sample Size Calculations in Astronaut Biodosimetry** *Benjamin Puzantian (Carleton University | Health Canada, Canada)*
P41 (S5-4)	**Simulating a Lymphocyte Cell in Geant4-DNA to Model Radiation Response in Space** *Dinindu Gunasekara (Carleton University | Health Canada, Canada)*
P42	**Impact of the obesity hormone leptin in combination with estrogen on frequencies of radiation-induced micronuclei in peripheral blood mononuclear cells of a health donor Checkpoint Bypass** *Andrzej Wojcik (Stockholm University, Sweden)*
P43	**EPR Study of Dose-rate Dependent Radiolytic Radical Formation during Ultra-high Dose-rate Irradiation In Vitro and In Vivo** *Johana Pelivan (Karlsruhe Institute of Technology, Germany)*
P46	**Evaluation of Neutron-Induced Bystander Effects in Human Colon Cancer Cells** *Wei Liu (McMaster University, Canada)*
P50	**Alanine Dosimetry for Determination of Radiation Exposure during Emergencies** *Jon Eakins (United Kingdom Health Security Agency, United Kingdom)*
P51	**Investigation of Alanine Dosimetry by EPR for Ultra-High-Dose-rate Electron FLASH Radiation Therapy** *Julia Sherman (Duke University, United States)*
P52 (S7-2)	**Alanine Spectrum Decomposition for Simultaneous Dosimetry and LET Estimations** *Erwan Martinet-Gerphagnon (Institut Curie | Autorité de sûreté nucléaire et de radioprotection, France)*
P53	**Alanine/EPR Dosimetry in the kGy/s Scale** *Kyssylla Monnyelle Araujo Silva (Universidade de São Paulo, Brazil)*

Poster ID	Title & Presenter
P54	**Alanine for Proton Therapy Bragg Peak Dosimetry** *Erwan Martinet-Gerphagnon (Institut Curie | Autorité de sûreté nucléaire et de radioprotection, France)*
P55	**Radiation quality effects on the EPR/alanine signal: spectrum decomposition and the radicals' contribution** *Maurizio Marrale (University of Palermo, Italy)*
P56	**Traceable alanine EPR dosimetry for low- and high-dose applications** *Nathaniel Woodall (National Physical Laboratory, United Kingdom)*
P57	**Dose heterogeneity assessment in 137 Cs-irradiator using alanine/ESR and glass dosimetry** *Kyssylla Monnyelle Araujo Silva (University of São Paulo, Brazil)*
P59	**Multi-Year Performance of Canadian Biodosimetry Interlaboratory Comparisons** *Ruth Wilkins (Health Canada, Canada)*
P60	**Capacity and capability of the RENEB network for large scale radiological and nuclear emergencies: Survey results 2025** *Stephen Barnard (United Kingdom Health Security Agency, United Kingdom)*
P61	**Development and Educational Evaluation of e-CLIK, a Biodosimetry Training Program for the Dicentric Chromosome Assay** *Susan Yang (Korea Institute of Radiological & Medical Sciences, Republic of Korea)*
P62	**Mixed-Field (Fast Neutrons and Gamma Rays) Cytogenetic Biodosimetry Inter-Laboratory Comparison Exercise** *William Blakely (Armed Forces Radiobiology Research Institute, United States)*
P63	**Evaluating Raman spectroscopy and multivariate analysis for blood plasma biodosimetry** *Josh Kazi (Carleton University, Canada)*
P64	**Robustness in Emergencies: Do Culture Types, Scorer Experience, and Reagent Substitutions Affect Dose Estimation by Dicentric Chromosome Assay?** *Shu Xian Teo (Singapore Nuclear Research and Safety Institute, Singapore)*
P65	**Multiple scorers not essential for triage dose estimations with manual dicentric scoring** *Valerie Swee Ting Goh (Singapore Nuclear Research and Safety Institute, Singapore)*
P67	**Transition of EAST H-ARS Tools using In-house Non-code App Solutions** *Lalitha Kurada (Henry M. Jackson Foundation, United States)*
P69	**Application of Electron Spin Resonance (ESR) Dating to a Fossil Tooth from Chuí Creek, Southern Brazil** *Oswaldo Baffa(Universidade de São Paulo, Brazil)*

Poster ID	Title & Presenter
P70	**Mitigating effect of piperazine derivatives after gamma irradiation** *Vojtech Chmil (University of Defence, Czech Republic)*
P71	**Evaluation of Interleukin Supplementation for Optimizing Dicentric Chromosome Assay Performance in Cryopreserved Whole Blood** *Marcela Milanová (University of Defence, Czech Republic)*
P72	**Impact of Low-Dose Ionizing Radiation on Lens Protein Expression and Cataractogenesis in Chornobyl Voles** *Paul Grzegorczyk (McMaster University, Canada)*
P73	**Emerging Roles of Multiparametric Biodosimetry and Artificial Intelligence in Advancing Radiation Dose Assessment for Triage** *Adayabalam Balajee (Oak Ridge Institute for Science and Education, United States)*
P74	**Application of Artificial Intelligence to Metaphase Finder and Automated Chromosome Aberration Finding (Second Report)** *Akira Furukawa (National Institute for Quantum Science and Technology, Japan)*
P75	**Development of an artificial neural network-based dose reconstruction and conversion model for predicting bone and blood doses in external exposure scenarios** *Hyoungtaek Kim (Korea Atomic Energy Research Institute, Republic of Korea)*
P76	**MATHELDE: Machine Learning Approach for Tooth Enamel Dose Estimation** *Maurizio Marrale (University of Palermo, Italy)*
P77	**Evaluation of Multi-Dosimeter Approaches for Posture- and Geometry-Dependent Dose Assessment** *Min Chae Kim (Korea Atomic Energy Research Institute, Republic of Korea)*
P78	**AI-Driven Triage Classification at the 1 Gy threshold Using Fortuitous OSL Materials and Portable Dosimetry** *Nadica Maltar-Strmečki (Ruđer Bošković Institute, Croatia)*
P79	**The Future of Biological Dosimetry: Integrating Automation and Artificial Intelligence for High-Precision Radiation Dose Assessment** *Oleg Belyakov (International Atomic Energy Agency, United Nations)*
P80	**Towards the development of a novel system for microdosimetry and cellular radiation response** *Prarthana Pasricha (Carleton University, Canada)*
P81	**A technical advance in automated cytokinesis-block micronucleus assay using AI-based instance segmentation** *Yohei Fujishima (Hirosaki University, Japan)*
P82	**An Oral Multi-Agent Countermeasure Against IR-Induced Injury That Preserves or Enhances Radiotherapy Efficacy** *Alegria Montoro (La Fe University and Polytechnic Hospital, Spain)*

Round Table Session

Monday, August 31st (15:30 – 17:30)

The Round Table Session will feature a unique discussion centered on the theme:

Artificial Intelligence in Retrospective Biological and Physical Dosimetry: From Emergency Triage to Integrated Dose Assessment

Moderators

Maurizio Marrale	*University of Palermo, Italy*
Adayabalam Balajee	*Oak Ridge Institute for Science & Education, United States*

Panelists

Matthias Port	*Bundeswehr Medical Academy, Germany*
Zhanat Kenbayeva	*World Health Organization, Switzerland*
Yohei Fujishima	*Hirosaki University, Japan*
Nadica Maltar-Strmečki	*Ruđer Bošković Institute, Croatia*
Kim Hyoungtaek	*Korea Atomic Energy Research Institute, Republic of Korea*
Prarthana Pasricha	*Carleton University, Canada*
Mohamed Amine Benadjaoud	*Autorité de sûreté nucléaire et de radioprotection, France*

Abstracts

Keynote Lectures

KL1

Perspectives in Multiple-Parameter Biological, Biophysical, and Physical Dosimetry

Blakely, W.F.

Multiple-Parameter Radiation Biodosimetry Laboratory, Armed Forces Radiobiology Research Institute, Uniformed Services of the Health Sciences, 4555 South Palmer Road, Bethesda, MD, USA 20889
william.blakely@usuhs.edu

The initial development of concepts related to correlating biological changes to radiation exposure occurred in the 1940-50's.[1] In the 1960s the use of cytogenetic biodosimetry was introduced by Dr. Michael A. Bender using the lymphocyte dicentric chromosome aberration assay to evaluate radiation exposure in a radiation accident patient. The use of multiple-parameter biological, biophysical, and physical dosimetry is justified since no single assay is sufficient to meet the requirements of initial triage, potential mass-casualty incidents, and definitive dose and injury assessment for both early-phase accidents and retrospective radiation dose assessment. The use of multiple assays can enhance the accuracy of radiation assessment. The sole use of a dose-centric process for reporting assessment is reported to have limited utility in the clinical management of severe radiation exposure.[2] METROPOL's response category injury-centric approach has provided a framework to report radiation injury severity. The dual use of both dose- and injury-centric reporting of multiple-parameter biological, biophysical, and physical dosimetry findings can provide significant benefits. A review of the maturation of new assays reveals that biomarkers used either for retrospective dosimetry or early-phase clinical outcome prediction requires intensive research covering the bioassay identification, characterization, validation, exercises, and automation. In addition, harmonization via adoption of standardization using the International Organization for Standardization is critical to make these bioassays and services reliable, safe, and of high quality. Further, networking is essential for a single laboratory or nation to overcome potential surge requests. [The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy or position of the Department of War, AFRRI, Uniformed Services University of the Health Sciences, or the U.S. Government. Funding support was provided by AFRRI (AFR-B2-10664 and RAB32165)].

Keywords Biodosimetry, biophysical and physical dosimetry, exercises, automation, dose assessment, networking.

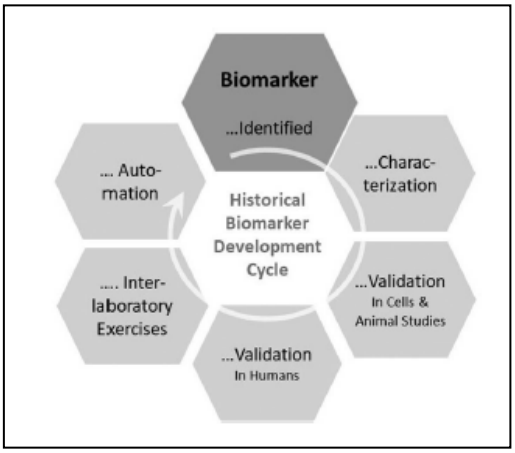


Figure 1 Historical biomarker development cycle (Blakely et al. 2014).

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Cutaneous Radiation Injury: Clinical Analysis and EPR Biodosimetry for Local Exposure Assessment

Cho, M.

National Radiation Emergency Medical Center, Korea Institute of Radiological and Medical Sciences, Seoul, Republic of Korea
choms@kirams.re.kr

Cutaneous radiation injury (CRI) refers to deterministic damage to the skin and underlying tissues from acute external radiation exposure. CRI can occur when a body part is exposed to an unshielded area of X-ray generating devices or radioactive materials. The erythema threshold is approximately 2 Gy, with severity escalating at higher doses. Historical cases have been associated with unsecured sources from food irradiators, radiotherapy equipment, well-logging gauges, and prolonged fluoroscopy [1].

CRI follows a characteristic cyclical course distinguishing it from chemical or thermal burns. Transient erythema with pruritus may appear within hours, followed by a latent phase lasting days to weeks, after which intense erythema, blistering, and ulceration develop. Subsequent waves of erythema may recur over months to years. High-dose exposure can cause permanent alopecia, glandular destruction, fibrosis, pigmentary changes, and tissue necrosis. Early recognition requires careful history-taking, as prodromal erythema is easily overlooked due to its transient nature [1,2].

Electron Paramagnetic Resonance (EPR) biodosimetry provides critical local dose assessment by measuring radiation-induced free radicals in biological samples. Fingernail EPR is particularly valuable for hand-region exposures—the most common local exposure scenario—offering non-invasive sampling and quantitative correlation with clinical severity. Tooth enamel EPR serves as a whole-body dose surrogate. Simultaneous measurement of both enables differentiation between local and whole-body dose distributions based on the inverse square law: high fingernail EPR with low tooth enamel EPR indicates local exposure, whereas elevated values in both suggest whole-body involvement. In cases of hand CRI, during the latent phase when prodromal erythema has resolved, EPR measurement may be the only quantitative indicator based on biological specimens for predicting subsequent severe skin damage.

Integrating EPR-measured doses with clinical findings—onset timing, extent, and intensity of erythema—enables objective severity assessment, prognosis prediction, and evidence-based treatment planning. Incorporating EPR dosimetry into national local exposure accident response protocols is essential for radiation worker health protection and optimized medical intervention.

Keywords Cutaneous radiation injury; EPR biodosimetry; Local dose assessment; Fingernail dosimetry; Radiation emergency response

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The Maturation of EPR Biodosimetry for Mass Casualty Response

Sidabras, J.W.

Department of Biophysics, Medical College of Wisconsin, 8701 Watertown Plank Rd, Milwaukee, WI, 53226, USA
jwsidabras@mcw.edu

In an ionizing radiation mass casualty event, the ability to rapidly and accurately assess absorbed dose across thousands of individuals is not a scientific aspiration, it is a triage imperative. Electron paramagnetic resonance (EPR) biodosimetry has long held the physical promise to meet that imperative, yet translating laboratory capability into deployable clinical tools has remained an enduring challenge for the field.

The scientific foundation for EPR-based biodosimetry was established through the pioneering work of Prof. Harold M. Swartz, whose systematic characterization of radiation-induced radicals in tooth enamel opened a new paradigm for retrospective dosimetry. Equally important contributions from Motoji Ikeya and others refined both the physical understanding and measurement methodology, which established the core toolkit. Over the past decade, our group has worked in direct and ongoing collaboration with Prof. Swartz to extend this foundation, driving it toward the clinical and operational realities of mass casualty response.

Three challenges have historically separated EPR biodosimetry from deployment: sensitivity in the clinically actionable dose range, measurement throughput at mass casualty scale, and instrument portability for field use. Through coordinated advances in resonator design, detection methodology, and signal processing, our team has demonstrated dose resolution across a 0–10 Gy triage-relevant window, with a target resolution of 0.5 Gy. We have developed a three-tier measurement framework: *i)* triage assessment (5–10 minutes, with dose homogeneity evaluation), *ii)* medical evaluation (15–25 minutes), and *iii)* retrospective dosimetry (applicable weeks to years post-incident). These measurements map directly onto operational response timelines.

Today, EPR biodosimetry is no longer just a research tool. The field-deployable L-band *in vivo* tooth enamel platform is operationally ready [1], and compliments traditional biologically-based dosimetry workflows. In parallel, supported by our NIH U01 program, we are advancing a novel resonator design for non-invasive nail and toenail dosimetry [2]. Measuring nail and toenails *in vivo* is an approach that eliminates sample extraction requirements and extends triage capability to unresponsive, pediatric, and field-assessed populations. New signal processing frameworks developed under this program are reducing environmental and system noise while accelerating acquisition across all *in vivo* dosimetry platforms, with a clear developmental path toward X-band nail dosimetry deployment in a follow-on program.

Keywords in vivo biodosimetry, electron paramagnetic resonance, technology development, ionizing radiation triage

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Oral Presentations

Biomarkers and Bioresponse following Internal Radiation Exposure

Woloschak, G.

Northwestern University
g-woloschak@northwestern.edu

The Northwestern University Radiation Archives (NURA) has a large collection of paraffin-embedded tissues and datasets from dogs, mice, rats, monkeys and other animals exposed to both external and internal radiation. Routes of administration for internal emitters include inhalation, injection and ingestion, and inhaled radionuclides were often encapsulated in microparticles of Si to mimic a nuclear incident. In recent work we have focused on dogs exposed to inhaled radionucldies including different isotopes and forms of Pu as well as Sr90, Y90, Y91 and others. Using X-ray fluorescence microscopy, we have detected the presence of the radionuclide or its daughter products in lungs and draining lymph nodes from the dogs. We have also used a variety of AI and statistical methods to examine elemental composition of these tissues for biosignatures of exposure. In molecular studies we have identified several miRNAs that appear to be specific for radiation exposure in general, being commonly found following external and internal exposures; studies to identify uniquely internally exposed miRNAs are underway.

New functionalities in Multiharmonic Analysis

Baranowski, M.[1,2]*, Czechowski, T.[1], Szczepanik, P.[1], Jurga, P.[1], Malinowski, P.[1]

[1]Novilet,1 Romana Maya, Poznań, Poland
[2]Department of Physics of Functional Materials, Faculty of Physics and Astronomy, Adam Mickiewicz University, Poznań, Poland
*Presenting Author: m.baranowski@novilet.eu

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POIR.01.02.00-0077/18

Multiharmonic analysis has been developed for years [1–5] and enables, among other things, the reconstruction of the unmodulated shape of CW EPR spectra regardless of the modulation amplitude used. Ultimately, the quality of the obtained signals is influenced by experimental parameters, hardware parameters, and the analysis algorithms used. Properly performed multiharmonic analysis requires consideration of a number of factors, in particular: the impact of the detection bandwidth on the recorded harmonics and the passage effect in the recorded data.

The solutions used in the new algorithm allow for the correction of these effects and the acquisition of spectra unaffected by these factors. Firstly, this results in signal reconstruction with the highest possible efficiency, even if the signal's bandwidth was wider than the detection bandwidth. Secondly, it is possible to perform multiharmonic analysis for radicals for which the passage effect known from the rapid scan technique is observed on the harmonics, which is corrected during the analysis. Additionally, the procedures used in the multiharmonic analysis algorithm to optimize the noise parameters of the spectrum provide an above-average increase in sensitivity. The use of multiharmonic analysis results in a multiple increase in the signal-tonoise ratio of the spectra, which, depending on the experimental conditions, can reach up to two orders of magnitude.

It should be emphasized that the development and practical implementation of multiharmonic analysis has been significantly advanced thanks to the work of Novilet, whose technological solutions and innovative algorithms played a key role in refining this technique. Multiharmonic analysis can be performed on Novilet ERI DUO imagers or as an add-on to existing or new Bruker EPR spectrometers.

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Biomarker Discovery in Minipigs Reveals Sex- and Survival-Associated Proteomic Signatures

Duracova, M.B.[1], Rehulka, P.[2], Vozandychova, V.[2], Tichy, A.[1]*

[1]Department of Radiobiology and [2]Department of Molecular Pathology and Biology, Military Faculty of Medicine, University of Defence, Trebesska 1575, 50001 Hradec Kralove, Czech Republic
*Presenting Author: ales.tichy@unob.cz

Purpose: Large-scale radiological or nuclear incidents underlie the need for reliable biomarkers for the rapid assessment of radiation exposure. Proteins represent promising candidates due to their dynamic response to ionizing radiation and their detectability in clinical settings. This study examined protein profiles associated with survival in minipigs and investigated sex-dependent differences following total body irradiation (TBI).
Methods: In this proteomic study, peripheral blood samples were collected from 27 Göttingen minipigs across three independent experiments. Samples were obtained at four time points: 3 days prior to irradiation and 1, 3, and 10 days following exposure to 2.1 Gy TBI. Label-free relative quantification was performed using an RP-nanoLC-ESI-MS/MS system coupled with a Q Exactive mass spectrometer (Thermo). Plasma was first separated from whole blood, and proteins were subsequently depleted, reduced, alkylated, and enzymatically digested with trypsin/LysC according to standard protocols. Protein identification and analysis were conducted using MaxQuant, Perseus, and RStudio. Statistical significance was assessed using a t-test, with $p \leq 0.05$ considered significant. The dataset is publicly available via ProteomeXchange under identifier PXD071774 (DOI: 10.6019/PXD071774).
Results: Comparative proteomic profiling revealed differential regulation of numerous proteins associated with radiation exposure, survival outcomes, and sex-specific responses. Eighteen top candidate proteins strongly associated with gamma radiation were identified. These proteins are involved in hemostasis, inflammatory signaling, immune regulation, and tissue repair. Validation across three independent studies confirmed the reproducibility of key biomarkers. Plasma proteomic analysis also demonstrated distinct sex-specific expression patterns following irradiation. In males, hemoglobin subunits alpha and beta (HBA/HBB) were significantly upregulated ($\log_2$ fold change > 2), while multiple immunoglobulin-like domain proteins and immunoglobulin heavy-chain components were consistently downregulated. In females, fibrinogen alpha and beta chains (FGA/FGB), complement C3, and apolipoprotein E (APOE) showed marked increases. Both sexes exhibited decreased levels of lysozyme C-3, serotransferrin, and von Willebrand factor, with a more pronounced reduction observed in females. Stratification of proteomic data by survival over a 30-day period revealed a distinct plasma signature associated with outcomes. Non-survivors exhibited elevated levels of periostin (the most prominent signal) and lipopolysaccharide-binding protein, whereas survivors showed higher levels of immune-related proteins, including immunoglobulin lambda chain and complement C1q subunits. These differences emerged shortly after irradiation, persisted over time, and remained consistent across experimental batches.
Conclusion: These findings support the use of minipigs as a translational model for radiation biomarker discovery. The identified proteins represent promising candidates for further validation as indicators of radiation injury and predictors of clinical outcomes.

Application of the Adverse Outcome Pathway Framework to Identify Multi-endpoint Biomarkers of Radiation Exposure

Vuong, N.Q.[1]*, Meir, M.[2], Rowan-Carroll, A.[2], Williams, A.[3], Bradford, L.[2], Wilkins, R.C.[4], Yauk, C.[5], and Chauhan, V.[4]

*Presenting Author: ngoc.q.vuong@hc-sc.gc.ca

[1]Healthy Environments and Consumer Safety Branch, Radiation Protection Bureau, Radiation Health Assessment Division (Ottawa, ON, Canada)
[2]Healthy Environments and Consumer Safety Branch, Environmental Health Science and Research Bureau, Mechanistic Studies Division (Ottawa, ON, Canada)
[3]Healthy Environments and Consumer Safety Branch, Environmental Health Science and Research Bureau, Population Studies Division (Ottawa, ON, Canada)
[4]Healthy Environments and Consumer Safety Branch, Consumer and Clinical Radiation Protection Bureau, Ionizing Radiation Health Sciences Division (Ottawa, ON, Canada)
[5]University of Ottawa, Department of Biology (Ottawa, ON, Canada)

Background/Issue and Objectives
Lymphocytes are among the most radiosensitive cells in the human body and therefore a valuable model for assessing biological responses to radiation exposure. This study characterized a panel of measurable molecular and cellular biomarkers in human lymphocytes that reflect the dose and time following exposure. In addition, the Adverse Outcome Pathway (AOP) framework was explored to contextualize these responses and link early molecular events to downstream adverse outcomes.

Design/Method/Description
Peripheral blood lymphocytes isolated from human donors were cultured and X-irradiated at 1 Gy/minute across nine doses (0-6 Gy). At multiple timepoints (0, 4, 24, 48, and 72 hours), cell number, membrane integrity (trypan blue exclusion), and ATP levels were measured. Additional endpoints, including pyruvate levels, mitochondrial function (MitoTracker™), and apoptosis (Annexin-V), were evaluated at 4, 24, and 48 hours. Transcriptomic profiling was undertaken to capture dose- and time-dependent changes in gene expression. Related AOPs in the AOP Wiki provided a framework for interpreting the biomarker data.

Results/Outputs
Radiation exposure resulted in clear dose- and time-dependent changes across multiple biological endpoints. Lymphocyte counts declined, accompanied by a reduction in mitochondrial function, ATP production, and pyruvate levels. In contrast, free radical, membrane damage, and apoptosis increased in a dose- and time-dependant manner. Transcriptomic analysis revealed alterations in pathways of oxidative stress response, metabolic processes, mitochondrial function, and cell death. The transcriptional and functional responses were integrated using the AOP approach and demonstrated that combining multiple endpoints provides a biologically meaningful characterization of radiation effects than any single biomarker alone.

Conclusions/Impacts/Outcomes/Implications/Next Steps
This study identifies a panel of cellular, metabolic, and transcriptional responses in human lymphocytes that show consistent dose- and time-dependent trends following radiation exposure. These findings highlight the potential of AOPs to support an integrated biomarker approach for characterizing radiation-induced biological effects.

Keywords: Radiation; Lymphocytes**;** Endpoints; Transcriptomic; Pathways

Molecular Echoes of High-Dose Radiation: Persistent Blood Transcriptome Remodelling in Rhesus Macaques

Fischer, S.[1]*, Schmid, N.[1], Singh, V. K.[2,3], Fatanmi, O.[2,3], Wise, S. Y.[2,3], Engelhard, C.[1], Stewart, S.[1], Orben, T., Port, M.[1], Eder, S[1], Ostheim, P.[1]

[1]Bundeswehr Institute of Radiobiology, Munich, Germany;
[2]Division of Radioprotectants, Department of Pharmacology and Molecular Therapeutics, F. Edward Hébert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, USA; [3]Armed Forces Radiobiology Research Institute, Uniformed Services University of the Health Sciences, Bethesda, MD, USA Bethesda, MD, USA;
[4]Armed Forces Radiobiology Research Institute, Bethesda, MD, USA
*Presenting Author: sabrina.f.fischer@gmail.com

The molecular basis of delayed effects of acute radiation exposure (DEARE) - including cancer and cardiovascular disease - remains poorly characterized. We performed comprehensive transcriptome profiling in peripheral blood of rhesus macaques (*Macaca mulatta*) exposed to 5.8, 6.5, or 7.2 Gy total-body irradiation to identify sustained radiation-induced molecular alterations.

Whole-transcriptome NGS across 25 animals at early (days 1-3) and sustained (days 35 and 60) post-exposure time points identified 74 genes with persistent deregulation throughout the full observation period. Independent qRT-PCR validation in 22 additional animals confirmed 22 differentially expressed genes (14 down-, 8 up-regulated; 2.0-50.9-fold), including 5 genes replicated across independent NHP cohorts.

Pathway enrichment analysis revealed a temporally structured immune program: an early innate inflammatory and myeloid burst transitioning toward adaptive immune reorganization by day 60. Disease ontology enrichment identified cancer-associated and cardiomyopathic pathways among the persistently dysregulated gene set - potential early molecular hallmarks of DEARE. Sex-stratified analysis identified 179 genes with divergent temporal trajectories, including a male-biased acute neutrophilic response and a female-specific delayed transcriptional program at day 35.

These findings demonstrate incomplete molecular recovery after high-dose irradiation and define a robust blood gene signature with relevance for biodosimetry and mechanistic understanding of long-term radiation sequelae.

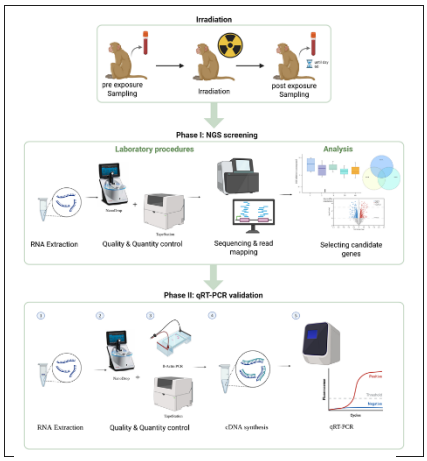

Figure 1: Study design

Keywords: Biomarker; clinical biodosimetry; *Macaca mulatta*; Radiobiology; Total-body radiation exposure
Disclaimer: The opinions or assertions contained herein are the private views of the authors and are not necessarily those of the Uniformed Services University of the Health Sciences, or the Department of War.
Conflicts of Interest: The authors report no conflicts of interest.

Transcriptional Signatures Along the Linear Energy Transfer Profile of Proton and Carbon-ion Tracks in Skin and Blood

Comoglio, Y.[1]*, Najim, M.[1], Petruta, M.[1], Facoetti, A.[2], Pullia, M.[2], Cruz-Garcia, L.[1], Badie, C.[1]

[1]Centre for Radiation, Chemical and Environmental Hazards, UK Health Security Agency, Chilton, Didcot OX11 0RQ, UK
[2]Centro Nazionale di Adroterapia Oncologica, Pavia, Italy
*Presenting Author: Yannick.comoglio@ukhsa.gov.uk

Radiotherapy has been an effective cancer treatment for more than a century, either as a stand-alone approach or in combination with chemotherapy. However, its effects are not restricted to malignant tissues. Ionising radiation can also damage DNA in surrounding healthy organs, leading to cell death and unwanted side effects. Particle based radiotherapy with protons or carbon ions offers an important advantage, since these particles deposit most of their energy at a specific depth in the tissue known as the Bragg peak [1]. This physical property has driven the rapid expansion of particle therapy centres worldwide.

Although transcriptomic studies are available for individual radiation qualities, very few investigations directly compare several particle types and their effect along their tracks within the same experimental conditions. To address this knowledge gap and in collaboration with the CNAO in Pavia, Italy, our project aims to identify gene expression signatures specific to different radiation qualities in human peripheral white blood cells (WBC) and ex vivo human skin, while also comparing the biological effects of exposures delivered before, within and after the Bragg peak to characterise how the radiation response may vary along the particle trajectory. Skin represents a primary normal tissue target in radiotherapy and can be used to assess individual radiosensitivity and predict normal tissue toxicity. White blood cells are frequently used in biodosimetry due to their accessibility and their high sensitivity to DNA damage signalling. Samples were irradiated with proton or carbon ions to deliver a 2 Gray dose at the Bragg peak and the RNA was collected twenty-four hours later and sequenced using nanopore technology. The results obtained will provide new insight into tissue specific responses along the particle trajectory and into how different tissues react to variations in radiation quality, supporting the development of new biomarkers that can be used to prevent radiation induced side effects. Moreover, it will provide transcriptional signatures specific for protons and carbon ions allowing to identify the radiation to which individuals have been exposed to.

Keywords: Radiation quality, Proton, Carbon Ions, biodosimetry, Bragg peak.

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Unmasking Radiation Signatures: Integrating Automated RNA Isolation and Globin-Depleted Nanopore Sequencing for Point-of-Care Biodosimetry

Stewart, S.[1,2]*, Polozov, S.[3,4], Goriacha, V.[3], Cruz-Garcia. L.[4], Badie, C.[4], Eder, S.[1], Ostheim, P.[1], Abend, M.[1], Fischer, S.[1]

[1]Bundeswehr Institute of Radiobiology affiliated to the University of Ulm, Germany
[2]Technical University Munich, Germany
[3] HQ Science Limited, United Kingdom, [4] UK Health Security Agency, United Kingdom
*Presenting Author: Samantha.stewart@outlook.de

Political conflicts in the 21st century have renewed concerns regarding large-scale radiological and nuclear (R/N) incidents, where damaged infrastructure may hinder the transport of blood samples to specialized laboratories. To enable rapid assessment of potentially irradiated individuals, the aim is to establish a multimodal point-of-care (PoC) platform integrating (1) microfluidic RNA extraction and (2) portable nanopore sequencing (NPS) for gene expression-based biodosimetry.

Firstly, a microfluidic module forming the basis of a lab-on-a-chip system was developed to miniaturize whole-blood RNA extraction. Compared with standard column-based methods, it halved processing time, eliminated bulky equipment, reduced blood input tenfold (250 µL vs. 2.5 mL), prevented DNA contamination, and doubled RNA yield after input normalization (12.0 ± 5.8 µg vs. 6.6 ± 3.2 µg). Despite lower RNA integrity (RIN: 3.3 ± 0.8 vs. 9.0 ± 0.4), qRT-PCR analysis of radiation-responsive genes (*FDXR, DDB2, POU2AF1,* and *WNT3*) after *ex vivo* irradiation (0, 0.5, and 4 Gy) remained comparable, supporting its suitability for field diagnostics.

Portable NPS offers a low-infrastructure alternative for transcriptomic biodosimetry, but globin transcripts account for 72–75% of whole-blood reads. Globin depletion removed ~98% of these transcripts and increased detectable genes threefold, while NPS expression measurements strongly correlated with qRT-PCR ($R^2 = 0.94$, $p < 0.001$). However, depletion reduced read length (631 to 465 bp) and mapping rate (91% to 66%), partially masking radiation effects. RUVg correction reduced globin-related variance from 58% to 0.2%, restoring radiation-associated signals. Pathway enrichment remained consistent, and analysis of 101 radiation-associated genes showed similar enrichment at 4 Gy (NES = 2.66, padj = 4.78×10^{-15} vs. NES = 2.43, padj = 1.44×10^{-12}). At the single-gene level*, FDXR, PHPT1*, and *DDB2* exhibited 2.08-, 1.65-, and 1.53-fold increases in read counts, respectively.

Overall, the primary microfluidic extraction module provides a robust upstream component for on-site diagnostics, while NPS, combined with globin depletion and bias correction, enhances transcriptomic sensitivity. Additional investigations exploring these findings in greater detail will be presented at the conference.

Keywords: Nanopore sequencing, point-of-care, radiation exposure, gene expression, globin depletion

Acknowledgments: The microfluidic module was funded by the European Union. Views and opinions expressed are, however, those of the author(s) only and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the granting authority can be held responsible for them (RESILIENCE- R- 2023), project No. 101168024.

CLOCK: meChanisms of the transcriptional cOntrol of the Circadian clocK

Lopez-Riego, M.[1]*, O'Brien, G.[1], Davies, P.R.[1], Freestone, D.[1], Comoglio, Y.[1], Najim, M.[1], Polozova, M.[1,2], Mancey, H.[1], Moquet, J.[1], Ashworth-Donn, L.[1], Morvay, P.[1], Ashok, A.[1], Barnard, S.[1], Cruz-Garcia, L.[1], Badie, C.[1]

[1]Radiation, Chemical, Climate and Environmental Hazards, UK Health Security Agency, Chilton, UK
[2]HQ Science Limited, London, UK
*Presenting Author: milagrosa.lopezriego@ukhsa.gov.uk

Shift work is a probable human carcinogen [1], but the mechanism of increased hematopoietic cancers observed in nightshift workers [2] is unclear. Shift work may lead to circadian disturbances, and CLOCK components intersect the DNA damage response, source of genomic instability upon disruption. This project aims to elucidate how circadian factors modulate carcinogenesis. Our hypothesis is that circadian dysregulation initiates/promotes carcinogenesis through DNA damage, exacerbated by IR. Considering the growing shift-work prevalence and use of radiotherapy and CT scanning, a significant proportion of shift workers will undergo IR exposure in their lifetime.

Phase 1 comprises a circadian biomarker discovery and characterization step using a multi-omics approach on blood samples from UK Health Security Agency (UKHSA) day shift workers and both day and night shift workers from the Norfolk and Norwich University Hospitals NHS Foundation trust (UK). Blood samples have been collected at 7AM and 7PM on three different occasions for each group. Preliminary data based on γ-H2AX levels suggest a less effective repair capability in night shift workers, at least in PM samples, in agreement with published results from simulated night shift workers [3]. Transcriptionally, a downregulation of PER1 in PM compared to AM samples has been detected in the UKHSA cohort. Sample analysis is ongoing and results will be validated in additional human cohorts and integrated with a mechanistic study using an animal model of IR-induced Acute Myeloid Leukaemia (rAML) [4]. This study is expected to advance knowledge on the biological impact of circadian dysregulation on radiation-induced leukemogenesis to ultimately inform health risk assessment. Simultaneously, biomarkers of IR exposure used in biodosimetry will be challenged to test their accuracy in dose estimation at different times of the day and under disturbed circadian conditions.

Keywords: circadian clock, rAML, shift work, ionizing radiation, DNA damage.

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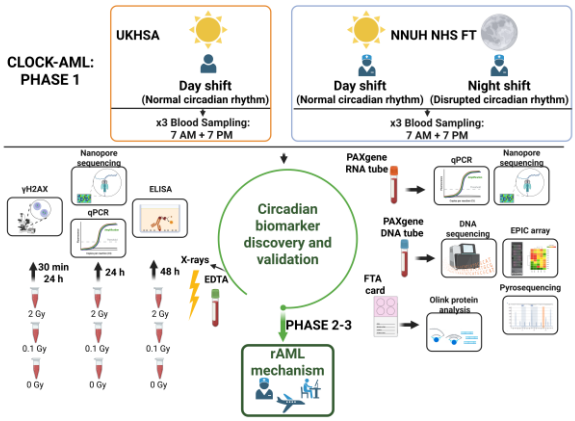

Figure 1. Experimental plan. Created in BioRender.

Identifying Urinary Exosomes and their Content as Biomarkers of Radiation Exposure

Armitage-Brown, B.[1], Bester, M.[2], Lai, V.[2], Bertucci, A.[2]*

[1]Biological Research Facility, Canadian Nuclear Laboratories
[2]Nuclear Response and Analysis, Canadian Nuclear Laboratories
*Presenting Author: antonella.bertucci@cnl.ca

Early and accurate assessment of radiation injury by radiation-responsive biomarkers is critical for triage and early intervention in large scale radiological or nuclear events. In these scenarios, the ability to rapidly distinguish between exposed and non-exposed individuals, as well as to stratify exposure severity, is critical for effective triage, allocation of limited medical resources, and timely therapeutic intervention. Current biodosimetry approaches, including cytogenetic assays and clinical symptom assessment, are often time-consuming, labor-intensive, or insufficiently sensitive during the early post-exposure window. As a result, there is a pressing need for rapid, accurate, and minimally invasive diagnostic tools that can be deployed at scale under emergency conditions.

Diagnostic biomarkers that can be detected in biofluids, such as urine, are ideal for early assessment of absorbed doses of radiation. Urine offers practical advantages: it is non-invasive, easily repeatable, and well-suited for large-scale screening without the need for specialized personnel or infrastructure.

This study focuses on the potential of urinary exosomes and exosomes' content as a novel and practical source of radiation-responsive biomarkers. Exosomes are a subset of extracellular vesicles, typically 30–150 nm in diameter, released by virtually all cell types into circulation and subsequently filtered into various biofluids, including urine. They play an important role in intercellular communication by transporting a diverse array of molecular cargo—such as proteins, lipids, messenger RNA, microRNAs, and other non-coding RNAs—that reflect the physiological and pathological state of their cells of origin. Importantly, exosomes are not merely passive carriers; their contents are selectively packaged through regulated cellular processes, making them highly informative indicators of cellular stress responses, including those induced by ionizing radiation.

Building on this rationale, we will present a comprehensive in vivo experimental design, along with standardized protocols for urinary exosome isolation and characterization, and preliminary results demonstrating the feasibility and sensitivity of this approach for radiation biomarker discovery. Together, these elements aim to establish a translational pathway toward rapid, deployable biodosimetry tools for use in mass-casualty and emergency response settings.

Keywords: Urine biomarkers; exosomes; biodosimetry; ionizing radiation.

Current Challenges and Future Perspectives in Cytogenetic Biodosimetry

Miura, T.

Institute of Radiation Emergency Medicine, Hirosaki University, Hirosaki, Japan
tomisato@hirosaki-u.ac.jp

Biological dosimetry in radiation medicine estimates absorbed radiation dose by quantifying radiation-induced biological responses. Major approaches include cytogenetic biodosimetry based on chromosome aberration analysis and electron spin resonance (ESR) dosimetry. This presentation highlights recent advances in cytogenetic biodosimetry.

Cytogenetic biodosimetry methods, including the dicentric chromosome assay, premature chromosome condensation assay, cytokinesis-block micronucleus assay, and FISH translocation assay, are widely used for radiation dose assessment. Blood culture-independent methods, such as the γ-H2AX assay and gene expression assay, are also under development. Advances in automation, artificial intelligence, and chromosome detection technologies have improved scoring efficiency and throughput while reducing reliance on specialized personnel. However, bottlenecks remain, including prolonged culture time, low mitotic index (MI), limited metaphase yield, and the need for standardized laboratory procedures.

At Hirosaki University, we improved MI and metaphase yield by optimizing blood concentration and metaphase enrichment. We also investigated whether fetal bovine serum (FBS) is required for lymphocyte culture. Peripheral bloods were cultured in RPMI 1640 or human plasma-like medium with or without FBS. Serum-free cultures showed significantly higher MI values, whereas dicentric chromosome and micronucleus frequencies remained unchanged. These findings indicate that FBS is not essential for chromosome aberration analysis of peripheral blood lymphocytes. Serum-free culture provides a practical alternative that supports standardization, biosafety, and animal-origin-free testing.

Although biodosimetry has advanced considerably, technical challenges remain. Continued collaboration among IABERD members will be essential to address these challenges and pass biodosimetry knowledge and technologies to the next generation.

Keywords Cytogenetic biodosimetry, Metaphase yield, Mitotic index, Serum-free blood culture

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Forensic Electron Paramagnetic Resonance Dosimetry for Former US Nuclear Workers

Romanyukha, A.[1]*, Tolmachev, S.Y.[2]

[1]Armed Forces Radiobiology Research Institute, Uniformed Services University, Bethesda, MD, U.S.A.
[2]United States Transuranium and Uranium Registries, Washington State University, Richland, WA, U.S.A.
*Presenting Author: alexander.romanyukha@usuhs.edu

Forensic dosimetry plays a critical role in radiation protection and research. While often associated with legal or criminal investigations, its primary importance lies in verifying the accuracy of official worksite dose records, which is essential for radio-epidemiological studies.

Current occupational radiation limits are largely based on studies of Hiroshima atomic bomb survivors. Because these individuals did not wear dosimeters, their doses were calculated and verified using retrospective techniques such as Electron Paramagnetic Resonance (EPR), Optically Stimulated Luminescence (OSL), and biodosimetry (FISH). Furthermore, while A-bomb survivors experienced acute exposure, contemporary research is primarily focused on chronic low-dose-rate exposure.

The United States Transuranium and Uranium Registries (USTUR) serves as a unique resource for verifying official worksite doses. By maintaining historical records alongside post-mortem tissue and bone samples, the USTUR allows for the comparison of official data with EPR dose measurements. Recent EPR dosimetry performed on tooth enamel [1, 2] and bone samples from USTUR donors have revealed significant discrepancies between measured doses and official records.

Furthermore, we have observed substantial dose variations among different samples from the same individual. This suggests high non-uniformity and potential localized exposure to the head and neck region that is not captured by personal dosimeters typically worn on the torso. These findings underscore the necessity of forensic dosimetry in ensuring the integrity of historical dose data and improving our understanding of radiation risks.

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Comparison of Cytogenetic Staining Methods Incorporating a New Modified C-Banding Technique

Anderson, D.A.[1]*, Takebayashi, K.[1], Sasaki, N.[1], Goh, V.S.T.[2], Nakayama, R.[3], Fujishima, Y.[1], Nakata, A.[4], Miura, T.[1]

[1]Institute of Radiation Emergency Medicine, Hirosaki University
[2]Singapore Nuclear Research and Safety Initiative, National University of Singapore
[3]Department of Radiation Life Sciences, Fukushima Medical University
[4]Faculty of Pharmaceutical Sciences, Department of Pharmacy, Hokkaido University of Science
*Presenting Author: ande4163@hirosaki-u.ac.jp

Rapid and reliable identification of dicentric chromosomes remains central to cytogenetic biodosimetry, particularly in mass-casualty scenarios where throughput and scoring consistency are critical [1]. In this study, we developed a modified C-banding protocol designed to enhance centromere visualization while reducing processing complexity and analysis time. Slides can be stained within 1.5 hours after preparation, compared to 1 week for the original method. Additionally, the method was evaluated against conventional Giemsa staining and peptide nucleic acid fluorescence in situ hybridization (PNA-FISH) to assess relative performance in terms of scoring accuracy and speed (Fig. 1).

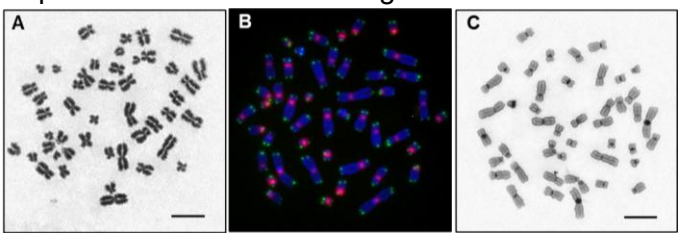

Figure 1. Cytogenetic stains (A) Giemsa, (B) PNA-FISH,(C) Modified C-banding.

The modified C-banding method significantly improved scoring consistency, reducing inter-scorer variability (coefficient of variation reduced from ~8–12% with Giemsa to ~1–4%), while maintaining comparable dicentric chromosome yields. Comparative analysis demonstrated that PNA-FISH provided the fastest image acquisition (approximately 65 seconds per metaphase); however, it requires fluorescence microscopy and specialized probe labelling, limiting its accessibility. The modified C-banding approach achieved comparable acquisition speed (approximately 70 seconds per metaphase) while maintaining compatibility with standard brightfield microscopy. Conventional Giemsa staining was slower (approximately 80 seconds per metaphase) and exhibited greater variability in centromere identification. These findings indicate that the modified protocol provides a practical balance between speed, accuracy, and accessibility.

Overall, this modified C-banding technique represents an alternative to fluorescence-based methods, offering improved reliability over conventional staining without the need for specialized equipment. Its applicability to archived slides further supports its utility for retrospective analysis, training, and large-scale biodosimetry applications.

Keywords Modified C-banding; PNA-FISH; Giemsa; Dicentric.

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Development of an Automated Calyculin-A Induced Premature Chromosome Condensation (PCC) Assay for Biodosimetry

Adams, H.[1,2,3]*, Rogers, E.[1,2], Wilkins R.C.[1], Beaton-Green, L.A[1,2]

[1]Consumer and Clinical Radiation Protection Bureau, Health Canada
[2]Department of Biology, Carleton University, Ottawa, Canada
[3]Department of Biology, University of Ottawa, Ottawa, Canada
*Presenting Author: hailey.adams@hc-sc.gc.ca

Ionizing radiation exposure can damage critical biomolecules, including DNA. Biodosimetry measures these biomarkers to estimate radiation dose. The dicentric chromosome assay (DCA), the current gold standard, is limited by its reliance on metaphase chromosomes and manual, microscope-based scoring, resulting in a labour-intensive assay that is limited to doses under 5Gy. The premature chromosome condensation (PCC) assay is a promising alternative. Chemically induced PCC uses calyculin-A to condense interphase chromatin, enabling aberration analysis in non-mitotic cells.

In this study, a G2-PCC protocol for primary human lymphocytes was established and optimized for biodosimetry. Incorporating fluorescence *in situ* hybridization probes targeting centromeres and telomeres enabled precise classification of chromosomal aberrations. Dose-response analysis across 0-10 Gy of X-rays showed a strong fit for both brightfield (R^2=0.99) and fluorescence (R^2=1.00) microscopy methods, with the fluorescence-based assay showing higher accuracy than DCA at 5Gy (Figure 1).

Imaging flow cytometry was explored to pilot an automated PCC assay. Base-specific DNA stains are being tested to detect dose-response intensity shifts. Preliminary results confirm the ability to detect changes in the chromosome sizes. These results demonstrate the feasibility of PCC-based biodosimetry and support further development toward higher-throughput approaches, particularly for high-dose scenarios where traditional methods are limited.

Keywords: Premature Chromosome Condensation, Biodosimetry, Automation

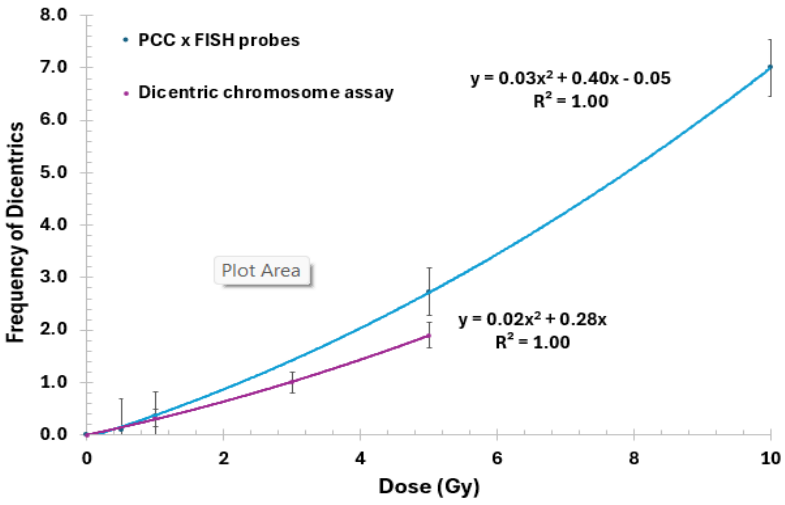

Figure 1. Dose-response curve showing ionizing radiation-induced dicentrics in PCC DNA stained with FISH probes. Compared to DCA method.

Development of Radiation Injury Triage and Assessment System (RITAS): A High-Throughput Biodosimetry Platform for Radiation Emergency Response

Turner, H.C.[1]*, Babb, D.[1], Wang, E.[1], Shuryak I.[1], Kleiman, N.[2], Kowalski, R.[3]

[1]Center for Radiological Research, Columbia University, New York, NY 10032
[2]Department of Environmental Health Sciences, Columbia University, New York, NY 10032
3. NextPhaseDx, Locust Grove, VA 22508
*Presenting Author: ht2231@cumc.columbia.edu

Following a mass casualty radiological/nuclear incident, there is a critical need to rapidly identify and treat individuals who have been exposed to damaging amounts of radiation and are at high risk of developing life-threatening radiation injuries, such as the hematopoietic and gastrointestinal acute radiation sub-syndromes. At the Center for Radiological Research at the Columbia University, we are developing RITAS (Radiation Injury Triage and Assessment System) designed to bridge the gap between dose estimation and clinical triage decision-making decisions after moderate (> 2 Gy) to high-dose (> 5 Gy) radiation exposure. RITAS is a laboratory-based in vitro diagnostic system that uses an integrative proteomic biomarker approach based on Meso Scale Discovery MULTI-ARRAY technology to quantify targeted blood plasma analytes designed to stratify exposure risk and provide an early indication of acute health effects. The multiplexed proteomic signature is comprised of a broad panel of blood plasma biomarkers associated with tissue damage, immune system response, apoptosis and bone marrow suppression. Towards pre-clinical validation studies, we have used the highly translational non-human primate (NHP) model to evaluate biomarker expression levels on days 2, 5, 7 and 14 after total body irradiation (TBI doses: 1, 2, 4, 6, and 8/10 Gy; n = 4-6 animals per dose).

In unpublished work, we have developed a 3-protein biomarker panel which can be used to successfully support binary classification and distinguish between unirradiated and irradiated NHPs at the clinically actionable 2 Gy threshold up to a week after exposure (AUC ≥ 0.95). To extend the panel to include surrogate blood biomarkers of acute radiation injury, we have screened ~40 plasma proteins that represent different yet complementary biological pathways that are responsive to ionizing radiation through distinct mechanisms. To date, we have identified a novel panel of eight proteins that are significantly upregulated on days 2, 5, and 7 after TBI and nine proteins that respond early (day 2) or later (day 7) during the week after exposure ($p < 0.05$ to 0.0001). Out of these 17 proteins, nine biomarkers were significantly upregulated on day 14 ($p < 0.05$ to 0.0001). Studies are ongoing to develop the RITAS bioinformatics model using advanced AI/machine learning (ML) algorithms for retrospective dose reconstruction and radiation injury classification. In summary, RITAS represents a simple and rapid blood test that can be used for both dose and acute injury assessment to support radiation emergency response within the first week post-exposure.

Keywords: proteomics; multiplex; biodosimetry; injury; AI/ML

Electron Paramagnetic Resonance for Retrospective and Accidental Dose Assessment: Biological and Fortuitous Materials

Tuner, H.

Department of Physics, Faculty of Art and Science, Balikesir University, Balıkesir, Turkiye
htuner@balikesir.edu.tr/hasantuner@gmail.com

Following a radiological or nuclear incident, the absorbed doses received by exposed individuals often need to be determined. When personal dosimeters are unavailable or were not worn at the time of exposure, retrospective dosimetry provides important information for medical management, triage, epidemiological investigations, and long-term health risk assessment. Among the available techniques for retrospective and accidental dose assessment, Electron Paramagnetic Resonance (EPR) spectroscopy enables absorbed dose determination by detecting and quantifying radiation-induced radicals.

Over the past decades, EPR-based retrospective dosimetry has been applied to a wide range of biological and fortuitous materials. Tooth enamel, fingernails, and bone have been extensively investigated, while pharmaceuticals, plastic cards, buttons, and other personal belongings have attracted considerable interest as fortuitous dosimetric materials. Several of these materials have been the subject of international intercomparison exercises and retrospective dosimetry studies, including applications related to radiation accidents and environmental exposure scenarios.

Recent research has increasingly focused on glass materials. Studies involving watch glass, optical lenses, mobile phone display glass (Gorilla Glass), screen protectors, and smartwatch glass have broadened the range of materials available for EPR dose assessment. The growing interest in these materials reflects a shift toward the use of widely available technological products and indicates an increasing role for such materials in future retrospective dosimetry and radiation emergency response.

Keywords EPR dosimetry; retrospective dosimetry; biological materials; fortuitous materials; glass dosimetry.

Recent Achievements and Current Challenges in EPR Dosimetry using Fingernails

Azariasl, S.*, Yasuda, H.

Department of Radiation Biophysics, Research Institute for Radiation Biology and Medicine (RIRBM),Hiroshima University
*Presenting Author: d220190@hiroshima-u.ac.jp

Fingernail EPR dosimetry has been widely investigated as a rapid and non-invasive method for retrospective dose assessment in radiological emergencies. However, its practical application remains limited, mainly due to inter-individual variability and complex overlapping signal components [1,2].

This work presents a comprehensive overview of research conducted during a PhD project aimed at addressing these challenges through a multi-scale approach. Fingernail samples from donors of different ages were systematically studied using X-band EPR spectroscopy under controlled irradiation conditions. The contributions and behaviours of radiation-induced signals (RIS), mechanically induced signals (MIS), and background signals (BGS) were investigated, along with the effects of sample treatment and measurement conditions. The results demonstrate that donor-dependent factors, particularly age, strongly influence both baseline and radiation-induced EPR signals. Experimental strategies such as water treatment significantly reduced background variability and improved reproducibility. In addition, detailed spectral analysis revealed distinct dose-dependent behaviours among overlapping signal components, highlighting limitations of conventional peak-to-peak evaluation methods. To further elucidate the origin of this variability, complementary Raman spectroscopy was employed to investigate radiation-induced molecular structural changes in fingernail keratin. The obtained results revealed age-dependent modifications in disulfide bond structures, providing a mechanistic basis for differences in EPR signal sensitivity [3]. These findings indicate that a multi-scale approach combining signal correction and molecular analysis improves the understanding and reliability of fingernail EPR dosimetry.

Currently, we plan to develop more practical and universal protocols for emergency dose assessment, focusing on the effects of different lifestyles, separation of overlapping peaks [4], and application of more intense magnetic field (Q-band EPR spectrometer) [5].

Keywords: Fingernail dosimetry**,** Retrospective dosimetry**,** Raman spectroscopy**,** Standardization

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Investigating the use of Alanine-based Chip Cards for Emergency Personal Dosimetry following Ionizing Radiation Exposures

Beazley, J.[1,2]*, Ardavan, A.[1], Liu, J.[1], Eakins, J.[2], Tanner, R.[2], Fallon, C.[2], Ainsbury, E.[2], Gougelet, R.[3]

[1]Department of Condensed Matter Physics, University of Oxford, United Kingdom
[2]United Kingdom Health Security Agency, Chilton, Oxfordshire, United Kingdom
[3]Global Resonance Technologies, United States of America
*Presenting Author: tel +44 7496 688535, email joel.beazley@physics.ox.ac.uk

Alanine dosimetry enables precise dose estimation by quantifying radiation-induced radicals via Electron Paramagnetic Resonance (EPR) spectroscopy. While well-established in clinical settings, miniaturizing this technology would provide a viable field-triage tool for large-scale scenarios where traditional biodosimetry resources are overstretched.

Integrating alanine pellets into identity cards ('alanine cards') offers a solution for emergency preparedness, provided a reader with sufficient sensitivity is available. This PhD project investigated measurement uncertainties through Monte-Carlo radiation transport simulations, and the development of an EPR reader capable of measuring clinically relevant doses from alanine cards.

MCNP simulations using the ICRP 110 male voxel phantom modelled the uncertainty in dose estimates from a worn alanine card that result from bodily shielding of the pellet from the source. We simulated the relative change in dose received by a pellet as a function of source orientation around the phantom for: 1) plane-parallel sources (modelling short-duration exposures like those from improvised nuclear devices) and 2) isotropic/rotational sources (modelling longer-duration exposures like those from radiation dispersal devices).

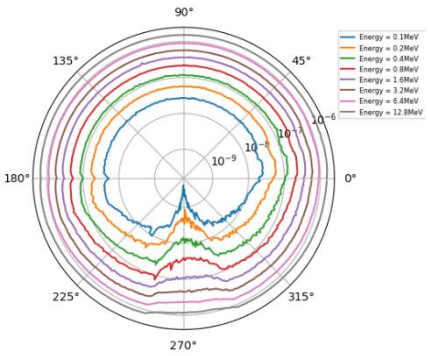

Figure 1: Polar plot of dose vs source orientation angle (horizontal exposures)

For isotropic and rotational exposures, body shielding resulted in an energy-dependent attenuation factor ranging from 1.8 at low photon energies to 1.4 at higher energies. In contrast, short-duration horizontal exposures led to a maximum attenuation factor of up to 100 at low photon energies (0.1 MeV), when the phantom maximally shielded the pellet. These results suggest that while a single-point dosemeter is potentially unreliable for highly directional and short-timescale exposures, a collection of measurements could be used for spatial dose mapping. For isotropic exposures, a correction factor can be applied to dose estimates to ensure individuals in need of care are not missed.

Current work focuses on the development of a portable EPR spectrometer. We will present the design and preliminary sensitivity data, demonstrating progress toward measuring clinically relevant doses.

Keywords: Alanine dosimetry; EPR spectroscopy; Emergency triage; Monte-Carlo simulation

Improvement of Sugar-EPR Dosimetry for Emergency Use

Yasuda, H.[1]*, Azariasl, S.[1], Della Monaca, S.[2], Fattibene, P.[2]

[1]Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan
[2]Istituto Superiore di Sanità, Rome, Italy
*Presenting Author: hyasuda@hiroshima-u.ac.jp

Sugar, mainly sucrose, is expected to be an effective EPR dosimeter for retrospective dose assessment after a radiological accident [1,2]. The preferable features of sugar, such as universality, low cost, and safety on human health, make this sugar-EPR dosimetry method promising for use by the general population in large-scale nuclear events. However, table sugar is usually placed in a kitchen or dining room, and the surrounding thermal conditions can change significantly over time. As a sugar EPR spectra include multiple peaks with different stabilities [3], time-dependent changes in the surrounding thermal environment can degrade the accuracy of sugar-EPR dosimetry.

Therefore, in this study, we systematically investigated the effects of post-irradiation temperature on the stability of sucrose EPR signals. Fine grains of sucrose (0.13 g) were put in a cellulose capsule (φ5.2 × 16 mm^2) and irradiated with 1, 4, and 10 Gy X-rays (160 kVp, 6.3 mA). The samples were stored at different temperatures (-19, 5, 15, 30, and 40 °C) immediately after irradiation, and the EPR signals were measured using an X-band EPR spectrometer (JES-FA100, JEOL Ltd.) at 2, 4, 22, and 46 h after irradiation.

As results, the samples stored at lower temperatures (≤15 °C) exhibited notable changes during two days post-irradiation, whereas the EPR spectra of the samples stored at higher temperatures (≥30 °C) became stable within four hours, as shown in Figure 1. Based on these findings, it is highly recommended that sugar-EPR dosimeters will be placed close to the body surface (approximately 36 °C) or preheated at 30-40 °C temperature range for a few hours before EPR measurement for accurate individual dose assessments in radiological emergencies.

Keywords: sugar, sucrose, EPR, radiological emergency, preheating

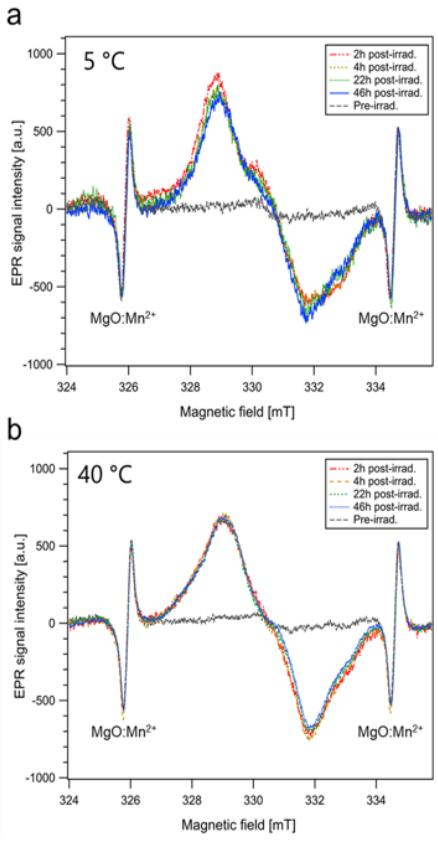

Figure 1. Changes in the EPR spectra of sucrose over time after irradiation with X-rays (160 kVp, 6.3 mA) at 10 Gy. The irradiated samples were stored immediately after irradiation in a refrigerator at 5 °C (a) and in an oven at 40 °C (b).

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EPR Dosimetry of *Sprague-Dawley* Rat Teeth

Bos, E.[1], Hassan, A.[2]*, Armitage-Brown, B.[3], Przbyla, H.[1], Waller, E.[1], Bertucci, A.[2]

[1]Department of Nuclear and Energy Science, Ontario Tech University.
[2]Nuclear Response and Analysis, Canadian Nuclear Laboratories.
[3]Biology Research and Development Facility, Canadian Nuclear Laboratories.
*Presenting Author: amna.hassan@cnl.ca

In the event of a large-scale radiological or nuclear accident, conventional dosimeters may not be available and biodosimetry might not be possible for logistical reasons to provide dose estimates to exposed populations. Consequently, there is a need for reliable methods capable of performing retrospective dose assessments to affected populations and the surrounding environment. Electron Paramagnetic Resonance (EPR) spectroscopy has been widely established as a technique for retrospective dosimetry through the detection and measurement of radiation-induced paramagnetic centres in tissues, such as teeth, bones, and nails. This study investigates the feasibility of using *Sprague-Dawley* rat teeth as a biological dosimeter for retrospective dose assessments following a nuclear or radiological accident. Species-specific sample preparation procedures were developed, which included sterilization, separation of dentin from enamel, and grinding of samples to suitable grain sizes for EPR measurements. In addition, a robust measurement protocol was developed to ensure repeatable and reproducible measurements were possible with high degree, and key measurement parameters were optimized to ensure accurate and non-saturated signals were obtained. A dose-response calibration curve was developed by irradiating prepared samples with a ^{137}Cs gamma source across a dose range of 0 to 10 Gy. Reference dosimetry was performed with alanine powder. The resulting EPR signal intensity showed a strong linear correlation with absorbed dose, indicating this species to be suitable for dose assessments within this range. Future work will focus on validating the calibration dose-response curve by measuring samples of unknown dose as well as assessing the fading of the radiation-induced signal. Further optimization of spectrum acquisition and analysis methods will also be investigated to improve dose reconstruction accuracy.

Keywords EPR dosimetry; retrospective dosimetry; nuclear accident; *Sprague-Dawley;* biodosimeter.

In vivo EPR Tooth Dosimetry for a Healthcare Worker Detects the Signal Composed of Medical and Occupational Radiation Exposure

Yamaguchi, I.[1]*, Morota, K.[1], and Miyake, M.[2]

[1]Dept. of Environmental Heath, National Institute of Public Health, Wako, Japan
[2]Department of Oral and Maxillofacial Surgery, Kagawa Univ., Miki, Japan
*Presenting Author: yamaguchi.i.aa@niph.go.jp

The electron paramagnetic resonance (EPR) can detect unpaired electrons. Since radiation exposure generates unpaired electrons that are stably retained in teeth, the amount of radiation can be estimated using teeth. Methods using extracted teeth have been used to estimate radiation doses in atomic bomb survivors and victims of accidents at nuclear facilities. In-vivo measurements have confirmed radiation exposure in patients who have undergone radiation therapy and workers engaged in radiation-related medical practices.
To verify whether this method is useful for assessing medical and occupational exposure, we conducted a measurement on a radiological technologist.

Using a L-band EPR spectrometer, in vivo tooth dosimetry was performed on one medical radiological technologist who has a history of both medical and occupational exposure.
This spectrometer operates in continuous wave (CW) mode with homodyne detection at an excitation frequency near 1.15 GHz (L-band) using a 41 mT dipole magnet weight 30 kg with 17 cm pole separation. All measurements are performed using surface loop resonators which were originally developed in the EPR Center at Dartmouth.
One to three sets of data were collected in vivo using the following instrumental settings: scan time 3 s, 30 sweeps, scan range 2.5 mT, time constant 0.03 s, modulation amplitude 0.4 mT at 20 kHz and an incident microwave power of 50 mW. This required the volunteer to keep his/her mouth open for 90 s for acquisition of one set of data. From three sets of individual scans collected in this period, median values at each field-point were calculated and analyzed to estimate the amplitude of the RIS component.

The figure shows an EPR spectrum. The horizontal axis represents the magnetic field [mT], and the vertical axis represents the derivative of radiofrequency absorption[a.u.]. Estimated radiation dose is 0.9 Gy including both medical exposure and occupational. The large signal at around 1.3 mT is due to the standard substance. The signal with a negative slope of approximately 0.4 mT at around 0.2 mT is attributed to radiation-induced radicals.
Although the exact contributions of medical exposure and occupational exposure have not been clearly established, it is possible that they account for roughly equal shares since not only this health care worker has been involved in fluoroscopy for many years, but also, he has a history of undergoing fluoroscopy of the head. In this presentation, we will present the dose reconstruction results, along with other similar cases.

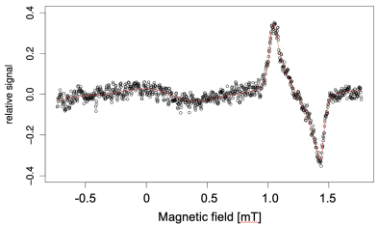

Figure 1. The EPR Spectrum

Keywords Electron paramagnetic resonance; in vivo, tooth, radiation measurement

Emerging Technologies in Radiation Biodosimetry: Harnessing Novel Approaches for a Radiological/Nuclear Public Health Emergency Toolkit

Satyamitra, M.M.
Radiation and Nuclear Countermeasures Program (RNCP), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Rockville, MD
merriline.satyamitra@nih.gov

Established in 2004, the Radiation and Nuclear Countermeasures Program (RNCP), within the NIAID/NIH has the central mission to advance medical countermeasure mitigators/therapeutics, and biomarkers and technologies to assess, triage, and inform medical management of patients experiencing acute radiation syndrome and/or the delayed effects of acute radiation exposure. The RNCP biodosimetry mission space encompasses: (1) basic research to elucidate novel approaches for rapid and accurate assessment of radiation exposure, (2) studies to support advanced development for US Food and Drug Administration (FDA) clearance of promising triage or treatment devices/approaches, (3) characterization of biomarkers and/or assays to determine degree of tissue or organ dose that can predict outcome of radiation injuries (i.e., organ failure, morbidity, and/or mortality), and (4) outreach efforts to facilitate interactions with researchers developing cutting edge biodosimetry approaches.

To date, no biodosimetry device has been cleared or approved by the US Food and Drug Administration. However, since the inception of the RNCP program, the study of radiation biodosimetry has advanced significantly, with expansion into the fields of multiparametric biomarker panels in addition to proteomics, metabolomics, lipidomics and transcriptomics, and non-invasive electron parametric resonance approaches. These technologies cover the gaps resulting from the traditional biodosimetry approaches that are time and labor intensive to meet every aspect of a radiological emergency response- triage, dose assessment, and/or outcome prediction. In addition, expansion of traditional cytogenetic assessment methods using automated platforms, and development of laboratory surge capacity networks have helped to advance biodefense preparedness. For all approaches, the advent of big data and the burgeoning field of artificial intelligence and machine learning has ignited rapid advances in key biodosimetry signatures. The ultimate goal of the RNCP biodosimetry program is to develop and establish accurate and reliable biodosimetry tools that will improve radiation preparedness and ultimately save lives during a radiological or nuclear incident.

Keywords: Radiation response, concept of operations, biodosimetry tests, continuum of care

A Simple, Rapid, Portable and Cost-Effective PCR Method for Radiation Biodosimetry

Ashok, A.[1]*, Comoglio, Y.[1], O'Brien, G.[1], Alaverdyan, J.[2], Vávra, J.[2], Cruz-Garcia, L.[1], Badie, C.[1]

[1]Centre for Radiation, Chemical and Environmental Hazards, UK Health Security Agency, Chilton, Didcot, OX11 0RQ, UK
[2]National Radiation Protection Institute (NRPI), Bartoškova 1450/28, 140 00 Prague 4, Czech Republic
*Presenting Author: Ashveny.Ashok@ukhsa.gov.uk

Rapid biodosimetry is essential for effective medical triage following a radiological emergency; however, access to low-cost and highly scalable tools remains limited, particularly in a low resource settings and during large scale incidents. Many existing gene expression-based approaches rely on quantitative PCR or high throughput sequencing platforms, which require specialised equipment, substantial financial investment and highly trained personnel, limiting their practical deployment in emergency response scenarios. In this study, we describe the development and optimisation of a low-cost, semi-quantitative, gel-based PCR assay for radiation exposure detection using human blood samples. The assay targets ferredoxin reductase (FDXR), a gene previously validated as a radiation responsive gene due to its strong radiation responsiveness, sensitivity across a broad dose range, and its accuracy in dose estimation in both ex vivo and in vivo exposures. The method developed includes optimised RNA extraction, cDNA synthesis, PCR cycling conditions, enzyme selection, and agarose gel electrophoresis with particular emphasis on implementation using a low-cost PCR setup and widely available laboratory infrastructure. The resulting protocol is reproducible, straightforward to perform, and compatible with basic molecular laboratories in hospitals. While not intended for precise dose reconstruction, the assay is designed to support rapid screening and triage during radiological emergencies. In addition, the assay may severe as a useful supplementary tool for evaluating suspected accidental high-dose exposures in occupational settings when further biodosimetric verification is required.

Keywords: Biodosimetry; Ionising radiation; PCR; FDXR; Radiological emergencies

Recent Developments in the Field of High Dose Biodosimetry in India

Yadav, U[1,2]*. Mungse, U.S.[1]., Khan, A.[1,2], Sapra, B.K.[1,2], Bhat, N.N.[1,2]

[1]Radiological Physics Advisory Division,Bhabha Atomic Research Centre, Mumbai, India
[2]Homi Bhabha National Institute, Training School Complex, Anushaktinagar, Mumbai, India-400094
[3]Associate Director, Medical Group, Head, Radiological Physics & Advisory Division Bhabha Atomic Research Centre, Mumbai, India-400094
*Presenting Author: yusha@barc.gov.in

Biodosimetry of high-dose accidental exposures are particularly challenging, as they often involve partial body exposures (PBEs). For high doses, Premature Chromosome Condensation (PCC) techniques (G0-/G2-PCC) are known to be more suitable than conventional gold standard dicentric assay. While G2-PCC has been the focus of many studies, G0-PCC is considered more suitable for PBEs. In India, the central biodosimetry lab has conducted comprehensive research on the G0-PCC technique to derive useful data and methodologies for rapid high dose and partial body dosimetry. Our initial work focused on gross fragment-based dosimetry using Giemsa staining, followed by G0-PCC-Fluorescence In-situ Hybridization (FISH) with whole chromosome paints for chromosomes 1, 2, and 4. This effort led to the first broad-range multi-donor calibration curve (0.25–15 Gy). Over 50 simulated partial body exposure samples were used to identify three key parameters that can distinguish PBEs from whole-body exposures, providing accurate dose estimates. It was observed that G0-PCC-FISH offers higher accuracy than G0-PCC-Fragment assay. Further, a 24-donor study was conducted to understand age and sex variation in baseline and radiation response. Age and sex showed no significant impact on chromosomal radiosensitivity, confirming the robustness of pooled biodosimetric calibration curves for risk assessment. Chromosome-specific radiosensitivity is strongly correlated with gene density. The tool was also utilized for clinical applications. Recently, we have simplified the G_0-PCC from mitotic cell fusion induced to chemical induced PCCs.

Keywords Biodosimetry, G0-PCC, Premature chromosome condensation, FISH Radiation

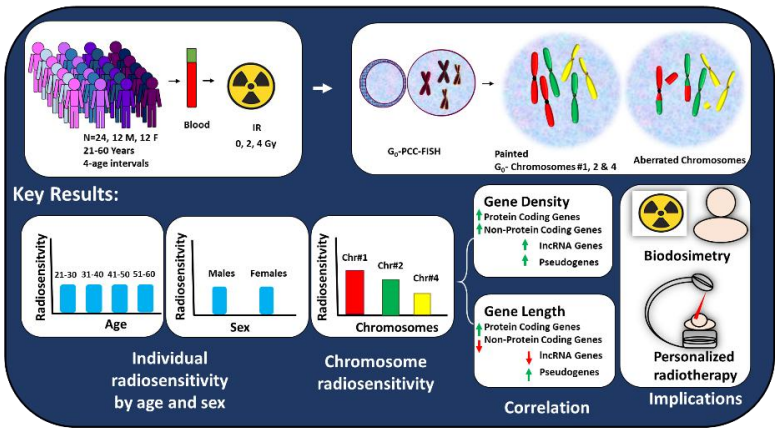

Fig. A 24-donor study to assess baseline and radiation response age and sex. Additionally substantial data analysed to assess radiosensitivity of chromosomes and its biological basis.

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The Impact of Inter- and Intra- Individual Variability on the DNA damage Response to Ionising Radiation

Mancey, H.[1]*, Davies, P.[1], Einbeck, J.[2], Barnard, S.[1]

[1]Radiation Effects Department, UKHSA
[2]Department of Mathematical Sciences, Durham University
*Presenting Author: Hannah.Mancey@ukhsa.gov.uk

Phosphorylation of the ser-139 residue of the histone variant H2AX, which then forms γH2AX, is an early cellular response to the induction of DNA double-strand breaks caused by ionising radiation, and can be detected and quantified by immunofluorescent staining under fluorescent microscopy (1). γH2AX can be detected manually as foci, or using automated methods such as flow cytometry, both of which form a clear dose-response relationship (1). This method provides faster dose estimates than the 'gold standard' dicentric assay, in as little as 4 hours. However, dose estimates tend to be less accurate, with lower specificity of DNA DSBs to ionising radiation. Several other factors are documented to cause DNA DSBs, including age, smoking status, genetics, and stress (2, 3), leading to variation in the DNA damage response both between and within people. This inter- and intra- individual variability is currently accounted for in dose estimation as overdispersion (4); however, no studies have directly measured or modelled these factors as part of a dose estimation procedure. This review aims to assess the impact of inter- and intra- individual variation on the DNA damage response to ionising radiation, and how this could affect dose and uncertainty estimation.

Keywords Biodosimetry; dose estimation; variability; uncertainty

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Space Radiation Environment Challenges for Biodosimetry

Vazquez, M.E.

Radiobiology Branch, Canadian Nuclear Laboratories
marcelo.vaquez@cnl.ca

Human health risks that may originate from exposure to ionizing radiation (IR) during long-term exploration-class missions is both uncertain and a major concern. Missions beyond low-Earth orbit (BLEO) will expose astronauts to IR from two primary sources including galactic cosmic radiation (GCR) and solar particle events (SPEs). The GCR environment consist mainly of protons (~87%), alpha particles (~12%), and a small fraction (~1%) of high charge and energy (HZE) particles (Z = 3 to 28) at very low-fluences and very high energies (on the order of GeV per nucleon). The HZE component of GCR can deliver significant dose and produce substantial biological effects because of the HZEs biophysical characteristics (radiation quality) and their high radiotoxicity per unit absorbed dose compared to the relatively abundant protons. GCR also interact with the spacecraft shielding material and generate secondary radiations, such as neutrons. Secondary neutrons are estimated to be responsible for ~30% of the dose that members of the crew receive onboard the International Space Station (ISS). In BLEO, neutrons are expected to be similarly significant; however, this again depends largely on the nature of shielding conditions. Unlike GCR, SPEs are largely made up of protons reaching hundreds of MeV. Each SPE is unique, and therefore there is no single description that is appropriate [1]. Compared to GCR, SPEs are relatively easily shielded. Physical doses are measured during flight using passive and active detectors. Physical dosimetry is routinely performed in manned space mission; however, biodosimetry is also necessary for a precise assessment of biologically relevant radiation risk. Recent measurements obtained during Artemis I and robotic spacecrafts on the Moon and Mars surface will provide an adequate context to evaluate the challenges and utility of future biodosimetry methods to evaluate the radiation risks in deep and long-term human space exploration.

Keywords:
Radiation; space; spaceflight; risk; biodosimetry

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Astronaut Biodosimetry in Canada

Wilkins, R.C.*, Beaton-Green, L.A.

Health Canada, 775 Brookfield Road, Ottawa, Canada
*Presenting Author: ruth.wilkins@hc-sc.gc.ca

Human exploration of space presents unique radiation protection challenges that differ substantially from terrestrial exposures. Astronauts are exposed to a complex mixed radiation field composed of galactic cosmic rays, solar particle events, and secondary radiation generated within spacecraft and planetary habitats. As international space agencies prepare for longer-duration missions beyond low Earth orbit, there is increasing interest in developing biodosimetric approaches capable of assessing individual biological responses to space radiation and supporting evidence-based health risk management.

Health Canada is responsible for assessing the biological effects of exposure to ionizing radiation. This work includes maintaining biodosimetry capabilities to determine the dose of radiation received by individual by measuring damage in biological samples. This service is used for nuclear energy workers, astronauts and for potentially exposed individuals during a large-scale event involving radiological or nuclear material. Health Canada has developed a robust biodosimetry program that, providing a strong scientific foundation for participation in astronaut health monitoring and space radiation research. Biodosimetry offers an important complement to physical dosimetry by measuring biological effects directly in exposed individuals. Cytogenetic techniques, including dicentric chromosome analysis, micronucleus assays, and fluorescence in situ hybridization (FISH)-based chromosomal translocation analysis, remain among the most established biodosimetric tools for retrospective dose assessment. More recently, advances in molecular biodosimetry, multi-omics technologies, gene expression profiling, and biomarker discovery have expanded opportunities for evaluating individual radiosensitivity and biological response to complex radiation environments.

This presentation will provide an overview of Canadian capabilities and activities relevant to astronaut biodosimetry, with particular emphasis on the work of the Canadian Cytogenetic Biodosimetry Laboratory and collaborating research groups. Current efforts focus on adapting terrestrial biodosimetry methods to the unique conditions encountered in human spaceflight, including chronic low-dose-rate exposure, mixed radiation quality fields, and cumulative mission-related radiation burdens. The integration of cytogenetic biomarkers with emerging molecular and computational approaches offers the potential to improve the assessment of biological dose, characterize inter-individual variability in radiation response, and support personalized radiation risk evaluation.

The presentation will also discuss opportunities for international collaboration involving biodosimetry networks, space agencies, and radiation research organizations. Harmonization of methodologies, development of reference datasets, and validation of biomarkers under space-relevant exposure conditions will be essential to ensure reliable application in future exploration missions. Canadian expertise in emergency-response biodosimetry, radiation health science, and biomarker research is well positioned to contribute to these global efforts.

As human space exploration enters a new era of lunar and deep-space missions, astronaut biodosimetry will play an increasingly important role in understanding radiation effects, protecting crew health, and informing operational decision-making. Canadian research and laboratory capabilities can provide valuable contributions to the development of integrated biodosimetric frameworks that support safe and sustainable exploration beyond Earth.

Machine Learning and Sample Size Calculations in Astronaut Biodosimetry

Puzantian, B.[1,2]*, Wilkins, R.C.[1,2], Beaton-Green, L. A.[1,2]

[1]Environmental and Radiation Health Sciences Directorate, Health Canada, 775 Brookfield Rd, K1A 1C1, Ottawa, Ontario, Canada
[2]Department of Physics, Carleton University, K1S 5B6, Ottawa, Ontario, Canada
*Presenting Author: Benjamin.Puzantian@hc-sc.gc.ca

Ionizing radiation in the space environment is an occupational hazard for astronauts participating in space exploration. Several astronauts that flew on International Space Station expeditions have undergone radiation biodosimetry assessments by whole chromosome painting with the multi-colour Fluorescent *in situ* Hybridization (FISH) assay. Astronaut blood samples were drawn shortly before, and 1-2 weeks after, flight and then 6-18 months later. The frequency of translocations was measured in cultured lymphocytes from these blood samples. Using the first post-flight sample, dose estimates have been derived from each astronaut's own calibration curve (CC), generated from the chromosome translocations quantified in pre-flight whole blood exposed *ex vivo* to X-rays or γ-rays between 0 to 2 Gy (lowest irradiated sample at 0.1 Gy).

A comparative analysis has been performed on the biodosimetry results from 51 astronaut samples across 3 cohorts (American, European, and Canadian) and two laboratories (41 samples from NASA and 10 samples from Health Canada) [1]. Statistical analysis of damage measured pre- and post-flight revealed significant inter-individual and inter-agency differences for several endpoints. The analysis confirmed that, while the yields of these endpoints generally increase significantly after spaceflight, the measurements near the detection threshold of the FISH assay [1,2], which can make the biodosimetry challenging. Addressing this limitation requires the use of sophisticated statistical techniques like machine learning. To this end, the feasibility of applying a supervised machine learning approach using the GradientBoosting algorithm to astronaut biodosimetry has been explored. This presentation highlights the dose estimates determined using this machine learning approach and describes how adding lower doses in the preflight analysis through surrogate data modelling could improve the results. The method uses the coefficients from a laboratory-averaged-CC to calculate the expected yield of translocations to 0.075 Gy of X-rays, and then generates the number of translocation events following the Poisson statistics. The process continues at higher dose points to generate the artificial damage. The GradientBoosting model is retrained using this damage and then assessed on the reduction of uncertainty on the dose estimation. We will report on the number of biodosimetry experiments on individual astronaut blood samples that would be needed to achieve statistical power. The number of cells to be scored for a detectable signal from the 0.075 Gy *ex vivo* irradiation will also be discussed.

Keywords Astronaut biodosimetry, Space radiation, Translocations, Machine Learning

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Simulating a Lymphocyte Cell in Geant4-DNA to Model Radiation Response in Space

Gunasekara, D.[1,2]*, Wilkins, R.C.[1,2], Beaton-Green, L.A.[1,2]

[1]Department of Physics, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, K1S 5B6, Canada
[2]Clinical Consumer and Radiation Protection Bureau, Health Canada, 775 Brookfield Road, A.L. 6302C, Ottawa, Ontario, K1A 1C1
*Presenting Author: dinindugunasekara@cmail.carleton.ca

Exposure to ionizing radiation is an inherent risk of spaceflight and must be accurately quantified to protect astronaut health. Physical dosimetry can provide a direct measure of absorbed dose, but it does not account for biological factors and stressors. Biodosimetric techniques can provide more insight on these factors by utilizing an individual's biological response to quantify the amount of radiation absorbed. This is accomplished by irradiating a pre-flight blood sample to a range of reference doses, in this case from an X-ray source, and plotting this against the observed biological damage, producing an individualized calibration curve. Using this curve, an X-ray-equivalent post-flight dose can be determined. However, during extended stays on the International Space Station (ISS), astronauts are exposed to a broad range of particles with varying energies. The resulting complex mixed field of radiation is not ideally approximated by a simple X-ray reference dose. To improve this approximation, a computational model can be used to assess the impact of employing a more realistic source of radiation to create calibration curves. Modelling of radiation transport and energy deposition can be done using the Monte Carlo code Geant4-DNA [1]. The molecular DNA [2] advanced example scores double strand breaks in the Standard for DNA damage (SDD) format in a given geometry. Using the Fractal DNA python package, a lymphocyte cell model can be generated. The SDD files are then used as an input for the Mechanistic DNA Repair and Survival (MEDRAS) model [3]. This model calculates the repair and misrepair of chromosome damage to isolate the endpoints of interest for the biodosimetry techniques considered here. In this case, dicentric chromosomes and translocations were calculated with MEDRAS to produce calibration curves for 250 keV X-rays. The space radiation environment was characterized inside a human-equivalent phantom inside a cylinder representing an ISS module in a previous study. Using the fluences recorded in that study, a space radiation source will be produced to simulate a space-equivalent calibration curve. The simulated curves will be compared to assess the impact of this new source and determine a conversion coefficient between the curves. This coefficient can then be used to adjust X-ray curves produced in the laboratory to better reflect the space radiation environment.

Keywords: radiobiology; biodosimetry; Monte Carlo method; dicentric chromosome assay; lymphocytes

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Biological Dosimetry for Low Dose and Medical Exposures – Where Are We And Where Are We Heading?

Wojcik, A.

Centre for Radiation Protection Research, Department of Molecular Biosciences, The Wenner-Grenn Institute, Stockholm University, Stockholm, Sweden.
andrzej.wojcik@su.se

Biological dosimetry has been developed to assess radiation exposures following accidents and emergencies involving largely high doses to large parts of the body. Currently, medical uses of ionising radiation constitute the largest source of man-made radiation exposure to the population and offer the possibility of testing the sensitivity of biodosimetry methods to a wide range of radiation doses involving partial body and internal exposure scenarios. Also, the aims of biodosimetry shift from the original pure dose assessment to precision radiation medicine, where it is hoped that biomarkers of exposure and biological response can contribute to the interpretation of individual radiation effects and risks.
Evidence accumulated from studies of radiotherapy patients confirms that biological dosimetry can characterize partial-body and fractionated exposures, estimate the absorbed dose, compare treatment modalities and, to some extent, provide insight into individual exposure patterns. At the same time, investigations involving diagnostic imaging reveal both the opportunities and limitations of current approaches. While several biomarkers appear sensitive to detect radiation-induced changes after low-dose exposures, the magnitude of the response is often small and can be influenced by biological variability and factors such as health status, inflammation, lifestyle factors, age and concomitant medical treatments.
Future research should aim to improve assay sensitivity and specificity in the low-dose range, establish robust baseline datasets for diverse populations, identify factors influencing radiation-induced biological responses and develop multiparametric approaches combining cytogenetic, molecular and genomic endpoints. Particular attention should be paid to repeated and fractionated exposures, internal exposures and the long-term persistence of biodosimetric signals. Emerging opportunities include the integration of biodosimetric markers with omics technologies, mechanistic modelling and artificial intelligence approaches. Together, these developments may facilitate the transition of biological dosimetry from a tool for pure retrospective dose assessment to a component of precision radiation medicine.

Keywords: biological dosimetry, low doses, medical exposure

Microdosimetric Considerations for Uncertainty Quantification in Cytogenetic Dosimetry

Dodgson, F.E.*, Thomson, R.M.

Department of Physics, Carleton University, 1125 Colonel By Drive, Ottawa, ON, K1S 5B6
*Presenting Author: finndodgson@cmail.carleton.ca

Previous characterizations of uncertainty in cytogenetic dose estimates have failed to account for microscopic variations in energy deposition [1]. This works seeks to quantify the dispersion in specific energy (the stochastic analogue of absorbed dose) in cell-sized targets (~ 1 to 10 μm) across the range of absorbed doses considered in biodosimetry, typically between 0 and 5 Gy. The ultimate objective is to quantify the contribution of microdosimetric effects to the combined uncertainties in calibration curves and dose estimates.

Monte Carlo simulations are performed using EGSnrc. Photons are sampled from a 250 kVp x-ray spectrum. The specific energy (z) deposited in cubic voxels within an all-water phantom is binned into a histogram to provide a surrogate for the probability density of z for a given dose D, $\mathrm{f(z;D)}$. The relative standard deviation ($\sigma_z/\bar{z}$) is determined for different dose levels and voxel sizes.

The specific energy distribution in the smallest targets considered (s = 1 μm) exhibits considerable variation across the entire dose range (see figure). By comparison, the relative standard deviation in z among voxels with the volume of human lymphocytes (s = 5.9 μm) [2], is small at 5 Gy (4%) but more considerable (38%) at 50 mGy; $\sigma_z/\bar{z}$ is smaller still for voxels with s = 10 μm. These results suggest that the variance of z in lymphocytes is substantial, especially at the limits of detection of cytogenetic assays. Future work will develop simulations representative of experimental and calibration conditions at Health Canada. This will allow the uncertainty due to microdosimetric effects to be incorporated in dose estimates.

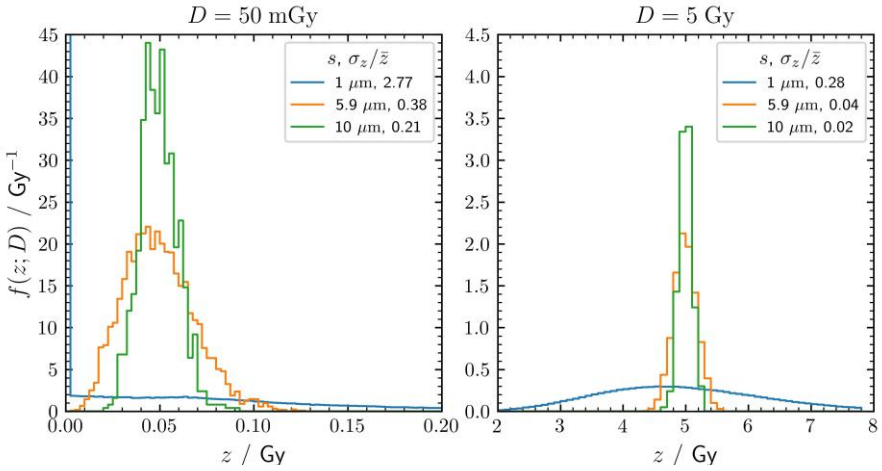

Figure. Comparison of $\mathrm{f(z;D)}$ at 2 doses: 50 mGy (left) and 5 Gy (right). The voxel side length (s) and relative standard deviation ($\sigma_z/\bar{z}$) for each distribution is included in the legend.

Keywords Microdosimetry; Monte Carlo; biodosimetry

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EPR Study of Dose-rate Dependent Radiolytic Radical Formation during Ultra-high Dose-rate Irradiation In Vitro and In Vivo

Pehlivan, J.[1]*, Govorukhin, A.[2], Holzapfel, B.[3], Horst, F.[4,5], Jäkel, O.[6,7], Leichtle, D.[1], Leßmann, E.[2], Pawelke, J.[4,5], Beyreuther, E.[2,4]

[1]Institute for Neutron Physics and Reactor Technology, Karlsruhe Institute of Technology, Germany
[2]Institute of Radiation Physics, Helmholtz-Zentrum Dresden-Rossendorf, Germany
[3]Institute for Technical Physics, Karlsruhe Institute of Technology, Germany
[4]OncoRay-National Center for Radiation Research in Oncology, Dresden University of Technology; Faculty of Medicine and University Hospital Carl Gustav Carus, Helmholtz-Zentrum Dresden-Rossendorf, Germany
[5]Institute of Radiooncology-OncoRay, Helmholtz-Zentrum Dresden-Rossendorf, Germany
[6]Division of Medical Physics in Radiation Oncology, German Cancer Research Center, Germany
[7]Heidelberg Ion Beam Therapy Center at Heidelberg University Hospital, Germany
*Presenting Author: johanna.pehlivan@kit.edu

The FLASH effect, observed during irradiation with ultra-high dose rates (UHDR), has been associated with reduced normal tissue toxicity while maintaining tumour control. The underlying mechanisms remain unclear but likely involve radiochemical processes during radiolysis and reactive oxygen species (ROS) formation. Electron Paramagnetic Resonance (EPR) spectroscopy, combined with spin trapping and spin probing, was used to investigate dose-rate dependent radiolytic radical formation.
E3 zebrafish medium supplemented with spin traps (DMPO, DEPMPO, BMPO) and redox-sensitive spin probes (CMH, TMTH, CAT1H) was irradiated under controlled oxygen conditions at the ELBE accelerator (Helmholtz-Zentrum Dresden-Rossendorf) using 30 MeV electrons at conventional and ultra-high dose rates. Oxygen concentration was monitored during irradiation and EPR spectra recorded post-irradiation. Complementary in vivo experiments in zebrafish embryos (ZFE) used cell-permeable spin probes to assess intracellular oxidative processes. Spin trapping showed reduced radical yields with decreasing oxygen levels but no significant dose-rate dependence, whereas spin probe oxidation exhibited clear and consistent dose-rate effects accompanied by oxygen depletion (Pehlivan et al., 2025). In ZFE, intracellular probe oxidation indicated dose-rate dependent differences between UHDR and conventional irradiation, modulated by oxygen conditions, with similar trends observed in morphological endpoints.

Keywords
FLASH Effect; Ultra High Dose Rate (UHDR); Radiolysis; Reactive Oxygen Species (ROS); Zebrafish Embryo (ZFE)

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Investigating the γ-H2AX Response for Low Dose and Low Energy-range X-ray Exposures

Beaton-Green, L.A.*, Cuddy-Walsh S.G., Courcelles, X., Flores, D., Wilkins, R.C.

Consumer and Clinical Radiation Protection Bureau, Health Canada, Ottawa, Canada
*Presenting Author: lindsay.beaton@hc-sc.gc.ca

Biodosimetry plays a critical role in both routine radiation protection and emergency preparedness. Whether exposures arise from occupational settings, environmental sources, or radiological incidents, many real-world scenarios involve low-dose ionizing radiation. Accurate quantification of low-dose ionizing radiation exposure remains a central challenge in biodosimetry, particularly for low doses from low energy photons, where doses can be well below the 100 mGy limitation of cytogenetic assays. To address this, we conducted a proof-of-concept study evaluating whether γ-H2AX endpoints measured by imaging flow cytometry can discriminate low-dose exposures.

Human whole-blood samples were irradiated, on ice, at 90 kVp (HVL = 12 mm Al) to doses of 0, 50, 100, 250, and 500 mGy. Delivered doses were quantified with a national standard-calibrated ion chamber co-located during sample irradiation. γ-H2AX endpoints, including spot count per cell and mean cell fluorescence intensity (MFI), were measured 1 h post-irradiation using an established imaging flow cytometry method [2].

Preliminary results demonstrated that, overall, γ-H2AX endpoints increased with dose across the 50–500 mGy range. The mean spot count per cell showed the strongest linearity, with an R^2=0.99 (Figure 1). The MFI response ≤100 mGy was not significantly different from 0 Gy whereas a linear response was observed above 100 mGy (data not shown) as found in previous studies where cells were irradiated between 1-10 Gy [2].

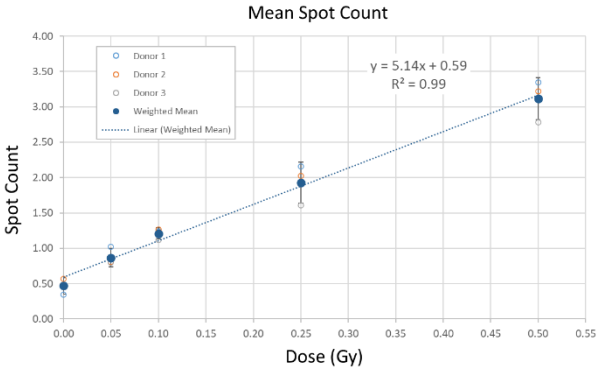

Figure 2. *Mean spot count per cell as a function of dose for the γ-H2AX response to 90 kVp x-rays*

These findings indicate that γ-H2AX can detect radiation-induced damage at doses as low as 50 mGy. This work will support future low dose and low energy-range biodosimetry studies and contribute to defining the minimum dose at which γ-H2AX can discriminate biological response in human samples.

Keywords: γ-H2AX; biodosimetry; low-dose x-rays; imaging flow cytometry

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Establishment of CBMN Dose-Response Curves with Age- and Sex-Adjusted Background Correction for Biodosimetry

Alkebsi, L.[1]*, Nashiki, R.[2], Anderson, D.[3,4], Fujishima, Y.[4], Goh, V.S.T.[5], Ishii, K.[1], Takashima, Y.[1], Nakayama, R.[6], Kurihara, O.[1], Miura, T.[4]

[1]Department of Radiation Measurement and Dose Assessment, Institute for Radiological Science, National Institutes for Quantum Science and Technology, Chiba, Japan
[2]Department of Radiation Emergency Management, Institute for Radiological Science, National Institutes for Quantum Science and Technology, Chiba, Japan
[3]Department of International Cooperation and Collaborative Research, Institute of Radiation Emergency Medicine, Hirosaki University, Hirosaki, Japan
[4]Department of Risk Analysis and Biodosimetry, Institute of Radiation Emergency Medicine, Hirosaki University, Japan
[5]Department of Radiobiology, Singapore Nuclear Research and Safety Institute, National University of Singapore, Singapore
[6]Department of Radiation Life Sciences, Fukushima Medical University, Fukushima, Japan
*Presenting Author: alkebsi.lobna@qst.go.jp

Purpose: Rapid and accurate radiation dose estimation is essential for effective medical response in radiation emergencies. In this study, we developed a refined cytokinesis-block micronucleus (CBMN) biodosimetry approach by integrating age-, sex-, and culture time-dependent background modelling with newly established γ-ray-induced CBMN dose-response curves (DRCs).

Materials and Methods: Background micronucleus (MN) frequencies were assessed in 21 Japanese donors with informed consent stratified into three age groups (20–25, 26–50, and >50 years), including both male and female participants. MN frequencies in binucleated cells (MN/BNC) were evaluated under two culture conditions (48-h and 72-h). Three male donors aged 33, 36, and 43 years from the 26–50-year group were used for DRC construction. Age-dependent exponential models were applied to estimate donor-specific baseline MN frequencies.

Results: Significant age- and sex-related differences in background MN/BNC frequencies were observed under both culture conditions, demonstrating the necessity of individualized background correction. Age-dependent exponential models enabled donor-specific baseline estimation and reduced low-dose variability when applied to DRC analysis. Validation using one external donor confirmed accurate dose estimation with narrower uncertainty intervals following individualized background correction.

Conclusion: These findings support the application of this framework for reliable CBMN-based dose estimation in both standard and emergency biodosimetry.

Key words: Dose-response curve; CBMN assay; Biodosimetry; Background frequency; Age- and sex-adjustment.

Multimodal Nonlinear Optical Microscopy Detection of In Vivo Radiation Exposure in Murine Skin

Riarh, P.[1]*, Buragina, T.[1], Gagnon, J.R.[1], Inman, J.L.[2], Murugkar, S.[1]

[1] Department of Physics, Carleton University, Ottawa, Canada
[2] Biological Systems and Engineering, Lawrence Berkeley National Lab (LBNL), Berkeley, USA
*Presenting Author: parminderriarh@cmail.carleton.ca

Accurate biodosimetry is required following radiological or nuclear exposure, yet current methods remain time consuming, expensive and not portable. Skin is a readily accessible tissue that exhibits radiation-induced structural and biochemical changes in the epidermis, dermal collagen, and hair follicles [1]. Multimodal nonlinear optical microscopy is a non-invasive technique combining second harmonic generation (SHG), two-photon excited fluorescence (TPEF), and stimulated Raman scattering (SRS) imaging. It enables high-resolution (1 μm) mapping of the biochemical as well as structural composition of the extracellular matrix and cellular constituents without adding external contrast agents (*i.e.,* label-free) and provides intrinsic 3D-sectioning capability.

Despite its strong potential for biodosimetry, nonlinear optical microscopy-based characterization of the radiation response in cells and tissue remains limited, with only a small number of studies focusing on high (≥10–40 Gy) radiation doses that produce clear visible skin tissue injury [2], leaving intermediate dose (2-6 Gy) responses relatively unexplored. We address this gap by studying radiation-induced changes at 4 Gy whole-body X-ray irradiation (~2× the clinical fraction dose), where early, subtle biomarkers are harder to detect.

Male C57BL/6 mice (n=18/per dose, total =36) were exposed to 0 Gy or 4 Gy X-ray irradiation and skin tissue was collected at Days 1, 6, and 14. Frozen 5 μm sections were imaged using a custom-built nonlinear optical microscope integrating SRS, TPEF and SHG microscopy. As shown in figure 1, this approach enabled visualization of epidermal lipid-rich structures (red, SRS at 2850 cm^{-1}), autofluorescent hair follicles (blue, TPEF), and dermal collagen architecture (green, SHG). Quantitative image analysis was performed using segmentation, optical intensity measurements, texture-feature extraction, statistical testing and supervised machine-learning classifiers. Preliminary results indicate that multimodal nonlinear optical microscopy, combined with quantitative image analysis and machine learning, can detect radiation-associated changes in skin after intermediate-dose exposure. These findings support the potential of label-free optical imaging as a platform for rapid, tissue-based for biodosimetry.

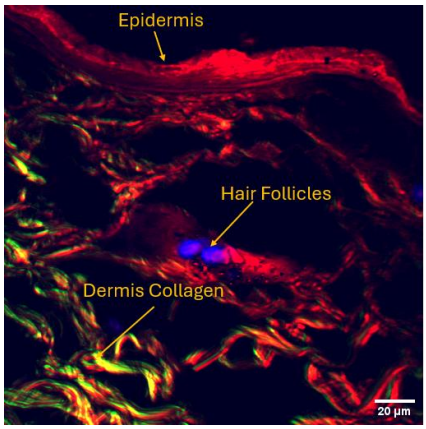

Figure 1. SRS at 2850 cm^{-1} (red), TPEF (blue), and SHG (Green)

Keywords: nonlinear optical microscopy; biodosimetry; radiation exposure; mouse skin; SHG; SRS; TPEF

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Dosimetry for Biological Studies: State of the Art, Recent Advances and Challenges

Dos Santos, M.

ASNR/SERAMED/LRMed, 31 avenue de la division Leclerc, 92260 Fontenay-aux-Roses, France
morgane.dossantos@asnr.fr

The importance of dosimetry in radiobiology is well established and has been recognized as a major concern for many years. Accurately estimating the doses delivered during irradiation continues to be a challenge that requires the use of appropriate tools and methodologies. Factors such as experimental conditions, the characteristics of irradiation facilities, field dimensions, and the nature of the irradiated samples often complicate the selection of the most suitable approach. Additionally, the implementation and validation of dosimetric protocols in radiobiology can be limited due to a lack of suitable tools.

Although reference protocols are available in the literature, such as those from the IAEA technical Reports Series (TRS), they are generally not directly applicable because irradiation geometries and biological models used in radiobiology differ substantially from those encountered in clinical practice. Nevertheless, guidelines for radiobiology experiments have been proposed by European working groups (e.g., Zoetelief, Broerse, and Davies, 1985) and American working groups (e.g., Almond et al., 1999; Ma et al., 2001). More recently, several studies have reported dedicated protocols for irradiator characterization (Tatu et al., 2020; Bucher et al., 2021), cell irradiation (Tatu et al., 2020; Dos Santos, Paget, et al., 2021; Dos Santos et al., 2018a), or rodent studies (Zoetelief, J. J. Broerse, F. A. I., 1997; Noblet et al., 2014; Vaniqui et al., 2019). Together with the development of detectors capable of measuring very small radiation fields, these advances have improved the accuracy of dose estimation. However, selecting the most appropriate protocol and establishing reliable reference measurements remain challenging.

The aim of this study is to review the current state of dosimetry in radiobiology, assess the strengths and limitations of existing experimental approaches, and identify emerging challenges in the field. Particular attention is given to the accuracy and reproducibility of dose measurements, the impact of different irradiation conditions, and the need for standardized methodologies to ensure reliable and comparable biological outcomes.

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Alanine Spectrum Decomposition For Simultaneous Dosimetry And Let Estimations

Martinet-Gerphagnon E.[1,2]*, Trompier F.[2], De Marzi L.[1]

[1]Institut Curie - Centre de Protonthérapie d'Orsay,[2]ASNR, Fontenay aux roses, France
*Presenting Author: Erwan.martinet-gerphagnon@curie.fr

Radiotherapy remains as of today a cornerstone of cancer treatment. Typically based on photon irradiations, innovative modalities such as **proton therapy** or **proton boron capture therapy (PBCT)** bring distinct healthy tissue sparing advantages. However, physical parameters such as Linear Energy Transfer (LET), which affect relative biological effectiveness, are still considered constant in modern proton therapy treatment planning. The EURADOS Working Group 9 has proposed several methods to estimate LET in proton fields, including alanine pellet dosimeters. Their ability to distinguish between gamma and alpha particle doses was demonstrated in [1] using electron paramagnetic resonance (EPR) spectra, a property particularly useful for PBCT and mixed-field dosimetry.

In this work, we explore the potential use of L-α-alanine as a control dosimeter for new radiotherapy modalities and its ability to simultaneously measure dose and LET using EPR methods. We extend the work presented in [2] by performing EasySpin simulations of alanine radical EPR spectra and decomposing experimental spectra using least-squares fitting.

Alanine pellets were irradiated with clinical proton beams of initial energies of 76, 100, and 180 MeV in water, corresponding to LET values ranging from 1 to 10 keV/µm (estimated using Monte Carlo simulations). Gamma/neutron mixed-field irradiations with varying proportions were also performed using AFRRI TRIGA reactor to assess high-LET differences. The EPR response of the three radicals generated in alanine was simulated using hyperfine coupling values from the literature and EasySpin, enabling spectral decomposition and extraction of radical proportions.

The simulation and spectral decomposition showed good agreement with experimental data (R^2 up to 0.97). Radical proportions remained constant for LET values between 1 and 10 keV/µm, but showed significant differences in gamma/neutron mixed fields.

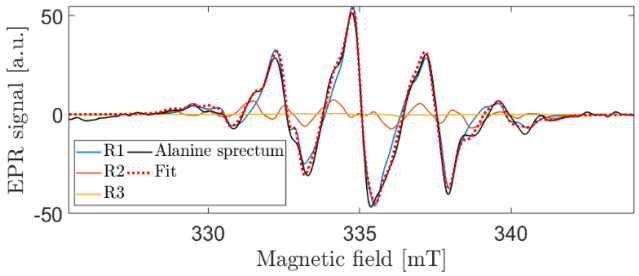

Figure 1 *Alanine spectrum decomposition using radicals' spectra simulated with Easyspin*

Our results demonstrate the potential of alanine as both a dosimeter and an indicator of LET in mixed-field irradiations. Further experiments are needed to confirm its applicability in PBCT and reactor dosimetry, where LET-based dose discrimination is essential.

Keywords : Alanine, LET, EPR Spectrum simulation, Mixed field dosimetry, Easyspin

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Validating Reference Dosimetry in Magnetic Fields using Alanine Dosimeters – a pan-Canadian study

McEwen, M.R.*, Muir, B.R.

National Research Council Canada, Ottawa, ON
*Presenting Author: Malcolm.mcewen@nrc-cnrc.gc.ca

MR-linacs, combining a linear accelerator with an MRI scanner, present a unique challenge in reference dosimetry. The magnetic field, typically 0.35 T to 1.5 T, alters the paths of charged particles and therefore the dose deposition. The National Research Council of Canada has, in recent years, developed a capability for using alanine dosimeters to measure absorbed dose to water at therapy dose levels in Co-60 and MV photon beams[1]. This system was applied to two types of MR-linac installed across Canada to verify the output calibration.

A calibration curve for a single batch of alanine dosimeters was established through reference 6 MV irradiations at the NRC over the range 10-50 Gy. For measurements at the cancer centres, the dosimeters were loaded into POM holders with the same external dimensions as a standard ionization chamber. Each dosimeter was irradiated to a dose in the range 15 Gy to 25 Gy, as determined using the centre's standard calibration method, and then returned to the NRC for read-out and analysis.

The standard uncertainty in the dose measured using alanine was 0.9%. The difference between the delivered and measured dose was less than 1% in all cases, consistent with the measurement uncertainties, and there was no significant difference between alanine irradiations in water or in solid phantom. This work enables accurate dose verification measurements for MR-linacs, ensuring confidence in treatment delivery and patient safety for these state-of-the-art devices.

Keywords alanine dosimeters; radiation therapy, MR-linac; dosimetry audit .

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Figure 1. Elekta Unity MR-linac, B = 1.5 T

	Phantom	Mean dose (Gy)	D_{read}/D_{stated}
MR-linac #1	Water	15.14	1.009
MR-linac #1	Solid Water	15.06	1.004
MR-linac #2	Water	14.82	0.999
MR-linac #3	Water	15.01	1.001
MR-linac #3	Plastic Water	15.19	1.001
MR-linac #4	Water	14.85	0.990
MR-linac #5	Water	14.92	1.002

Figure 2. Results from five units across Canada

X-ray Microdosimetry in Aluminium Oxide

McNairn, C.[1#*], Karmali, A.[2#], de Vries, R.[1], Chi, H.[2], McEwen, M.[3], Murugkar, S.[1]

[1]Department of Physics, Carleton University
[2]Department of Physics, University of Ottawa
[3]Metrology Research Centre, National Research Council Canada
*Presenting Author: connormcnairn@cmail.carleton.ca
[#]Equal contributions

Microdosimetry systems with superior sensitivity to low doses (<0.1 Gy) and high spatial resolution are particularly desirable for radiation therapy and protection applications. Here we demonstrate a novel platform leveraging optical spectroscopy read-out in aluminium oxide samples irradiated with X-rays from a 6 MV Elekta Synergy linear accelerator with doses of 0 – 5 Gy. A custom-built setup using a 785 nm continuous wave excitation laser was used to collect the data from the samples. For each sample, the data were collected over a 10 × 10 point grid within a 100 × 100 μm^2 region of interest at post-irradiation timepoint of 24 and 108 hr, respectively. Dose-response curves based on the intensity of the signal read-out reveal similar non-linear dependence for post-irradiation timepoints of 24 and 108 hr. Each dose, including the lowest 0.1 Gy, is clearly discernible from the 0 Gy control sample. The relative standard deviation over the region of interest is ~2% at doses above 1 Gy. This is significantly lower than the value of ~11% in radiochromic films bearing inherent inhomogeneity [1]. The relative standard deviation decreases monotonically from 9% at 0.1 Gy to 2% at 5 Gy, which is consistent with expectations of stochastic nature of energy deposition at low doses (<1 Gy) [2]. As such, optical spectroscopic read-out of aluminum oxide shows great potential as an alternative for microdosimetry.

Keywords Microdosimetry; aluminium oxide

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Networking, Exercises, and Emergency Response

Barnard, S.

UK Health Security Agency, Radiation Chemicals Climate and Environmental Hazards Directorate, Chilton, Oxfordshire, UK.
Stephen.barnard@ukhsa.gov.uk

The importance of networking between and within biological dosimetry assistance networks, whether national, regional, or internationally, has demonstrated several benefits during the last decade or so. It is important to recognise the continued benefits of such activities to remain progressive in our quest to further refine and define emergency response to radiological accidents through intercomparison exercises, logistical considerations and adapting to an increasing need for digital data sharing, developments in automation and the future role(s) of artificial intelligence. This lecture provides an overview on networking, exercising, and emergency response activities across physical and biological retrospective dosimetry.

Keywords: Biological and retrospective dosimetry, dosimetry networks; radiological emergency response

Development of a National Biological Dosimetry Protocol in Spain: Interlaboratory Comparisons and Future Network Implementation

Alcaraz, M.[1], Armengol, G.[2], Barquinero, J.F.[2], Burgos, A.M.[3], Carrasco, P.[5], Domínguez, I.[6], Fernández, C.[7], Fernández, C.[8], Fernández, J.L.[7], Francès-Abellán, A.[2], García, J.A.[1], Gomis-Ferraz, J.[4], Gyingiri, D.[1], López, I.[7], Lozano, M.A.[8], Luis, F.J.[9], Mariño, A.[7], Mercado, A.M.[1], Montoro, A.[4]*, Moreno, M.[8], Navarro, J.L.[10], Olivares, A.[1], Pastor, N.M.[5], Prieto, M.J.[8], Ruiz, M.J.[9], Sierra, F.[8], Villaescusa, J.I.[4]

[1]Universidad de Murcia, [2]Universidad Autònoma de Barcelona, [3]Universidad de Málaga, [4]Hospital Universitario y Politécnico La Fe de Valencia, [5]Hospital de la Santa Creu i Sant Pau de Barcelona, [6]Universidad de Sevilla, [7]Centro Oncológico de Galicia, [8]Hospital General Universitario Gregorio Marañón de Madrid, [9]Hospital Virgen del Rocío de Sevilla, [10]Hospital Clínico Universitario Virgen de la Arrixaca de Murcia

*Presenting Author: montoro_ale@gva.es

In the context of radiological emergency preparedness, biological dosimetry networks play a key role in ensuring rapid and reliable dose assessment [1]. Spain has multiple laboratories with biodosimetry capabilities; however, a harmonized national framework has not yet been fully established. This work aims to present the development of a national biological dosimetry protocol in Spain within a research project supported by the Spanish Nuclear Safety Council (CSN), and to evaluate interlaboratory performance as a basis for future network implementation. Two interlaboratory comparison exercises (ILCs) were conducted involving six Spanish laboratories using the dicentric chromosome assay (DCA) and the cytokinesis-block micronucleus (CBMN) assay [2]. Whole-body and simulated partial-body exposures were analysed under both triage and full-scoring conditions. All participating laboratories achieved satisfactory dose estimations for whole-body exposures, demonstrating strong consistency across centres. In triage conditions, scoring 20–50 cells allowed reliable classification of high-dose exposures (>2 Gy), while higher cell numbers improved accuracy [3]. For partial-body exposures, results highlighted the need for increased scoring (>50 cells), especially at doses below 3 Gy. While CBMN showed good performance for whole-body exposure assessment, limitations were observed for partial-body detection. These results demonstrate the feasibility of harmonizing biological dosimetry capabilities across Spanish laboratories and provide a robust scientific basis for the establishment of a national protocol. The ongoing study, supports the integration of existing capacities into a coordinated response system. This initiative represents a key step toward the creation of a Spanish biological dosimetry network, enhancing national preparedness and contributing to international biodosimetry frameworks.

Keywords: Biological dosimetry; Dicentric chromosome assay; Cytokinesis-block micronucleus assay; Interlaboratory comparison; Radiological emergency preparedness

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Enhanced Operational Capacity for a Laboratory using Triage-mode Based Biodosimetry: Implementation and Training

Martinez, J.S.[1]*, Fernandez, P.[1], Baldeyron, C.[1], Dugué, D.[1], Grégoire, E.[1], L'Homme, B.[1], Thoër, G.[1], Baillon, C.[2], Descazaux, J.[2], Goldstein, S.[2], Bobyk, L.[3], Valente, M.[3] and Gruel, G.[4]

[1]Authority for Nuclear Safety and Radiation Protection (ASNR), PSE-SANTE/SERAMED/LRAcc, Fontenay-aux-Roses, France and [2]PSE-SANTE/SM2IV/LBMA, Le Vésinet, France
[3]French Armed Forces Biomedical Research Institute (IRBA), Radiation Biological Effects Department, Brétigny-sur-Orge, France
[4]Authority for Nuclear Safety and Radiation Protection (ASNR), PSE-SANTE/SERAMED, Fontenay-aux-Roses, France
*Presenting Author: juan.martinez@asnr.fr

During a radiological emergency of accidental or terrorist nature, a large number of potential victims could be involved. Rapid and correct classification in terms of radiological exposure level would be crucial, not only for those that require specific medical treatment, but also for non-exposed individuals. Our goal was to develop a system allowing to classify as many potential victims as possible in our lab by using the reference cytogenetic biodosimetry assay.

We developed an organizational scheme based on the classification of victims into different dose classes. The system has the potential to classify 320 individuals 13 days after sample reception, with a classification rate of 32 victims every 24 hours starting on the fourth day, by using a triage-mode dicentric chromosome assay (DCA). It is based on 4 independent teams of 2 members each, working in complete autonomy. A first exercise was performed in partial-capacity mode where 120 victims were classified. Pre-planned logistics were applied as instructed and classification results were compiled with a strict time limit. Following previous developments from our laboratory [1], three radiation exposure grading scales of 5, 4 and 3 classes were applied. Correct classification ranged from 85 to 92%, according to the scale used, which contributed to the validation of the newly developed organization [2].

We performed 2 additional exercises, where external partners participated. They had trained on our developed system and adapted their lab-specific methods in order to produce harmonized results. In both cases, a total of 240 blood samples were analyzed, and as in the previous exercise, lab operators were asked to treat and analyze the blindly coded samples by applying time-optimizing protocols and to respect an established deadline. Both exercises allowed us to treat a higher number of samples and to maintain a similar level of correct classification. In addition, they were rich in lessons that will enhance the efficacy of our scheme. Current efforts include maximizing correct classification, harmonizing methods with external partners and maintaining our operational capacity to better respond to an eventual radiological emergency.

Keywords Radiological emergency; triage; retrospective biological dosimetry; dicentric chromosome assay.

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UKHSA RCCE Emergency Response Exercise 2025 - Biological and Physical Dosimetry. Comparison of Techniques for Full and Triage Dose Estimations in the Event of a Mass Casualty Nuclear Incident

Ashworth-Donn, L.[1]*, Freestone, D.[1], Moquet, J.[1], O'Brien, G.[1], Humphrey, K. [1], Eakins, J.[1], Zmijewska, K.[1], Polozov, S.[1,3], Polozova, M.[1,3], Beazley, J.[2], Fallon, C.[1], Barnard, S.[1]

[1]UK Health Security Agency, Radiation, Chemical, Climate and Environmental Hazards Directorate, Chilton, Didcot, OX11 0RQ
[2]Wolfson College, Oxford University, Oxford, UK
[3]HQ Science Limited, London, UK
*Presenting Author: Lauren Ashworth-Donn: lauren.ashworth-donn@ukhsa.gov.uk

Under the Civil Contingencies Act (CCA) 2004, the UK Health Security Agency is designated as a Category 1 responder. In alignment with this role, a series of comprehensive emergency response exercises were conducted to evaluate preparedness for an ionising radiation (IR) incident. To assess the performance of various biodosimetry assays, coded blood samples were irradiated to simulate real-world exposure scenarios. Multiple research groups employed a range of assays to evaluate their ability to triage and categorise potentially overexposed individuals in a timely and effective manner. Standard triage categories in radiation emergencies are low (<1 Gy), medium (1–2 Gy), and high (>2 Gy) [1]. Biological dosimetry methods included the 'gold standard' dicentric chromosome assay (DCA), the γ-H2AX assay [2], a rapid approach suitable for initial triage, and FDXR gene expression analysis, a molecular biomarker of IR exposure. Additionally, Tachyon, a portable molecular diagnostic device, was deployed to assess radiation exposure in real time using nanopore sequencing and advanced bioinformatics, offering a rapid and scalable solution in emergency contexts. In parallel, physical dosimetry approaches included Thermoluminescent Dosimeters (TLDs), smartphone-based dosimetry, and alanine chip dosimetry. Results, an example is shown in Figure 1, confirmed the accuracy and effectiveness of each method in assigning individuals to triage categories based on estimated dose. These exercises underline the importance of integrating multiple dosimetry approaches to strengthen emergency preparedness and ensure robust public health responses in the event of radiological incidents [3].

Keywords Biodosimetry; emergency response; triage; emergency preparedness, radiological incidents

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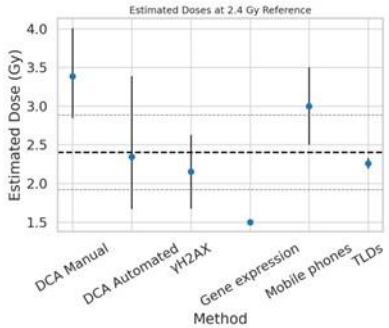

Figure 1. Estimated radiation doses across multiple dosimetry methods at reference dose 2.4 Gy. Each point represents an individual dose obtained by specific method.

Poster Presentations

In silico Identification of Circulating miRNA for Radiation Exposure Assessment

Ko, H.Y.*, Kim H.J., Cho, M.

Department of Radiation Dose Assessment, National Radiation Emergency Medical Center (NREMC), Korea Institute of Radiological & Medical Sciences (KIRAMS), Republic of Korea
*Presenting Author: hyko@kirams.re.kr

Rapid and accurate assessment of radiation exposure is essential for effective medical response in radiological emergencies. Recently, circulating microRNAs (miRNAs), present in extracellular body fluids, have emerged as promising minimally invasive biomarkers. This study aims to identify biologically relevant circulating miRNAs targeting radiation-responsive genes for biodosimetry applications. Seven radiation-responsive genes (FDXR, BAX, CDKN1A, GDF15, DDB2, MDM2, and GADD45A), known to be the most sensitive indicators of DNA damage, were selected as target genes. miRNAs targeting these genes were identified using miRNet, a database of experimentally validated miRNA-target interactions, resulting in 46 candidates including well-known radiation-responsive miRNAs such as miR-16-5p, the miR-19 family, and the let-7 family. Among them, 44 circulating miRNAs were selected based on evidence of their presence in plasma or serum from miRandola database and PubMed literature. To further refine candidate selection, TargetScan and miRDB were applied to evaluate direct binding potential and provide additional predictive support. This integrated approach identified a total of 10 high-confidence circulating miRNAs, including miR-34a-5p, miR-16-5p/15b-5p, miR-19a/b-3p, miR-146a-5p, and miR-148a/b-3p, etc. Most of these miRNAs play a central role in regulating key biological pathways associated with radiation exposure. This integrated in silico approach combining multiple database-based strategies identified robust circulating miRNA biomarkers for biodosimetry, which may enable rapid and minimally invasive radiation exposure assessment. With further validation using cell, blood, and animal models, these candidates could be developed into biosensor-based detection systems for biodosimetry.

Keywords Circulating microRNA(miRNA), Biodosimetry, Radiation-responsive genes, miRNA-gene interaction, In silico analysis

P05

A Saliva-Based Microfluidic Sensor for Point-of-Care Diagnosis of Acute Radiation Syndrome

Fischer, S.*, Stewart, S., Eder, S., Ostheim, P.

Bundeswehr Institute of Radiobiology affiliated to the University of Ulm, Munich, Germany
*Presenting Author: Sabrina2Fischer@bundeswehr.org

iMEDCAP is a European Defence Fund flagship project that brings together 24 organisations from 9 countries, coordinated by Technische Universität München, to develop an autonomous rescue chain for gravely injured personnel. The concept covers the full sequence from autonomous detection of casualties, through automated definition of an evacuation strategy, to initial treatment inside an interoperable patient box under remote control during transport. A central requirement is that this chain remains functional in contaminated environments, including chemical, biological, radiological and nuclear (CBRN) scenarios, where conventional evacuation and diagnostic solutions reach their limits and the medical paradigm of the golden hour is otherwise unattainable.

Radiological scenarios pose a particular diagnostic problem. Estimating the severity of Acute Radiation Syndrome (ARS) currently relies on laboratory-bound biodosimetry that takes hours to days and cannot be performed in the field, yet triage and treatment decisions must be made far earlier. Our contribution to iMEDCAP addresses this gap with a field-deployable microfluidic sensor that quantifies radiation-responsive mRNA biomarkers by PCR directly at the point of care. Saliva is used as the primary matrix because it can be collected non-invasively and without trained personnel, which matters when casualties are handled autonomously. The same analytical concept is implemented on a second cartridge for whole blood, so that diagnosis does not depend on which sample type is obtainable. Sample preparation, RNA extraction and amplification run automatically on chip, removing any need for laboratory infrastructure.

A first prototype of the automated RNA extraction device for blood has been delivered. For saliva, extraction currently still runs on the upstream pre-module, and its transfer into the integrated cartridge is the next development step. The cartridge itself is being redesigned to accommodate the distinct rheology of saliva, and a dedicated saliva buffer system has been optimised and is under independent verification. The device is designed to operate inside and outside the iMEDCAP patient box, feeding ARS severity data directly into the triage and treatment workflow.

Keywords Biodosimetry; Acute Radiation Syndrome; Saliva; Point-of-Care Diagnostics; Microfluidics; mRNA Biomarkers

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Application of the TGx-DDI Biomarker to Detect Radiation-Induced DNA Damage Responses in Human Lymphocytes

Khilji, S.[1]*, Chauhan, V.[1], Williams, A.[2], Koebel, C.[2], Yauk, C.L.[3]

[1]Consumer and Clinical Radiation Protection Bureau, Health Canada, Ottawa, ON, Canada
[2]Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada
[3]Department of Biology, University of Ottawa, Ottawa, ON, Canada
*Presenting Author: skhil083@uottawa.ca

Transcriptomic biomarkers offer a mechanistically informative approach for detecting radiation-induced biological responses and may complement established biodosimetry methods. The TGx-DDI transcriptomic biomarker is a 64-gene signature, originally developed in TK6 lymphoblastoid cells to distinguish DNA damage-inducing from non-DNA damage-inducing agents based on coordinated activation of DNA damage response pathways [1]. Although TGx-DDI has been validated primarily for chemical genotoxicants, its application to ionizing radiation remains limited. Here, we evaluated whether TGx-DDI can classify radiation-induced DNA damage response-associated transcriptional profiles across dose and dose-rate conditions. Primary human lymphocytes from 14 healthy donors were exposed to 0.05 – 6 Gy X-rays at a lower-dose-rate (0.05 Gy/min) or higher-dose-rate (1 Gy/min), and TempO-Seq transcriptomic data were analyzed 24 h post-exposure [2]. TGx-DDI classifications were assessed using probability analysis, principal component analysis, and hierarchical clustering, with ATP-based cytotoxicity measures used to exclude cytotoxic samples.

TGx-DDI classifications reflected dose-dependent activation of DNA damage response transcriptional profiles following X-ray exposure. At higher doses, particularly ≥1–2 Gy, nearly all non-cytotoxic samples were classified as DNA damage-inducing across donors and dose-rate conditions. Low-dose exposures showed greater variability, with some donors exhibiting detectable responses at 0.05 Gy, while others transitioned to DNA damage-inducing classifications only at higher doses. This variability was most evident at low doses, where transcriptional responses were closer to the classification threshold. The radiation-associated signal reflected coordinated changes across the 64-gene panel, including contributions from canonical DNA damage response genes such as CDKN1A, DDB2, GADD45A, MDM2, and AEN. This proof-of-concept study demonstrates that TGx-DDI can detect radiation-associated transcriptional activation of DNA damage response pathways in primary human lymphocytes. Future validation in larger donor cohorts, across additional post-exposure timepoints, and against established biodosimetry endpoints will help define its utility for individual-level radiation response profiling and biodosimetry applications.

Keywords: TGx-DDI; transcriptomics; DNA damage response; ionizing radiation

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A Highly Efficient and Sensitive Approach for Detecting Chromosome Aberrations in Cytogenetic Biodosimetry Using Checkpoint Bypass

Okimoto, A.[1,2]*, Anderson, D.[1], Kamakura, S.[1,2], Fujishima, Y.[1], Miura, T.[1]

[1]Institute of Radiation Emergency Medicine, Hirosaki University, Japan
[2]Graduate School of Health Science, Hirosaki University, Japan
*Presenting Author: h25mh304@hirosaki-u.ac.jp

In cytogenetic biodosimetry, the frequency of chromosome aberrations in peripheral blood lymphocytes from irradiated individuals is quantified to estimate absorbed dose. However, particularly at high radiation doses, exposure activates cell cycle checkpoints (G1, G2/M, and spindle assembly), resulting in cell cycle arrest [1]. As a result, damaged cells fail to progress to stages suitable for cytogenetic analysis and are excluded from evaluation, potentially reducing assay sensitivity. In this study, we investigated whether the dedifferentiation agent can bypass radiation-induced cell cycle arrest, with the aim of improving the efficiency and sensitivity of cytogenetic biodosimetry.

Peripheral blood from healthy donors with informed consent was irradiated with X-rays, incubated for 2 h at 37°C, and cultured for 24 to 48 hours with or without the dedifferentiation agent. Chromosomal aberrations were analyzed using the dicentric chromosome (Dic) assay, cytokinesis-block micronucleus (CBMN) assay, and premature chromosome condensation (PCC) assay. First, treatment conditions were optimized by evaluating PCC induction. The highest frequency of G2/M-PCC cells was observed at a concentration of 0.25 μM. Although 48-h culture is generally recommended, the G2/M-PCC index exceeded 50% at 42 h. Next, Dic analysis was performed under untreated conditions and under optimized treatment conditions. In the untreated group, a large number of metaphase cells were obtained for analysis. In contrast, few metaphase cells were obtained in the treated group, likely due to bypass of the spindle assembly checkpoint, making conventional Dic analysis difficult. Then, to analyze Dic, PCC was labeled using telomere and centromere peptide nucleic acid probe using fluorescence *in situ* hybridization (PNA-FISH). As a result, Dic was successfully detected in G2/M-PCC cells using PNA-FISH. Compared with untreated groups or treated with optimized condition for the dedifferentiation agent, treated group analyzed by PNA-FISH showed the highest frequency of Dic. In the CBMN assay, although the IAEA and ISO recommend 72-h blood culture [1, 2], it was confirmed that cells for analysis can be obtained even after 48-h culture. The frequency of micronuclei increased following treatment with the dedifferentiation agent.

These results suggest that the dedifferentiation agent enables more sensitive detection of chromosomal aberrations. This study was supported by Takahashi Industrial and Economic Research Foundation.

Keywords: PCC; Cell cycle arrest; PNA-FISH; Micronucleus; Biodosimetry

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P14

Use of the p21 Inhibitor UC2288 as a Strategy to Circumvent Cell Cycle Arrest during Proliferation for Dicentric Chromosome Analysis

Dakovic J.[1], Boateng G.K.[2], Eder S.F.[1], Beinke C.[1]*

[1]Bundeswehr Institute of Radiobiology affiliated to the University of Ulm, Munich, Germany
[2]PREOMICS GmbH, Part of Bruker Biognosys Group, Planegg/Martinsried, Germany
*Presenting Author: christinabeinke@bundeswehr.org

UC2288, a cell-permeable p21 attenuator, decreases p21 mRNA expression in various cell lines transcriptionally or post-transcriptionally [1]. The compound is toxic, induces DNA damage and promotes apoptosis in various cancer cell lines at higher concentrations, but has been found to be less toxic in non-cancer cells [2]. In theory, UC2288 can reverse cell cycle arrest because it lowers levels of p21, whose main role is to arrest the cell cycle in G1 or G2/M phase upon DNA damage. The idea is to inhibit p21 function by UC2288 in human lymphocytes to prevent cell cycle arrest especially in G2/M phase and thereby to accelerate cell cycle progression. This could shorten the culture time of lymphocytes of usually 48 hours for dicentric analysis, which would enable earlier provision of dose estimates.

For practical reasons, the (EBV) immortalized human lymphoblastoid cell line GM12878 has been used as a model for human lymphocytes. Experiments were performed with 10 µM UC2288 (in DMSO, approx. 50% growth inhibition) or DMSO as control at 4 and 24 hours after culture setting following sham irradiation (IR) or 3.0 Gy (240 kV/1.0 Gy/min). Viability, proliferation and toxicity was determined using the WST-8 assay, a highly sensitive, non-radioactive, colorimetric method and growth curve modelling. Downregulation of p21 was shown by western blot, immunofluorescence, and flow cytometry with specific p21-antibodies. Cell cycle behaviour was visualized by flow cytometry after BrdU incorporation and DNA staining. Apoptosis induction was observed by flow cytometry (subG1 fraction and annexin V-FITC/7AAD assay) and western blot analysis of total cell protein and cell fractions (caspase 3/8 cleavage, Cytochrome C release).

In summary, 10 µM UC2288 lead to a p21 attenuation, but unexpectedly induced mild apoptosis and an increased delay of the cell cycle in sham- and 3.0 Gy irradiated GM12878 cells. The desired effect was therefore not observed, at least in the GM12878 model cell line. Due to the described dependence of the opposing p21 effects (tumor-suppressive and oncogenic potential) on the cell type and cell context, the effect of 10 µM UC2288 on the proliferation of lymphocytes and thus on the number of metaphases after 24 hours is currently being investigated.

Keywords: UC2288, p21, cell cycle, dicentric analysis

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P15

Quantification of Golgi Morphology using Imaging Flow Cytometry for Radiation Biodosimetry

Lee, J.Y.[1,3], Cho, S.H.[2], Um, H.R.[2], Goo, C.W.[2], Kim, D-H.[3], Park, J.S.[1,3], Lee, Y.[1,2,3]*

[1]Department of Medical Sciences, Graduate School, Soonchunhyang University, Asan, Republic of Korea,
[2]Department of Biomedical Laboratory Science, College of Medical Sciences, Soonchunhyang University, Asan, Republic of Korea
[3]Institute for Molecular Metabolism Innovation, Soonchunhyang University, Asan, Republic of Korea
*Presenting Author: ylee0123@sch.ac.kr

In the event of a large-scale radiation accident, it is necessary to assess the radiation dose using a rapid and high-throughput method. Although classical cytogenetic biodosimetry assays have provided dose estimation in various histological radiation accidents [1], the development of new biodosimetry tools might be desired due to the limitations of conventional methods. Radiation-induced changes in cytoplasmic suborganelles have been relatively less investigated than nuclear DNA-related markers, and it was currently reported that DNA damage can trigger Golgi fragmentation [2]. We aimed to establish an imaging flow cytometry-based Golgi assessment tool and evaluate the changes in Golgi morphology after radiation exposure. The quantification tool for Golgi assessment was prepared according to Wortzel et al. [3] and modified using TK-6 cell images treated with Brefeldin A or ionizing radiation. We compared the changes in Golgi morphology with γ-H2AX foci levels, which is a sensitive radiation biomarker. TK-6 lymphoblastoid cells were exposed to γ-rays at various doses of 0, 0.25, 0.5, 1, and 2, 4 Gy, and fixed and stained with the Golgi marker GM130 or γ-H2AX antibody 24 h after irradiation. Cell images were acquired using a microscope and imaging flow cytometry. Imaging flow cytometry-based method allowed to acquire more than ten thousand of cell images within 10 min. As expected, it was difficult to observe a dose-dependent increase in γ-H2AX foci at 24 h post-irradiation due to rapid recovery; however, increased Golgi fragmentation was observed at the same time point after radiation exposure. The percentage of cells (%) with fragmented Golgi increased with the irradiation dose, which is similar to the results obtained using the conventional manual method with a microscope. Other quantification indices, such as the Golgi-to-nucleus area ratio and perimeter, also increased in a dose-dependent manner. In addition, the expression of Golgi structure-related proteins was increased in the irradiated TK-6 cells. Taken together, we suggest that Golgi fragmentation may be a promising candidate to represent radiation-induced DNA damage. Imaging flow cytometry-based Golgi assessment could enable rapid and high-throughput dose assessment, overcoming the limitations of conventional biodosimetry methods.

Keywords: Golgi fragmentation, γ-H2AX, Imaging Flow Cytometry, Biodosimetry, Radiation

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Serum-Free Culture Supports Reliable Chromosomal Aberration Analysis for Clinical Cytogenetics and Biodosimetry

Miura T.[1]*, Fujishima Y.[1], Anderson D.[1], Kamakura S.[1], Saito R.[1], Tsushima Y.[1], Okimoto A.[1], Goh V.S.T. [2]

[1]Institute of Radiation Emergency Medicine, Hirosaki University, Hirosaki, Japan,
[2]Singapore Nuclear Research and Safety Institute, Singapore
*Presenting Author: tomisato@hirosaki-u.ac.jp

The lymphocyte culture method for chromosomal aberration analysis has been widely used since the 1960s, with foetal bovine serum (FBS) routinely added to culture media. However, concerns over lot-to-lot variability, potential contamination, and the growing demand for animal-origin-free (AOF) systems highlight the need for reliable alternatives.

In this study, we evaluated the suitability of serum-free culture conditions for human peripheral blood chromosome analysis in both clinical cytogenetics and cytogenetic biodosimetry, including the dicentric chromosome (Dic) assay and the cytokinesis-block micronucleus (MN) assay. Phytohemagglutinin-stimulated peripheral blood cultures were established using conventional RPMI 1640 medium and human plasma-like medium, each with or without FBS. Cell proliferation indices (mitotic index, MI and binucleated cell frequency) as well as chromosomal aberration frequencies were compared across conditions.

Serum-free media showed significantly higher MI values than FBS-supplemented RPMI 1640, suggesting that FBS may suppress cell-cycle progression during 48- and 72-hour cultures (Figure 1). Despite the increased proliferation, the frequencies of Dics and MNs did not differ significantly between serum-free and serum-supplemented conditions.

These findings demonstrate that serum supplementation is not essential for peripheral blood lymphocyte culture in chromosomal aberration analysis [1]. Serum-free, AOF culture systems therefore represent a viable alternative, offering improved consistency, biosafety, and ethical advantages for applications in clinical cytogenetics and biodosimetry.

Keywords Cytogenetics, Chromosome, Blood culture, Animal-origin-free, Biodosimetry

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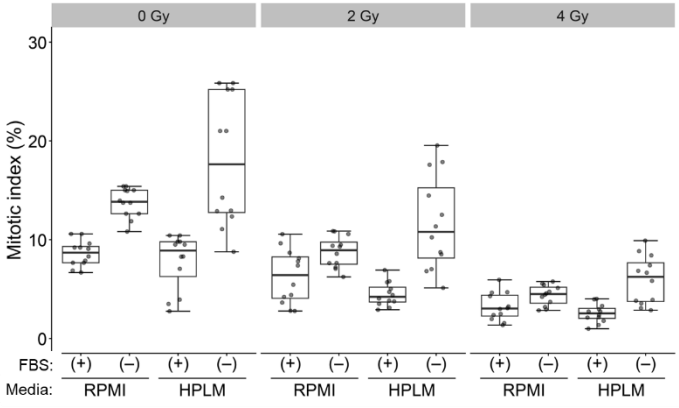

Figure 1. Comparison of mitotic index in whole blood culture for 48 h.

P17

EPR Dosimetry in Bone Samples of Former United States Nuclear Workers

Romanyukha A.[1]* and Tolmachev S. Y.[2]

[1]Armed Forces Radiobiology Research Institute, Uniformed Services University, Bethesda, MD 20889, U.S.A.
[2]College of Pharmacy and Pharmaceutical Sciences, Washington State University, Richland, WA 99354, U.S.A.
*Presenting Author: alexander.romanyukha@usuhs.edu

The United States Transuranium and Uranium Registries (USTUR) is a research program focused on actinide biokinetics in occupationally exposed individuals [1]. This work builds upon our previous studies [2, 3], in which EPR in tooth enamel was used to reconstruct external doses for nine USTUR registrants. Our prior results indicated that, in most cases, EPR doses in tooth enamel significantly exceeded worksite-reported doses without a clear explanation for the discrepancy. To investigate the cause of this variance, we conducted EPR dose measurements on bone samples collected from the same donors. Powdered bone samples were prepared using ultrasound treatment in a 20% potassium hydroxide solution and measured via EPR. To determine the radiation dose, the peak-to-peak amplitude of the radiation-induced signal was used as a relative measure of the received dose. This value was measured in each prepared bone sample before and after irradiation to a set of known doses from ^{60}Co, followed by linear back-extrapolation. The radiation doses obtained from the bone samples in this study were then compared with worksite-reported doses and the previously reconstructed tooth enamel EPR doses for the same individuals.

Keywords Bone; EPR; plutonium workers; USTUR.

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P18

Assessing Various Processing Methods to Optimize the Detection of the Stable Radiation Induced Signal (RIS) in Nail Clippings

Swarts, S.G.[1]*, Kobayashi, K.[2], Trompier, F.[3], Swartz, H.M.[2]

[1]Department of Radiation Oncology, University of Florida, Gainesville, Florida, USA
[2]Department of Radiology, The Geisel School of Medicine at Dartmouth, Hanover, New Hampshire, USA
[3]Authorité de Sûreté Nucléaire et de Radioprotection (ASNR), Paris, France
*Presenting Author: sgswarts@ufl.edu

In the event of a large-scale radiation disaster, there is likely to be hundreds to thousands of individuals exposed to unknown doses of radiation. Of these individuals there will be a subset of those who have received doses beyond a threshold of 2 Gy, requiring further medical evaluation and treatment. In one such method, the dose is estimated from nail clippings using electron paramagnetic resonance (EPR) where we have developed sample measurement and spectral analysis method to extract the radiation-induced signal (RIS) from an interfering mechanically-induced signal (MIS) in nail clippings. In previous studies, it was reported that 10 min nail soaking treatment would eliminate the unstable radiation-induced signal (RIS2) and MIS, leaving the RIS5 and background signal (BKS). However, our studies demonstrated that longer soak times (up to 20 min) are necessary to fully eliminate the RIS2 and MIS. In this study, we assess the changes in nail signal intensities with soak time in an attempt to find the appropriate soaking time to obtain the RIS5 and characterize the contributions to the BKS.

Nail sample sets from 34 donors containing clippings irradiated to doses of 0, 1, 2, 4 and 6 Gy, were previously measured by EPR, and then stored at -20°C for 1 to 5 months prior to use in this study. Donor nail samples sets were randomly divided into four soak time groups (5, 10, 15, and 20 min), measured by EPR to assess the stability of the irradiated clipped nail signal during the long-term storage at -20°C, soaked and air dried, then measured by EPR to measure the residual signal. Spectral fitting methods were used to calculate the amplitudes of the residual singlet signal (RIS5 + BKS). Linear regression analysis of the residual signal amplitudes was used to calculate the slope which is dose-response of the RIS5 + BKS. These slopes were compared as a function of soak time, and to the slope of the signal amplitudes of the RIS2 + RIS5 + BKS from the previous (pre-soak) EPR measurements. As expected, the slopes of the RIS5+BKS amplitudes decrease with increasing soak time of the nail clippings, with a minimum slope obtained with soak times between 15-20 min. At maximum soak times (20 min), the RIS5 formed at a dose of 6 Gy can be differentiated from 0 Gy.

The dose-response of the stable RIS5 in irradiated nail clippings can be measured in irradiated nail clippings at doses of $\leq$ 6 Gy using conventional X-band EPR instrumental methods, however, the variability of the BKS signal amplitude using the current measurement methods limits the accuracy of dose estimates calculated from the RIS5. This will be addressed in this work through identification and characterization of the contributions to the BKS signal.

Keywords: Dosimetry, fingernails, stable radiation induced signal, sample preparation, electron paramagnetic resonance spectrometry

P19

Feasibility Study of ESR Dosimetry in Turtle Shells

Harshman, A.M.[1]*, Romanyukha, A.[2], Patel, V.[2], Johnson, A.[3], Carter, E.T.[3], Gardner, S.[3,4]

[1]Environmental Protection Services Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA
[2]Armed Forces Radiobiology Research Institute, Uniformed Services University, Bethesda, MD, USA
[3]Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA
[4]School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University, Auburn, AL, USA
*Presenting Author: harshmanam@ornl.gov

Electron spin resonance (ESR) is a powerful tool for retrospective dosimetry; however, aside from widely studied materials such as tooth enamel and fingernails, its application to biological tissues remains limited [1]. This project investigates the feasibility of using tissues collected from turtles, namely keratin-based scutes and small fragments of underlying shell bone, as novel ESR dosimeters. There are several advantages associated with turtle shells for environmental dose reconstruction that include the ability to collect samples non-destructively, characteristics of the shell matrices [2], and the potential for the material to accumulate radiation exposures over long life spans. The Oak Ridge National Environmental Research Park (NERP) provides an ideal sampling landscape to evaluate the feasibility of ESR measurement in free-ranging wildlife owing to its established ecological research infrastructure and well-characterized radiological conditions.

Shell samples are collected using minimally invasive techniques to establish their suitability as an ESR dosimeter. Suitability was determined by evaluating ESR signal quality, dose-response, and signal stability using advanced spectral analysis techniques [3]. This work outlines the methodological framework, sampling strategy, and analytical approach developed and implemented to validate turtles as sentinel species and the usefulness of their shells as biological dosimeters for ESR dosimetry.

Keywords: electron spin resonance; biomonitoring; retrospective dose reconstruction

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The opinions, conclusions, and recommendations herein expressed are those of the authors and do not necessarily reflect the official policy or position of the Uniformed Services University of the Health Sciences or the Department of War.

EPR Spectral Classification of Smartphone Screen Glass: From Variability to Dosimetric Insight

Tuner, H.[1]*, Ollier, N.[2], Tepe Cam, S.[3], Trompier, F.[4]

[1]Department of Physics, Faculty of Art and Science, Balikesir University, Balıkesir, Turkiye
[2]Laboratoire des Solides Irradiés, Ecole Polytechnique, CNRS, CEA\\DRF\\IRAMIS, Institut Polytechnique de Paris, 91128, Palaiseau, France
[3]Academy and Publication Coordination Office, Turkish Energy, Nuclear and Mineral Research Agency, (TENMAK), Ankara, Turkiye
[4]Autorité de Sûreté Nucléaire et de Radioprotection (ASNR), Laboratoire de dosimétrie des rayonnements ionisants (LDRI), Fontenay-aux-Roses, France
*Presenting Author: htuner@balikesir.edu.tr/hasantuner@gmail.com

Smartphone screen glasses are widely considered promising materials for retrospective EPR dosimetry; however, their practical use is limited by variability in radiation-induced signals (RIS) that cannot be reliably predicted from the Gorilla® Glass (GG) generation reported for the corresponding smartphone models [1–3]. In this study, we examine whether RIS characteristics, supported by intrinsic/background signals and elemental composition, can provide a more robust basis for classifying smartphone screen glass. Samples collected from in-use, commercially circulated smartphones, were prepared by separating the glass layer from adhesive and electronic components using a heat gun and diamond wire, followed by irradiation with ^{60}Co γ-rays and measurement under standardized EPR conditions. The RIS exhibit pronounced and systematic variations in spectral structure, linewidth, and relative signal contributions, whereas unirradiated samples show only minor differences. Based on these observations, the samples were grouped into distinct spectral classes defined by characteristic RIS features. Elemental analysis was then used to validate and support this RIS-based classification, helping to confirm that glasses with similar RIS patterns indeed share comparable composition, while also enabling differentiation where RIS features alone were ambiguous. These results show that the GG generation reported for smartphone models is not a reliable predictor of EPR spectral behavior. Instead, RIS-based classification, supported by intrinsic and compositional information, provides a structured pathway from spectral variability toward dosimetric insight. However, more detailed research is needed to establish quantitative dose–response relationships for each RIS-based spectral class, to systematically assess the impact of UV exposure on signal stability, and to validate the framework using blinded samples from unknown devices.

Keywords EPR dosimetry; smartphone screen glass; radiation-induced signals; Gorilla Glass.

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Development of Preliminary STG-EPR Dosimetry Protocol for Radiological Emergency

Kim, J.S.*, Lee, B.M., Park, C., Park, M.S., Cho, M.

National Radiation Emergency Medical Center,
Korea Institute of Radiological and Medical Sciences, Seoul, Republic of Korea
*Presenting Author: kimzgo@kirams.re.kr

In radiological emergencies, retrospective dosimetry is essential for estimating absorbed doses when conventional dosimetric data are unavailable [1]. While various human-derived tissues and personal belongings have been utilized for retrospective dosimetry using electron paramagnetic resonance (EPR) techniques, biological samples often possess limitations such as invasive collection or high sensitivity to environmental factors [2]. Smartwatch touchscreen glass (STG) serves as a promising alternative for dose assessment due to its widespread usage and consistent proximity to the human body [3].

The evaluation of radiological characteristics of STG, derived from the Mi Band series, demonstrates outstanding performance for retrospective dosimetry. The intensity of radiation-induced signal (RIS) in STG exhibits a linear correlation ($R^2 = 0.9994$) with radiation doses ranging from 0 to 20 Gy. Time stability of RIS is observed in the STG, with an 8.48% decrease over 4 months. Thermal stability showed less than 3% variation at 50°C for 24 hours, and the variability of RIS induced by pretreatment was observed to be within 3%. The minimum detectable dose for STG was determined to be 0.93 Gy. Although ultraviolet light exposure induces signal fading, the resulting attenuation can be calibrated using RIS fading curves to prevent underestimation of the absorbed dose.

Assessment of radiological characteristics confirms the feasibility of achieving rapid and reliable dose estimation in radiological emergencies scenarios. Figure 1 illustrates a preliminary STG-based EPR dosimetry protocol proposed based on the evaluation of STG characteristics. In further study, the protocol will be improved through simulations and intercomparison with other retrospective dosimetry methods.

Keywords retrospective dosimetry, electron paramagnetic resonance, smartwatch touchscreen glass, radiological characteristics, radiological emergencies.

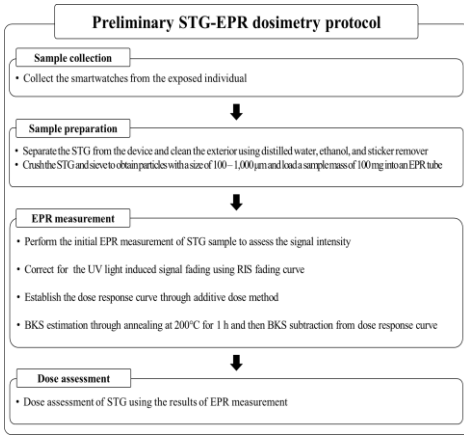

Figure 1. Schematic of preliminary STG-EPR dosimetry protocol

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Absorption dose ratio for fingernails and fingers by incident direction and energy of mono-energy photons

Yamaguchi,I.[1]*, Tadokoro, T.[2], Miyake, M.[2] and Yasuda,H[3]

[1] Dept of Environmental Heath, National Institute of Public Health, Wako, Japan
[2] Department of Oral and Maxillofacial Surgery, Kagawa Univ., Miki, Japan
[3] Research Institute for Radiation Biology and Medicine, Hiroshima Univ., Hiroshima, Japan
*Presenting Author: Yamaguchi.i.aa@niph.go.jp

As an indicator of radiation exposure, the absorbed dose is the amount of interaction between the radiation and a target object. Consequently, the ratio of absorbed dose between tissues within the body is influenced not only by the elemental composition and density of each tissue but also by the geometric effects of the irradiation. It is anticipated that the angle dependence will be greater when fingernails are used as samples for electron paramagnetic resonance (EPR) dosimetry. In this study, we investigated the ratio of absorbed dose between fingernails and fingers when the incident angle was varied, as well as the ratio of this absorbed dose to the air absorbed dose for the radiation field was present.

The calculations were performed using PHITS 3.350. The calculation conditions are as follows.

- The radiation source is a circle with a radius of 10 cm, located 30 cm from the origin and photons are emitted parallel to the z-axis.
- The finger is a cylindrical column of water with a radius of 0.8 cm and a length of 10 cm.
- The superficial layer is 2 mm thick within a 2 cm range from the tip of the finger and this region above the x-axis is defined as the nail and located around the origin.

Fig.1 shows the tracks of electrons following irradiation with 10 keV photons. Photons were being emitted from the left. The radiation is absorbed by the finger located on the z-axis.

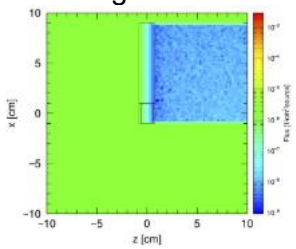

Table 1 Angle Dependence and Dose Ratio

Energy [keV]	Angle					nails to fingers dose ratio	nails to air dose ratio
	-90	-60	-30	0	180		
10	0.19	0.69	0.95	1.0	0.05	8.09	0.66
30	0.77	0.97	0.99	1.0	0.75	1.15	1.35
60	0.85	1.00	0.99	1.0	0.86	1.07	1.27

Using Fluorescence In Situ Hybridization (FISH) to Evaluate Multiple Biomarkers of Recent and Past Exposure to Ionizing Radiation

Lai, V.*, Pecoskie, S., Bertucci, A.

Nuclear Response and Analysis, Canadian Nuclear Laboratories.
*Presenting Author: vita.lai@cnl.ca

Accurate dose assessment after radiological and nuclear events is crucial for enabling effective emergency response and managing long-term, post-accident recovery. Biological and physical dosimetry, alongside clinical assessment, is paramount to determine the magnitude of individuals' exposure to ionizing radiation as quickly and precisely as possible. Various cytogenetic markers, including dicentrics, translocations, and chromosome fragments, are routinely used for dose estimation. Each marker is best suited to different scenarios.

The dicentric chromosome assay (DCA), which is considered a gold standard of biodosimetry, is somewhat limited by dicentric instability over long periods of time post-exposure [1]. In contrast, stable translocations persist, potentially throughout an individual's lifetime. Since these aberrations pass through cell cycle checkpoints, they can provide us with a dose estimate decades after exposure. A standardized method for evaluating multiple markers, for both recent and past exposures, from a single metaphase cell could be essential for increasing precision and reducing procedural variability.

This work investigated the possibility of using the fluorescence *in situ* hybridization (FISH) technique to evaluate cytogenetic damage, from multiple cytogenetic biomarkers, after exposure to ionizing radiation. In accordance with IAEA and ISO guidelines [1; 2], we established laboratory-specific protocols for staining and analysis of cytogenetic damage evaluation. Once protocols were established, we proceeded to develop calibration curves by exposing peripheral blood lymphocytes (PBL) to graded doses of γ-rays using a ^{60}Co source with a dose rate of ~0.8Gy/min, to quantify markers of radiation exposure. Validation of the method by estimating doses from blinded samples is in progress and results will be presented at the conference.

Keywords: biodosimetry; FISH; chromosomal translocation; calibration curves; dicentrics.

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Implementation of the γH2AX Assay as a Biodosimetry Tool for Radiation Emergencies

Vavra, J.*, Ekendahl, D., Sergunin, A., Pasek, D., Alaverdyan, J.

Dosimetry Department, National Radiation Protection Institute (SURO), Bartoskova 1450/28, 140 00 Prague 4, Czech Republic
*Presenting Author: jakub.vavra@suro.cz

Bioldosimetry methods can significantly contribute to the retrospective personal dose assessments in radiation emergencies, especially when the data from personal dosimeters is missing or need to be confirmed. The dicentric chromosome assay (DCA) is currently considered the “gold standard” biodosimetry method. However, DCA requires the 48 hours of blood samples cultivation to obtain peripheral blood lymphocytes in metaphase which causes a delay in dose reporting during the critical time period after suspected exposure [1]. Therefore, alternative biodosimetry methods such as the DNA double-strand repair foci assay [2] or the cell fusion-induced premature chromosome condensation assay [3] have been developed to overcome time-related limitations of DCA.

In this study, we implemented the γH2AX assay for the biodosimetry purposes at the National Radiation Protection Institute (SURO). Specifically, blood samples from four healthy volunteers were placed in a temperature-controlled water phantom (37 °C) and irradiated with a 1.4 MeV X-ray beam. In total eleven doses ranging from 0 to 5 Gy were applied. Following that the blood samples were placed in an incubator set at 37 °C and further processed at various time points after irradiation ranging from 30 minutes to 48 hours. Firstly, the blood leukocytes were separated, transferred onto microscopic slides and fixed with paraformaldehyde. Then, several steps of washing, permeabilization, blocking and incubation with primary and secondary antibodies were conducted. Finally, the cell nuclei were counterstained with DAPI and analysed using an automatic scanning microscopy system (Metafer) to determine the frequency of γH2AX foci. As a result, dose-response curves were established for the respective time points. Additionally, a validation experiment was conducted using blood samples irradiated with a priori blind doses to confirm the applicability of the laboratory-specific procedures and calibration curves established for the γH2AX assay at SURO.

Keywords Ionizing radiation; Radiation Emergencies; Biodosimetry; DNA repair foci; γH2AX assay

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P30

γH2AX as a Biodosimetric Marker of Ionizing Radiation: the Use of Metafer Systems and Flow Cytometry

Andrejsová, L.*, Filipová, A., Čížková, J., Šinkorová, Z., Milanová, M., Carrillo, A.

Department of radiobiology, Military Medical Faculty, University of Defence, Czech Republic
*Presenting Author: lenka.andrejsova@unob.cz

The phosphorylated form of histone H2AX (γH2AX) is one of the most sensitive biomarkers of double-strand breaks (DSB). The formation of DSB is typically followed within minutes of exposure to ionizing radiation by rapid phosphorylation of histone H2AX at serine 139 in the surrounding chromatin. The phosphorylation signal spreads to an area of several megabases around and leads to the formation of characteristic discrete foci known as γH2AX foci. These structures allow not only the detection of DNA damage but also its semi-quantitative assessment based on the number and intensity of the resulting foci. The introduction of specific antibodies against γH2AX has enabled the development of a wide range of immunological methods, such as immunofluorescence microscopy, flow cytometry, Western blotting, and the ELISA method, designed to analyse the extent of DNA damage.

Immunofluorescence microscopy for the detection of γH2AX is a well-established and widely used method for studying DNA damage. Microscopic analysis allows for the direct visualization of lesions forming in the cell nucleus and it is a sensitive method allowing retrospective dose determination. The determination of γH2AX by flow cytometry represents an alternative approach to microscopic evaluation. The output variable of this method is the median fluorescence intensity (MFI) of the γH2AX-positive cell population. The main advantage of this approach is the ability to analyze a large number of cells, while providing results within 4 hours. Compared to the microscopic method, the sensitivity of flow cytometry is lower, as it provides relative MFI data, whereas microscopic analysis yields absolute data on the number of γH2AX foci in individual cells. However, the upper range of detectable doses is not limited by the merging of foci, which allows for analysis even at doses exceeding 4 Gy. A limiting factor in the use of γH2AX as an effective biodosimetric marker is the activity of cellular repair mechanisms, which leads to rapid dephosphorylation of histone H2AX and a decrease in the detectable signal as early as approximately 30 minutes after exposure to ionizing radiation.

Within this study, a protocol for the detection and analysis of γH2AX foci using the Metafer automated image analysis system was standardized, and the methodology for determining γH2AX by flow cytometry was optimized. For the purposes of rapid triage, a standardized time for peripheral blood collection two hours after exposure was proposed, and rules for transporting samples to the laboratory in the presence of phosphatase inhibitors responsible for the dephosphorylation of histone H2AX were defined. For both methodological approaches, calibration curves were created describing the dependence of the monitored biological response on the absorbed dose of ionizing radiation, and based on these, dose thresholds were established in relation to the expected severity of acute radiation sickness. The created biodosimetric curves were successfully validated through a blind study.

Keywords γH2AX; Metafer; flow cytometry; gamma irradiation; triage.

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Cases with Suspected Exposure to Ionizing Radiation Referred to the Biological Dosimetry Laboratory of the Hospital General Universitario Gregorio Marañón, Madrid (Spain)

Prieto Rodríguez, M.J.[1]*, Fernández Martrin, C.[2], Muñóz Fernández, M.[3], Tavera Pomata, P.[3], González San Segundo, C.[3], Moreno Domene, M.[1]

[1]Biological Dosimetry Laboratory, Hospital General Universitario Gregorio Marañón
[2]Instituto de Investigación Sanitaria Gregorio Marañón
[3]Radiation Oncology Department, HGUGM
*Presenting Author: mprietor@salud.madrid.org

Biological dosimetry is a key component of radiological emergency response, providing reliable dose estimates when physical dosimetry is unavailable or uncertain. The Biological Dosimetry Laboratory at Hospital General Universitario Gregorio Marañón (HGUGM), accredited under ISO/IEC 17025:2017 and ISO 19238:2023, offers routine and emergency biodosimetry services to support medical decision making after suspected exposure to ionizing radiation.

The laboratory applies cytogenetic dosimetry based on dicentric chromosome and chromosomal translocation analyses, selected according to the exposure scenario and timing. Dose assessment relies on laboratory-specific calibration curves for low and high-LET radiation developed in line with IAEA recommendations, ensuring traceability and robustness for emergency use.

Since 1989, 143 individuals with suspected radiation overexposure have been evaluated, including healthcare workers, industrial radiography personnel, nuclear facility staff, and members of the public. Most cases were assessed within the first month after exposure. Three incidents involved doses above 0.5 Gy, including a confirmed exposure of 1.3 Gy from an Ir-192 source, directly informing clinical follow-up and patient management.

Experience from real emergency cases, together with regular participation in national and international intercomparison exercises, has strengthened the laboratory's operational readiness. This long-term experience highlights the critical role of biological dosimetry in complex exposure scenarios and underscores the importance of validated methodologies, trained personnel, and interlaboratory collaboration to ensure an effective and scalable emergency response

Keywords: Biological dosimetry; Dicentric chromosome assay; Radiological emergency preparedness.

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P32

Translocation Frequencies in Peripheral Blood Mononuclear Cells of Cancer Patients Undergoing External Beam Radiotherapy

Meher, P.K.[1]*. Nilsson, H-O.[1],Płódowska, M.[2], Lisowska, H.[2],Węgierek-Ciuk, A.[2], Braziewicz, J.[3],Ramadan, L.[4], Dayal, R.[4], Baeyens, A.[4], Vral, A.[4], Wojcik, A.[1,2]

[1] Centre for Radiation Protection Research, Department of Molecular Biosciences, The Wenner-Grenn Institute, Stockholm University, Stockholm, Sweden.
[2] Institute of Biology, Jan Kochanowski University, Kielce, Poland.
[3] Holy Cross Cancer Centre, Kielce, Poland.
[4] Radiobiology, Department of Human Structure and Repair, Ghent University, Ghent, Belgium.
*Presenting Author: prabodha.meher@su.se

Radiotherapy (RT) is standard treatment method for cancer where patients receive high doses of radiation in a fractionated manner, to different volumes of their tissue. RT induces chromosomal aberrations in peripheral blood mononuclear cells (PBMC) of the patients but the impact of such factors as tumor site, volume of tissue receiving a certain fraction dose and time between termination of RT and PBMC collection on the aberration frequency are not well understood. Within the SINFONIA project, we analysed translocations by chromosome painting in PBMC of patients treated for breast cancer, brain cancer and Hodgkin lymphoma. Blood samples were collected before RT and one or two time points after RT. The breast patients formed the largest group with more than 100 patients. Some patients were treated in the supine position and others in the prone position, resulting in big differences in the volume of normal tissue receiving a certain total dose. The time of blood collection after RT varied between 1 and 25 weeks, allowing the analysis of the impact of this factor on the frequency of translocations. Analyses of aberrations will be finalised by August 2026, so the final results will be presented.

This SINFONIA project has received funding from the Euratom research and training programme 2019-2020 under grant agreement No 945196.

Keywords: Cancer; Radiotherapy; Translocations; Individual variability

Validating an Imaging Flow Cytometry-based γ-H2AX Assay for Triage Screening in Case of a Radiological Event

Pecoskie, S.*, Bester, M., Lai, V., Boell, S., Bertucci, A.

Canadian Nuclear Laboratories, Chalk River, Ontario, Canada
*Presenting Author: steve.pecoskie@cnl.ca

In a large-scale radiological event, where physical dosimetry may not be available, various biodosimetry methods may be used for triage, dose assessment, and dose reconstruction. The γ-H2AX assay is a sensitive, molecular technique which can be used to detect and quantify DNA double-strand breaks following an exposure to ionizing radiation.

In this study, a rapid lyse/fix methodology for γ-H2AX processing [1] was adopted and optimized to analyse samples using imaging flow cytometry. Imaging flow cytometry offers the advantage of increasing throughput by utilizing principles of traditional flow cytometry while retaining the detailed spatial visualization of microscopy. Several gating schemes and scoring outputs were explored [2] before opting for a spot counting strategy similar to traditional γ-H2AX. Coupling rapid processing with imaging flow cytometry allows for complete batch analysis and dose estimates on the same day of sample receipt.

A dose-response calibration curve was constructed using a Cobalt-60 gamma source. The flow-based γ-H2AX assay and calibration curve were validated in a blind study with a comparison against the dicentric chromosome assay. It was further tested in a practical exercise scenario where the samples were exposed to up to 4 Gy absorbed dose utilizing 250 kVp x-rays.

Keywords γ-H2AX; validation; dicentric chromosome assay; exercise

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Accelerated Triage of Suspected Irradiated Individuals Based on Dicentric Chromosome Analysis of Human Peripheral Blood

Sinkorova, Z.[1]*, Milanova, M.[1], Andrejsova, L.[1], Filipová, A[1], Cizkova, J.[1], Vavra, J.[2], Ekendahl, D.[2], Davidkova, M.[2], Carrillo, A.[1]

[1]Department Radiobiology, Military Medical Faculty, University of Defence, Hradec Kralove, Czech Republic
[2]National Radiation Protection Institute, Prague, Czech Republic
*Presenting author: zuzana.sinkorova@unob.cz

Human peripheral blood (PB) presents a useful tool for biological dosimetry because the blood lymphocytes belong to the group of biological materials that can be used as a sensitive radiation detector. Changes in lymphocytes chromosomes are almost exclusively induced by ionizing radiation and their evaluation due to the dicentric chromosome analysis (DCA) (2) as a diagnostic triage tool for individual radiation dose assessment is widely used in radiobiology around the world. To speed up the evaluation of the method in the case of many analyzed samples, several approaches can be used. Most commonly, the approach based on reducing the number of evaluated metaphases suspected of being irradiated is used. PB represents a suitable biological material for radiation biodosimetry, as peripheral blood lymphocytes are highly sensitive to ionizing radiation and can serve as reliable biological radiation detectors. Chromosomal aberrations in lymphocytes, particularly dicentric chromosomes, are induced almost exclusively by ionizing radiation. Consequently, dicentric chromosome analysis (DCA) is widely used worldwide as a standard triage and diagnostic method for individual radiation dose assessment.
In situations involving many suspected irradiated individuals, rapid evaluation is essential. To accelerate DCA processing under such circumstances, several approaches can be applied. The most used approach is based on reducing the number of evaluated metaphases in samples suspected of irradiation.

In our laboratory, a methodology for accelerated DCA triage has been developed that is based solely on the detection of dicentric, tricentric, and tetracentric chromosomes in lymphocyte metaphases, without determining their exact frequencies or estimating absorbed dose using calibration curves. For individual dose-response categories—R0 (non-irradiated individuals, no therapeutic intervention), RC1 (general supportive care without specific therapy), RC2 (supportive care including substitution therapy), RC3 (stimulation therapy using growth factors), and RC4 (classification for stem cell transplantation)—the method relies on the absence of dicentric chromosomes or, conversely, on the presence of dicentrics alone or in combination with tricentrics and tetracentrics. Once chromosomal abnormalities are identified in analyzed metaphases, further metaphase evaluation is no longer required.

This triage-oriented approach is extremely rapid and enables the prompt classification of suspected irradiated individuals into appropriate triage categories, fulfilling the critical requirements of emergency response in scenarios involving mass radiation exposure.

Keywords dicentric chromosome analysis; human peripheral blood; gamma irradiation; triage; dicentric and multicentric chromosome.

Accurate Estimation of Clinically Significant Radiation Doses up to 5 Gy using qRT-PCR–based Gene Expression Analysis

Romero, I.[1]*, García, O.[2], Loren, P.[3], Gonzalez Mesa, J.E.[2], Alasá, Y.[4], Barquinero, J.-F.[5], Cerda, A.[1], Santibañez, M.[6], Saavedra, N.[1]

[1]Basic Science Department, University of La Frontera, Temuco, Chile, [2]Radiobiology Laboratory, Center for Radiation Protection and Hygiene (CPHR), La Habana, Cuba, [3]Medicine Faculty, Catholic University of Temuco, Temuco, Chile, [4]Natural Resources Faculty, University of Temuco, Temuco, Chile, [5]Unitat d'Antropologia Biològica, Departament de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, Catalonia, Spain, [6]Ionizing radiation Laboratory, Physics Science Department, University of La Frontera, Temuco, Chile
*Presenting Author: ivonne.havana@gmail.com

Gene expression biodosimetry has demonstrated strong performance within low-to-intermediate radiation dose ranges; however, the intermediate to high dose of transcriptional responses and its impact on dose reconstruction accuracy remain insufficiently characterized. *Purpose:* The main aim of this study is to evaluate the capability of the qRT-PCR based on *FDXR* and *DDB2* expression to accurately dose reconstruction over 2 Gy within clinically relevant exposure intervals.
Materials and methods: Whole blood samples from one donor were irradiated *in vitro* (0–25 Gy) and incubated for 24 h. Peripheral Blood Mononuclear Cells were isolated, from which total RNA was extracted, cDNA was synthetized and gene expression (*FDXR, DDB2*) relative to *18S* was quantified by qRT-PCR (ΔCt). Saturation dose was determined using a combined approach including effect-threshold analysis, local slope reduction, and four-parameter logistic (4PL) modelling. Three calibration models (asymptotic exponential, 4PL inverted logistic, and linear-log2) were fitted and compared using residual standard error (RSE), AIC and BIC. Dose estimations were performed from data obtained from an independent set of samples irradiated at 1, 2.5, 5 and 7.5 Gy. Dose estimation accuracy was assessed by 95% confidence intervals (CI), ±20% deviation from physical dose, and correct allocation into clinically relevant categories
Results: Both genes exhibited progressive ΔCt reduction with increasing dose followed by plateau formation. The median saturation dose was 6.9 Gy for *FDXR* and 7.5 Gy for *DDB2*, indicating diminishing sensitivity above approximately 7 Gy. Although the linear-log model provided the lowest RSE and most favorable AIC/BIC, it failed to identify the control (0 Gy). The 4PL model correctly identified unexposed samples and was therefore selected for dose estimation. *FDXR* achieved accurate reconstruction up to 5 Gy and correctly classified doses into relevant clinical intervals (Figure 1). Both genes demonstrated large uncertainty and overestimation at 7.5 Gy, consistent with saturation.
Conclusions: The 4PL model provides superior reconstruction validity incorporating biological asymptotes. Within 0–5 Gy, *FDXR* demonstrated clinically robust interval classification performance.

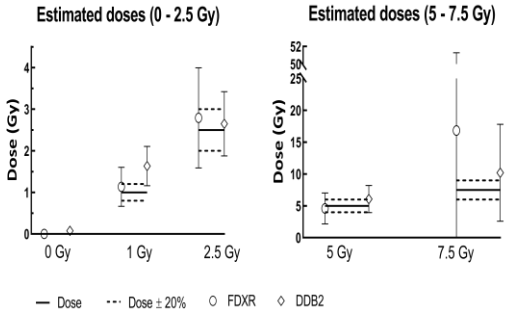

Figure 1. Dose estimations performed with the 4PL-H dose-response relationship for both FDXR (○) and DDB2 (◊). Straight lines represent the true Dose ( ) and the dashed lines represent the ± 20% interval of the true Dose ( ).

Keywords: Biodosimetry – Gene expression – qRT-PCR – accurate dose estimation – clinically relevant categories.

Impact of the Obesity Hormone Leptin in Combination with Estrogen on Frequencies of Radiation-Induced Micronuclei in Peripheral Blood Mononuclear Cells of a Health Donor

Baniotis, A., Dhakal, K., Kocibalova, Z., Meher, P.K., Wojcik, A.*

Centre for Radiation Protection Research, Department of Molecular Biosciences, The Wenner-Grenn Institute, Stockholm University, Stockholm, Sweden.
*Presenting Author: andrzej.wojcik@su.se

Biological dosimetry relies on the assessment of dose in peripheral blood mononucler cells (PBMC) of a victim. Variability in the individual radiosensitivity of PBMC has an impact on the precision of dose estimate and radiation-induced micronuclei show a strong individual variability. The understanding of individual-related factors responsible for the variability will contribute to an improved precision of dose estimate following a radiation emergency. A health factor which is related to an increased risk of cancer and possibly interacts with radiation is obesity. A comparison of the in vitro radiosensitivity of PBMC collected from people with normal and excessive body mass index (BMI) would be challenging. However, a hormone that is increased in obese people is leptin and experimental results suggest that it may potentiate the detrimental effect of radiation. The impact of leptin on radiation response of PBMC can be tested under in vitro conditions. With this in mind, we have initiated a study where PBMC of a healthy male donor with a normal BMI (between 18.5 and 24.9) were treated with 6.25 nM leptin (a concentration that is close to the equilibrium dissociation constant value of leptin for its receptor and corresponds to serum levels in an obese person) for 24 h and exposed to 2 and 4 Gy of gamma radiation. In addition, the impact of estrogen (10 nM) in combination with leptin was also tested, because of the possibility of an interaction between the hormones. Binucleation was induced by cytochalasin B and micronuclei were scored in binucleated cells stained with acridine organge.
Experiments are under way but preliminary results suggest a weak impact of leptin on spontaneous and radiation-induced micronuclei. Completed results wil be presented.
The study is part of the MIRAMARE project that has received funding from the Pianoforte partnership supported by the European Union’s “EURATOM” research and innovation program under the 101061037 grant agreement.

Keywords: Obesity, individual radiosensitivity, biological dosimetry

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Evaluation of Neutron-Induced Bystander Effects in Human Colon Cancer Cells

Liu, W.[1]*, Byun, S.H.[1], Seymour, C.[2], Mothersill, C.[2]

[1]Department of Physics and Astronomy, McMaster University, Hamilton, ON, L8S 4K1, Canada
[2]Department of Biology, McMaster University, Hamilton, ON, L8S 4K1, Canada.
*Presenting Author: Liu1324@mcmaster.ca

Dose-response relationships can be complicated by the radiation-induced bystander effect (RIBE), where cells directly exposed to radiation transmit signals and induce responses in cells that have not been directly irradiated. Although RIBE has been well documented for many radiation types, its presence and dose threshold for neutrons remain highly debated. Neutron exposures arise in nuclear reactors, accelerator-based facilities, space radiation environments, and certain medical applications, where they frequently occur as components of mixed radiation fields. Conflicting findings have left the existence and characteristics of neutron-induced RIBE unresolved, posing challenges for biological dosimetry and risk assessment in mixed radiation fields.

In this study, we investigated neutron-induced bystander response in a human colon cancer cell line (HCT 116, wild-type p53) using two neutron sources with different beam qualities: a fast neutron beam containing a measurable gamma component generated at Tandetron accelerator, and a clean thermal neutron beam with minimal gamma contamination produced at McMaster Nuclear Reactor. Conditioned-medium transfer was used to deliver bystander signals to unirradiated cells, and bystander responses were quantified using clonogenic survival assay. To further assess potential interactions in mixed-field exposures, a Cs-137 gamma source was included to evaluate how neutron irradiation may modulate gamma-induced bystander responses.

Although this cell line exhibited a clear bystander effect following gamma irradiation, our preliminary data indicate an absence of detectable bystander responses after exposure to thermal neutrons up to around 70 mGy or fast neutrons across the 300 mGy–2 Gy range, even in the presence of around 10% gamma-dose contamination. These findings suggest that neutron irradiation may not trigger the same intercellular signalling pathways responsible for RIBE observed with low-LET radiation. Ongoing analyses of bystander-related signalling molecules, including reactive oxygen species (ROS), are expected to provide mechanistic insight into differences between neutron- and photon-induced responses. Additional gamma-subtraction experiments are underway to further clarify the contribution of neutron exposure to bystander effects in mixed radiation fields. Overall, this work aims to improve our understanding of RIBE under neutron irradiation and to support more accurate modelling of biological responses in mixed-field environments relevant to biological dosimetry.

Keywords Radiation-induced bystander effect; Neutron; Clonogenic survival; Reactive oxygen species

P50

Alanine Dosimetry for Determination of Radiation Exposure during Emergencies

Eakins, J.[1]*, Ainsbury, E.[2], Ardavan, A.[3], Beazley, J.[1,3], Fallon, C.[1], Gougelet, R.[4], Speers, I.[4], Tanner, R.[1]

[1]United Kingdom Health Security Agency (UKHSA), Chilton, United Kingdom
[2]AWE Nuclear Security Technologies, Reading, United Kingdom
[3]Department of Physics, University of Oxford, United Kingdom
[4]Global Resonance Technologies (GRT), United States of America
*Presenting Author: Jonathan.Eakins@ukhsa.gov.uk

The risks to the public and responders from nuclear emergencies and malicious radiological attacks are well recognized (e.g. in the 2020 UK National Risk Register). Vital to any response is the need to accurately and quickly assess the doses that individuals have received, to aid triage categorization and provide appropriate medical care.

We hypothesise that a new alanine-based personal dosimetry system can be used to improve emergency response. This Emergency Radiation Dosimetry System (ERDS) utilizes a small alanine pellet that, when exposed to radiation, stores a signal directly proportional to the dose received. The pellets can be embedded easily into everyday objects routinely carried by individuals, such as identification cards, and will be read-out after radiation exposure using a portable reader in a self-service kiosk to provide estimated doses, with the added benefit of having a clear identification of the individual.

ERDS will contribute to establishing an inexpensive mitigation strategy based on retrospective dosimetry. Implementation will permit large numbers of individuals to be assessed quickly. This will improve patient care and help allocate limited medical resources, ultimately complementing and improving the existing UK, EU and US retrospective dosimetry capacities and capabilities.

The present poster summarizes the central concepts and methods underpinning ERDS, shows the progress to-date in bringing it to fruition, and highlights the potential applications and advantages of its success.

Investigation of Alanine Dosimetry by EPR for Ultra-High-Dose-rate Electron FLASH Radiation Therapy

Sherman, J.R.[1a]*, Gougelet, R.[2], Pazos, I.[3], Yokoyama, K.[1b], Wu, Y. [1c], Oldham, M.[1a]

[1]Duke University, [a]Medical Physics [b]Biochemistry [c]Physics
[2]Global Resonance Technologies
[3]Physical Measurement Laboratory, NIST
*Presenting Author: j.sherman@duke.edu

Ultra-high dose rate (FLASH) radiation therapy has shown potential for reduced normal tissue damage while preserving tumor killing. FLASH dosimetry at dose rates >40Gy/sec is extremely challenging. Gafchromic film (EBT-XD) is commonly used for FLASH dosimetry, but recent data suggests an over-response at extreme FLASH dose rates of >MGy/s. Alanine dosimetry is a promising independent approach for FLASH due to negligible known dose-rate dependence and high stability. This project investigates the feasibility of alanine dosimetry for a research FLASH beam (35MeV electrons with 30MGy/s dose rate) from the High Intensity Gamma Source (HIGS, Duke University, TUNL). Alanine measurements are directly compared to Ashland Gafchromic EBT-XD film measurements.

A calibration curve was created by irradiating groups of four alanine pellets (from Global Resonance Technologies) in 5 Gy increments from 5 – 40 Gy on a clinical Varian TrueBeam accelerator operated at 16 MeV at clinical dose rates (0.15 Gy/sec) with pellets at 0.5 cm depth in a solid water stack. Pellets were read out several weeks post-irradiation on a Bruker X-band EMXplus EPR spectrometer. FLASH alanine pellets were irradiated on the HIGS in a dosimetry cassette of dosimetry film and 1 cm bolus in front of and behind the pellets. Each pellet was targeted in the center of a 1.5 cm beam and received a single pulse and was read out 1 week post-irradiation. Film from the cassette was read out 24 hours post-irradiation following standard procedures. The spectra from the calibration and HIGS irradiated pellets were collected on the same day and quantified as the peak-to-trough intensity normalized to the pellet mass.

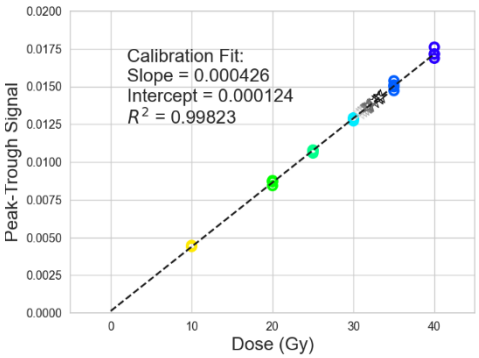


Figure 1. Peak-to-trough amplitude of calibration pellets (rainbow circles) and interpolated doses of HIGS irradiated pellets (grey stars).

A linear calibration curve was observed (Fig 1) with typical uncertainty between pellets on order of 1.8%. The doses from the 11 HIGS irradiated pellets were estimated from interpolating signal onto the calibration curve. Visual inspection of the film revealed strong alignment between of the beam center, alanine, and film. A direct comparison between film and alanine revealed that on average the HIGS alanine doses agree with EBT-XD film to within ~2.5%.In this preliminary study, good agreement was found between alanine and film from single pulses of ~ 30 Gy on the HIGS. Future work will extend to a wider range of doses and dose rates.

Keywords Alanine; FLASH

Alanine/EPR Dosimetry in the kGy/s Scale

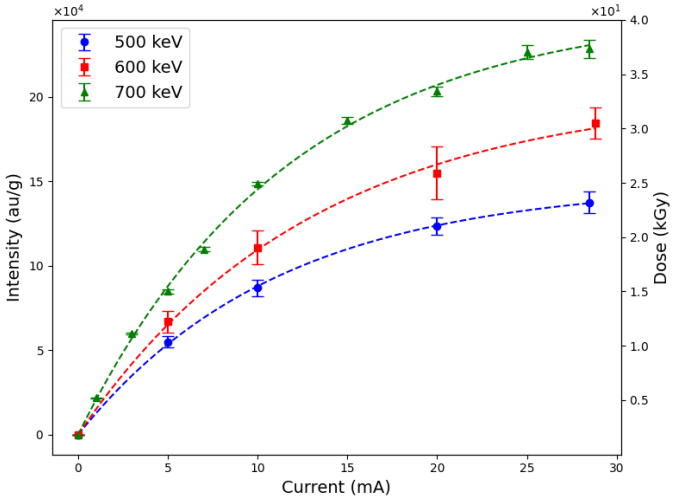
Silva, K.M.A.[1]*, Bueno, C.C.[2], Gonçalves, J.A.C.[2], Somessari, S.[2], Calvo, W.A.P.[2], Nicolucci, P.[1], Baffa, O.[1]

[1]Department of Physics - Faculty of Philosophy, Sciences and Letters - University of São Paulo, Brazil
[2]Institute for Energy and Nuclear Research (IPEN), Brazil
*Presenting Author: kyssyllamonny@usp.br

A mobile unit equipped with a linear accelerator operating with electron beams of 500, 600 and 700 keV, currents ranging from 1 to 28.5 mA, at high dose rates (kGy/s) was acquired through an Institute for Energy and Nuclear Research (Instituto de Pesquisas Energéticas e Nucleares - IPEN/CNEN) and the International Atomic Energy Agency (IAEA) cooperative project. This work presents the development of an alanine/EPR-based dosimetry system for this mobile accelerator. Alanine was chosen as the reference dosimeter due to its well-established response up to 10 kGy [1], and its ability to maintain accuracy, stability and reproducibility in the 10–100 kGy range [2].

A batch of 800 alanine cylindrical pellets (10% PTFE as a binder), 45 mg on average, 4 mm in diameter, and 2 mm thick, was produced. Calibration and testing of the batch were performed using a Cs-137 irradiator with 50 randomly selected pellets to a 1 kGy dose. The average EPR spectrum intensity after irradiation was (870 ± 43) au/g., resulting in a coefficient of variation of 5%.

Figure 1 shows the dose as a function of the applied current for the three beam energies. The beam profile was also determined by this dosimeter. Monte Carlo simulation is being implemented and validated by the experimental results to be used as a predictor of different experimental set ups, enabling the optimization of industrial processes with a strong focus on sustainability and environmental aspects.



Keywords: High-dose; Alanine/EPR; Electron beam.

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Alanine For Protontherapy Bragg Peak Dosimetry

Martinet-Gerphagnon E.[1,2]*, Trompier F.[2], De Marzi L.[1]

[1]Institut Curie - Centre de Protonthérapie d'Orsay, [2]Authority for Nuclear Safety and Radiation Protection (ASNR), Fontenay aux roses, France
*Presenting Author: erwan.martinet-gerphagnon@curie.fr

Radiotherapy remains as of today a cornerstone of cancer treatment. Typically based on photon irradiation, innovative modalities using protons (protontherapy) or heavier ions allows for better dose conformity in the tumor and therefore better treatment.

L-α-alanine dosimeters have been proposed as a secondary reference for clinical protontherapy due to its linear dose response, tissue-equivalence and dose rate independence. Those being especially useful in innovative ultra-high dose rate (UHDR) radiotherapy. However, these dosimeters are impacted by the exponential increase of linear energy transfer (LET) in the peak of dose deposit (Bragg peak), influencing dose estimations. Previous studies showed that alanine underestimates the dose in the Bragg Peak due to radical recombination [1]. While corrections models of LET effects have been proposed, they are yet to be tested in clinical conditions, and their precision is still to be estimated.

In this work, we explore the use of alanine as control dosimeter in the Bragg Peak region using electronic paramagnetic resonance (EPR) spectroscopy. We characterized and modeled the response of alanine as a function of dose, LET and energy. Clinical proton irradiation of alanine with 76/100/180 MeV initial energies were performed. EPR was used to develop an accurate and reproducible dosimetry protocol with uncertainty of less than 1% (k=1) for all measurement points.

Monte Carlo simulations were carried out to obtain LET and energy of protons in alanine and apply correction factors. New correction models using analytical microdosimetric calculations based on work from [2] were developed using literature data and points from our studies. Alanine showed an expected dose underestimation, reaching 30% in the Bragg peak. On the other hand previously published correction models showed doses overestimated by 5% and have noticeable difference in quality for different initial proton energy. Corrections obtained from microdosimetric models made it possible to reduce this error down to 2%, keeping similar correction quality for all initial energies.

To conclude, alanine dosimeters is a promising candidate for high energy UHDR proton beam dosimetry. It's signal nonetheless needs to be corrected in the Bragg Peak region. While semi-empirical models of LET and energy give reasonable results, their precision is far from being clinically applicable. Microdosimetric analytical models allow for more precise and robust dose estimations in the Bragg Peak, work is nonetheless still needed to increase its precision and physical meaningfulness.

Keywords: Alanine, LET, EPR, Bragg Peak, Microdosimetry, Monte Carlo

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Radiation Quality Effects on the EPR/Alanine signal: Spectrum Decomposition and the Radicals' Contribution

de Farias Soares, A.[1,2], D'Oca, M.C.[1,2,3], Ciocca, M.[4], Mantovani, L.[5], Di Liberto, R.[5], Mirandola, A.[4], Rossi, E.[4], Marrale, M.[1,2]*

[1]Department of Physics and Chemistry "Emilio Segrè", University of Palermo, Palermo, Italy.
[2]National Institute for Nuclear Physics (INFN), Catania Division, Catania, Italy.
[3]Advanced Technologies Network Centre, University of Palermo, Palermo, Italy.
[4]Medical Physics Unit, National Centre for Oncological Hadrontherapy, Pavia, Italy.
[5]Medical Physics Department, IRCCS Policlinico San Matteo, Pavia, Italy.
*Presenting Author: maurizio.marrale@unipa.it

Alanine dosimeters based on electron paramagnetic resonance (EPR) are widely used in radiotherapy due to their excellent dosimetric properties. Upon irradiation, three radicals are formed in the material, namely R1, R2 and R3 [1], whose concentrations are proportional to the absorbed dose. These radicals are known to be dependent on radiation quality, which induces changes in the EPR/alanine spectrum [2]. This property might be exploited, e.g., to obtain information on the linear energy transfer (LET). This study, therefore, aimed to investigate the effects of LET and microwave power on the individual radicals' contribution to the EPR/alanine. The dosimeters were irradiated with photons (6 MV X-rays), proton and carbon ion beams (pencil beam scanning), at the San Matteo Policlinico and CNAO centre, respectively (both in Pavia, Italy). For the latter two, the spread-out Bragg peak (SOBP) condition was used, with pellets inserted at depths related to the proximal, middle and distal positions. LET values covered from 0.5 keV/µm (for photons) up to 100 keV/µm (carbon ions). EPR spectra were acquired with an E580 Bruker spectrometer operating in the X-band. EasySpin MATLAB-based package with the *pepper* function was used to obtain the simulated spectra [3]. Literature-based g-values and hyperfine interaction constants were considered, in addition to broadening effects (Voigtian distribution). Those spectra were used to fit the experimental data. The goodness of the fitting was evaluated in terms of percentage root-mean-square-deviation (%RMSD). Results showed that at low-LET and low-microwave-power conditions, R1 and R2 are the main radicals contributing to the EPR/alanine signal, whilst R3 becomes predominant at either high-LET or high-microwave-power conditions (i.e., for a 100 keV/µm LET, its relative weight is more than double compared to the photon-irradiated case). Microwave saturation and inhomogeneous broadening analyses suggested that radiation quality affects not only the relative amounts of each radical but also the surrounding environment. In addition, R3 is more saturation-resistant. These findings confirm that the EPR response of alanine is sensitive to radiation quality and may be exploited for LET-related dosimetric applications.

Keywords: alanine radicals; dosimetry; EPR decomposition; LET effects; radiation quality.

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Traceable Alanine EPR Dosimetry for Low- and High-dose Applications

Woodall, N.*, Cox G., Crossley D.C., Sharpe P.H.G.

National Physical Laboratory, Hampton Road, Teddington, Middlesex, TW11 0LW
*Presenting Author: nathaniel.woodall@npl.co.uk

Accurate and traceable dose measurements are essential for applications spanning radiation processing and healthcare dosimetry. Since 1991, the National Physical Laboratory (NPL) has operated a mail-order alanine reference dosimetry service, with alanine dosimeters distributed worldwide and returned to NPL for measurement using electron paramagnetic resonance (EPR) spectroscopy. Initially, the service covered the dose range from 70 Gy to 70 kGy and primarily served the radiation processing industry, supporting the sterilisation of medical devices, and the irradiation of food and pharmaceuticals. Advances in EPR measurement techniques and signal analysis at NPL in the late 1990s led to significant reductions in measurement uncertainty, enabling the functional dose range to be extended down to 5 Gy [1]. This extension supports applications in radiotherapy, where accurate measurement at clinically relevant dose levels is required. In addition to its wide dose range, the service includes the measurement of alanine dosimeters irradiated in electron beams and mega-voltage X-ray beams, using beam-quality conversion factors of 1.014 and 1.006, respectively [2,3]. Each batch of alanine pellets used in the service is manufactured by Harwell Dosimeters Limited, using a formulation developed at NPL, and is calibrated by irradiating pellets in a cobalt-60 gamma-ray field. The dose rate at the point of irradiation is expressed in terms of absorbed dose to water and is directly traceable to the NPL primary standard graphite calorimeter. Customer-irradiated dosimeters are measured alongside monthly check dosimeters irradiated in the calibrated cobalt-60 field over the relevant dose range. These check dosimeters are used to normalise the EPR system calibration for each measurement run to ensure traceability to NPL standards. Ongoing developments, include automation of the EPR readout using robotic handling, aiming to enhance output, reproducibility and scalability of the service for routine and clinical audit applications.

Keywords Electron paramagnetic resonance (EPR) spectroscopy; Alanine dosimetry; Reference dosimetry; Signal analysis

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Dose Heterogeneity Assessment in ^{137}Cs-Irradiator using Alanine/ESR and Glass Dosimetry

Silva, K.M.A.[1]*, Travagin, P.C.[1], Kinoshita, A.[2], Viccari, C.[1], Nicolucci, P.[1], Baffa, O.[1]

[1]Department of Physics, Faculty of Philosophy, Sciences and Letters at Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil
[2]University of Western São Paulo – Unoeste, Presidente Prudente, Brazil
*Presenting Author: kyssyllamonny@usp.br

Self-shielded irradiators with ^{137}Cs sources are widely used for *in vitro* and *in vivo* radiobiological studies and irradiation of biological samples [1]. In recent years, regulatory requirements in Brazil lead to the decommissioning or relocation of some of these irradiators. In that process, one such irradiator (CIS Industries, model IBL-637 with two radioactive ^{137}Cs sources) was relocated from the Biosciences Institute to the Physics Department at University of São Paulo. The lack of information on the dose rate and dose distribution inside the irradiation chamber demanded the use of suitable dosimeters to study and characterize the doses in the irradiation chamber.

Alanine/ESR dosimetry was employed to obtain the reference dose rate of the irradiator, and the dose map in two planes at different distances from the sources position. Glass samples were then calibrated using the reference dose rate obtained with alanine to also investigate the dose map in a longitudinal plane extending from the bottom (closes to the sources) to the upper position (farther from the source) in the irradiation chamber.
The alanine pellets showed a 1.524 Gy/min dose rate in the centre of a plane at z = 17 cm (bottom plane of the cubic irradiation chamber), with a hot spot near the chamber door, as shown in Figure 1. The glass slabs employed have shown a 41% dose difference from the bottom of the chamber up to z = 20 cm.

The results show that alanine/ESR dosimetry was adequate for the high dose rate provided by the irradiator. Also, the dose maps obtained with alanine and glass allowed to assess the doe heterogeneity in the irradiation chamber, helping the planning of irradiation of different samples in the ^{137}Cs irradiator.

Keywords Dosimetry; alanine/ESR; glass; ^{137}Cs irradiator.

Acknowledgements INCT PICS funded by CNPq (408417/2024-2), Capes (88887.197686/2025-00 and 001), and FAPESP (grant no. 2025/26818-7).

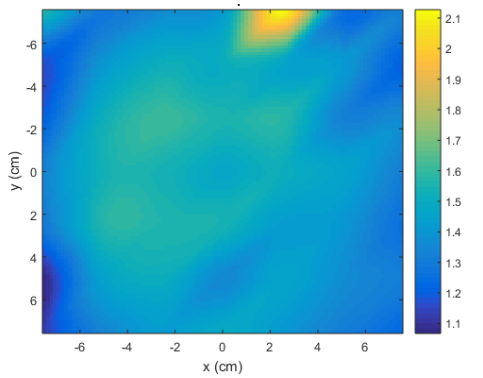

Figure 1. Normalized dose map at the z = 17 cm plane of the irradiator chamber.

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Multi-Year Performance of Canadian Biodosimetry Interlaboratory Comparisons

Wilkins, R.C.[1]*, Wu, K[2], Courcelles, X.[1], Beaton-Green, L.A.[1]

[1]Health Canada, 775 Brookfield Road, Ottawa, Canada
[2]Department of Physics, Carleton University, Ottawa, Canada
*Presenting Author: ruth.wilkins@hc-sc.gc.ca

The effective use of biodosimetry for large-scale emergency response depends on a robust network of laboratories capable of providing rapid dose estimates to support medical decision-making following radiation exposure. To function effectively as a network, participating laboratories must be validated to ensure comparability of dose estimates for similarly exposed samples. This consistency and comparability are best achieved through regular inter-laboratory comparisons (ILCs).

Health Canada, as the lead laboratory of the Canadian Biodosimetry Network, has a robust program of coordinated ILCs first established in 2007[1]. The program has since evolved to a standardized protocol, in use since 2011. Using this protocol, nine ILCs having been completed, each involving the distribution of ten blinded blood samples or microscope slides to between five and ten participating laboratories. Dose estimation was primarily conducted using the dicentric chromosome assay, supplemented with other assays available at individual laboratories. While initially focused on Canadian laboratories, the program has progressively expanded to include international partners from the United States, Taiwan, the Republic of Korea, Singapore, and Argentina.

The resulting multi-year dataset has enabled a detailed assessment of laboratory performance and provided the basis for refining acceptance criteria. In particular, a modified Z-score approach, based on established robust methods [2], was developed, incorporating predefined standard deviation values derived from historical data. This modification addresses the limitations of Z-scores at low dose levels, especially when analyses are based on only 50 cells.

In this study, data from all completed ILCs were reanalyzed using the modified Z-score methodology. Laboratory performance trends were assessed over multiple years to examine consistency, identify factors influencing performance, and evaluate the evolution of the ILC program itself. These findings provide valuable insights into the interpretation of ILC results and highlight areas for continued improvement, while confirming the readiness of participating laboratories to contribute effectively to rapid biodosimetry response during a mass casualty radiological event.

Keywords: Emergency response; Biodosimetry; interlaboratory comparisons;

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Capacity and Capability of the RENEB Network for Large Scale Radiological and Nuclear Emergencies: Survey Results 2025

Moquet, J.[1], Barnard, S.[1]*, Barquinero, J-F.[2], Bucher, M.[3], Kulka, U.[3], Martinez, J.S.[4], Port, M.[5], Wojcik, A.[6], Oestreicher, U.[2]

[1]UK Health Security Agency, Radiation Chemicals Climate and Environmental Hazards Directorate, Chilton, Oxfordshire, UK.
[2]Unitat d'Antropologia Biològica, Departament de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, Bellaterra E-08193, Catalonia, Spain.
[3]Federal Office for Radiation Protection, Department of Effects and Risks of Ionising and Non-Ionising Radiation, Oberschleissheim, Germany.
[4]Authority for Nuclear Safety and Radiation Protection (ASNR), PSE-SANTE/SERAMED/LRAcc, Fontenay-aux-Roses, France
[5]Bundeswehr Medical Academy, Munich, Germany
[6]Center for Radiation Protection Research, Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden.
*Presenting Author: Stephen.barnard@ukhsa.gov.uk

Since 2017 the RENEB network (Running the European Network of Biological and Retrospective Physical Dosimetry) is a group of specialised laboratories that can provide mutual assistance in individual dose estimation by biological and retrospective physical dosimetry for large scale radiological or nuclear emergencies in Europe and beyond. To ensure the operability of RENEB, Working Group "Infrastructures", regularly produce a survey to update partners contact details, collect information on laboratories emergency capacity / capabilities and sample transport. An online survey was developed and distributed to RENEB members and completed in May 2025. Replies were received from scientists working in 29 institutes and provide a clear picture of the RENEB networks capacity and capability. The data collected gives reassurance that RENEB is ready to react to a large-scale radiological event, as well as giving key information about operational aspects which will be essential in an emergency.

Keywords Emergency-response, biodosimetry, RENEB, survey

Development and Educational Evaluation of e-CLIK, a Biodosimetry Training Program for the Dicentric Chromosome Assay

Yang, S.S.[1]*, Kwon, S.W.[1], Kim, D.H.[1], Lee, Y.[1], Lee, Y.H.[2], Kim H.J.[1], Cho, M.[1]

[1]Laboratory of Biological Dosimetry, National Radiation Emergency Medical Center, Korea Institute of Radiological and Medical Sciences, Seoul, Republic of Korea
[2]Department of Biomedical Laboratory Science, College of Medical Sciences, Soonchunhyang University, Republic of Korea
*Presenting Author: ssussan725@kirams.re.kr

The dicentric chromosome assay (DCA) is widely regarded as a standard method for biological dosimetry [1]. However, since the DCA process depends on manual slide preparation and microscopic scoring, it can become a significant operational bottleneck during radiological mass-casualty incidents [2]. To address this limitation and help expand the surge capacity of dicentric scorers, we developed e-CLIK (Electronic Chromosome Learning IKON), a web-based training program for novice learners. This study evaluated the educational effectiveness of e-CLIK using a single-group pre- and post-test design among 44 undergraduate students with limited prior experience in cytogenetics and biodosimetry.

The e-CLIK program integrated theoretical instruction, visual review of standard operating procedures (SOPs), and digital scoring practice with high-resolution chromosome images. Practical competency was also assessed through laboratory performance and slide preparation quality to determine whether trainees could produce readable metaphase slides suitable for interpretation.

After training, the mean theoretical knowledge score rose from 45.7% to 75.4%. In the practical competency assessment, 39 of 44 participants (88.6%) produced readable slides that met the qualification criteria. Among these qualified participants, the mean scoring accuracy improved from 42.8 to 69.9, while the mean scoring time decreased from 16.2 to 11.8 minutes. In a more detailed analysis of achievement, 61.5% of qualified trainees improved in both scoring accuracy and speed, without a decline in either measure. Participant feedback was generally positive about the educational content, particularly the image-based scoring exercises and SOP review materials, though technical issues with system access and stability were also noted.

In conclusion, the findings demonstrate that e-CLIK serves as a robust digital framework for educating novices on the fundamental and practical aspects of the DCA. Subsequent technical enhancements to this platform will increase the capacity to deploy trained personnel during radiation emergencies.

Keywords Biodosimetry dosimetry; Dicentric chromosome assay; e-CLIK; Emergency preparedness; Surge capacity

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Mixed-Field (Fast Neutrons and Gamma Rays) Cytogenetic Biodosimetry Inter-Laboratory Comparison Exercise

Blakely, W.F.[1]*, Ashworth-Doon, L.[2], Balajee, A.[3], Barnard, S.[2], Beaton-Green, L.A.[4], Beinke, C.[5], Bolduc, D.L.[1], Brener, M.[1], Bucher, M.[6], Capaccio, C.[7], Chmil, V.[8], Cleveland, K.[1], Damer, K.[7], De Sanctis, S.[9], Eder, S.F.[5], Endesfelder, D.[6], Escalona, M.B.[3], Franchini, V.[9], Garty, G.10, Gregoire, E.[11], Kannan, N.[12], Martinez, J.S.[11], Milanova, M.[8], Miura, T.[13], Moquet, J.[2], Oestreicher, U.[6], Regalbuto, E.[9], Riley, K.[1], Royba, E.[10], Ryan, T.L.[3], Tichy, A.[8], Wilkins, R.C.[4]

[1]Multiple-Parameter Biodosimetry Laboratory, Armed Forces Radiobiology Research Institute, Uniformed Services of the Health Sciences, 4555 South Palmer Road, Bethesda, MD, USA 20889; [2]UK Health Security Agency; Radiation, Chemical, Climate and Environmental Hazards Directorate, Harwell Campus, Chilton, Didcot OX11 0RQ, United Kingdom; [3]Cytogenetic Biodosimetry Laboratory, Radiation Emergency Assistance Center/Training Site, Oak Ridge Institute for Science and Education, Oak Ridge Associated Universities, Oak Ridge, Tennessee, USA; [4]Consumer and Clinical Radiation Protection Bureau, Health Canada, Ottawa, Canada; [5]Bundeswehr Institute of Radiobiology affiliated to the University of Ulm, Neuherbergstrasse 11, 80937 Munich, Germany; [6]Bundesamt für Strahlenschutz, BfS, Oberschleissheim, Germany; [7]ASELL, LLC, 11515 Cronridge Drive, Suite Q, Owings Mills, MD 21117; [8]Cytogenetic Biodosimetry Laboratory, Department of Radiobiology, Faculty of Military Health Sciences, University of Defence, Hradec Králové, Czechia; [9]Army Medical and Veterinary Research Center, Defense Institute for Biomedical Sciences, Rome, Italy; [10]Center for Radiological Research, Columbia University, New York, NY, 10032, USA; [11]Autorité de Sûreté Nucléaire et de Radioprotection (ASNR), PSE-Sante/SERAMED/LRAcc, B.P.17 – 92262 Fontenay-aux-Roses, France; [12]Department of Human Genetics, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai, 600 116 Tamil Nadu, India; [13]Department of Risk Analysis and Biodosimetry Institute of Radiation Emergency Medicine, Hirosaki University, Hirosaki, Japan
*Presenting Author: william.blakely@usuhs.edu

The Cytogenetic Biodosimetry Laboratory of the Armed Forces Radiobiology Research Institute (AFRRI) coordinated a cytogenetic biodosimetry inter-laboratory exercise. Twelve laboratories participated in this exercise using AFRRI's TRIGA Reactor Source to evaluate mixed-field (fast neutrons and gamma rays) exposures. Two radiation fields were used, characterized by neutron fluence and lineal energy spectra representing approximately 94% and 30% neutrons. Ten samples with known doses were provided to create dose-response calibration curves, along with five blind-dose samples. Participating laboratories performed the dicentric chromosome aberration, cytokinesis-blocked micronuclei, and/or premature chromosome condensation assays. Results from this comparison will be reported. These efforts enhance the capacity of laboratories to provide radiation dose assessments for mixed-field exposures. [The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy or position of the Department of War, AFRRI, Uniformed Services University of the Health Sciences, or the U.S. Government. Funding support was provided by AFRRI (AFR-B2-10664 and RAB32165)].

Keywords Exercise, mixed field (fission neutrons/gamma rays), cytogenetic biodosimetry, inter-laboratory comparison, dose assessment, networking.

Evaluating Raman spectroscopy and Multivariate Analysis for Blood Plasma Biodosimetry

Kazi, J.[1]*, Ghasemi, M.[1], Vuong, N.[2], Inman, J.L.[3], Wilkins, R.C.[2], Chauhan, V.[2], Dang, S.[4], Murugkar, S.[1]

[1]Department of Physics, Carleton University, Ottawa, Canada
[2]Consumer and Clinical Radiation Protection Bureau, Health Canada
[3]Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, USA
[4]School of Mathematics and Statistics, Carleton University, Ottawa, Canada
*Presenting Author: joshkazi@cunet.carleton.ca

Biodosimetry is essential for triage and medical management following accidental radiation exposure, but current approaches are often labor-intensive and time-consuming for large-scale emergency responses. Raman spectroscopy (RS) is a rapid, label-free technique that provides a molecular fingerprint based on vibrational modes of molecules, enabling concurrent identification of chemical changes of protein, lipids, nucleic acids, and metabolites in cells or tissues. This approach has shown promise for discriminating irradiated samples using blood-based biopsies. Here, we evaluated the potential of RS with multivariate analysis to detect ionizing radiation-induced changes in blood plasma across two complementary models: *in vitro* irradiated human plasma and *in vivo* irradiated mouse plasma. For the mouse study, blood plasma was collected at the Day-6 timepoint from 27 male and female BALB/cJ and C57BL/6 mice exposed to 0 (control), 1, 2, or 4 Gy whole-body X-rays (196 mGy/min). For the human study, blood collected from 25 healthy donors was exposed *in vitro* to multiple X-ray doses (1 Gy/min), with the present comparison focused on the 0, 2, and 4 Gy subset at Day-1 post-irradiation. Raman spectra were acquired from thawed plasma using a 785 nm laser. After applying standard preprocessing techniques, Raman data was analyzed using partial least squares-discriminant analysis (PLS-DA) and linear mixed-effects covariate adjustment.

The control Raman spectra of both mouse and human plasma (Figure 1. (a)) are consistent with previous reports[1]. Across both plasma studies, RS detected reproducible radiation-associated spectral changes. In the mouse data, covariate adjustment (sex as a fixed effect, strain as a mixed effect) enhanced dose-group separation, with biomarkers corresponding to protein- and lipid-associated contributions in the Raman spectra, including phenylalanine, amide III, CH_2/CH_3 deformation, and amide I bands. Preliminary results from the human plasma data (Figure 1. (b)) underscore the importance of standardization of sample preparation and measurement protocols to reduce inter-donor variability. Overall, our results support the potential of blood plasma RS, combined with multivariate analysis, as a promising approach toward rapid biodosimetry.

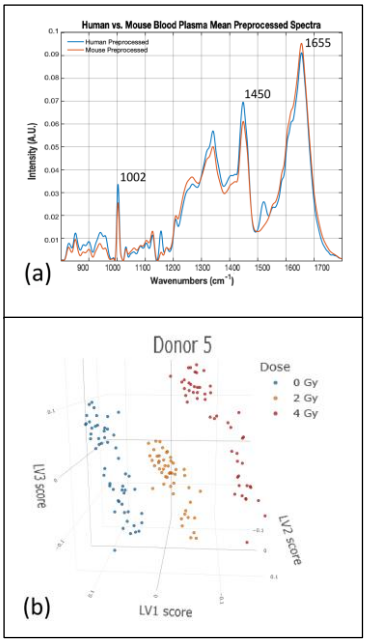

Figure 3. (a) Mean Raman spectra of human and mouse plasma (b) PLS-DA scores plot for a representative human donor

Keywords: Raman spectroscopy; biodosimetry; blood plasma

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Robustness in Emergencies: Do Culture Types, Scorer Experience, and Reagent Substitutions Affect Dose Estimation by Dicentric Chromosome Assay?

Teo, S.X.[1]*, Yeo, J.J.W.[1], Chew, Z.H.[1], Tay, L.L.T.[1], Tan, H.W. [1], Khng, Y.C.[1], Goh, V.S.T.[1], Beaton-Green, L.A.[2], Wilkins, R.C.[2], Chua, C.E.L.[1]

[1]Department of Radiobiology, Singapore Nuclear Research and Safety Institute (SNRSI), Singapore;
[2]Consumer and Clinical Radiation Protection Bureau, Health Canada, Ottawa, Canada
*Presenting Author: snrsxt@nus.edu.sg

Biodosimetry techniques such as dicentric chromosome assay (DCA) are often used for dose assessment during a nuclear or radiological incident. While each laboratory may have their own optimized protocols and dose-response curves (DRCs), an emergency scenario requiring quick response may challenge laboratories' ability to fully adhere to these procedures. Modifications of variables in the protocols may be needed hence laboratories should consider different options to ensure that accuracy and turnaround time are not compromised.

We studied several variables such as DRCs constructed with different cell culture types, dicentric scoring experience and reagent brands. Laboratories typically standardise the DCA with a specific culture protocol (whole blood (WB) or peripheral blood mononuclear cells (PBMCs)), that is aligned with their established DRC. However, when assisting other laboratories through fixed sample or slide sharing, the culture protocol does not always match that of the receiving laboratory. While dicentric frequencies between WB and PBMC cultures are theoretically similar [1], this interchangeability should not be assumed and requires validation for reliable dose estimation. Secondly, there may be a shortage of experienced scorers during an emergency. Therefore, less experienced scorers may be recruited, provided that accuracy is ensured. Lastly, logistical challenges may arise during an emergency, leading to supply disruption of original reagents which cannot always be stockpiled. This would affect the speed of response. Hence, the suitability of reagents from different brands should also be evaluated.

The abovementioned variables were investigated with blood samples of blinded doses, received as part of an inter-laboratory comparison (ILC) exercise conducted in 2025 by Health Canada. Samples were processed as WB DCA cultures. Two cultures for each sample were prepared, with each culture treated with phytohemagglutinin (PHA) and colcemid from a different brand. Five experienced scorers (>100 hours of training and with prior ILC experience) and two inexperienced scorers (<100 hours of training and no prior ILC experience) performed triage scoring. Doses were estimated with DRCs constructed from dicentric frequencies obtained from WB and PBMC cultures. Analysis of estimated doses and scoring times were performed and compared across all variables. Overall, no significant differences were detected.

Keywords Dicentric chromosome assay; triage; dose-response curve; dose estimates

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Multiple Scorers Not Essential for Triage Dose Estimations with Manual Dicentric Scoring

Goh, V.S.T.[1]*, Teo, S.X.[1], Chew, Z.H.[1], Yeo, J.J.W.[1], Tay, L.L.T.[1], Wilkins, R.C.[2], Beaton-Green, L.A.[2], Chua, C.E.L.[1]

[1]Department of Radiobiology, Singapore Nuclear Research and Safety Institute (SNRSI), Singapore;
[2]Consumer and Clinical Radiation Protection Bureau, Health Canada, Ottawa, Canada
*Presenting Author: valeriegoh@nus.edu.sg

Background and purpose: As recommended by ISO 21243:2022, triage dose estimation with the dicentric chromosome assay (DCA) can be performed in the event of a mass casualty radiation emergency. Due to the high radiation specificity of dicentric formation, a reduced number of cells and/or dicentrics can be manually scored (50 cells or 30 dicentrics), but the number of scorers was not specified. This study aims to investigate if validity and accuracy in estimated doses could be addressed with multiple scorers, especially when identifying the triage threshold dose of 2 Gy or doses above 2 Gy.

Methods: 10 blinded dose samples (0, 0.3, 0.7, 1.0, 1.2, 1.5, 1.8, 2.3, 2.7 and 3.5 Gy) were manually scored by six scorers (one beginner, five expert) in SNRSI as part of the 2023 Health Canada inter-laboratory comparison exercise (INTC09). Heparinized blood was irradiated with X-rays (250 kVp, 15 mA, 0.37 Gy/min) in Canada and shipped to Singapore. Both Quickscan and triage scoring were evaluated while scoring time was recorded. Metaphase images were obtained with Metafer 4. 500 raw images was used for Quickscan, while another 150 pre-selected images were used for triage scoring. Scoring was performed with SNRSI's in-house software, DotTheQuickScan and DotDotDicentric, respectively. Combinations of multiple scorers were obtained without repetition (Table 1). For single scorers, estimated doses were directly compared. For multiple scorers, averaged doses were obtained and compared.

Table 1: Combinations of six scorers (A to F) without repetition.

1 scorer (6 combinations)	2 scorers (15 combinations)			3 scorers (20 combinations)				4 scorers (15 combinations)			5 scorers (6 combinations)	6 scorers (1 combination)
A	A-B	B-C	C-E	A-B-C	A-C-E	B-C-D	B-E-F	A-B-C-D	A-B-E-F	B-C-D-E	A-B-C-D-E	A-B-C-D-E-F
B	A-C	B-D	C-F	A-B-D	A-C-F	B-C-E	C-D-E	A-B-C-E	A-C-D-E	B-C-D-F	A-B-C-D-F	
C	A-D	B-E	D-E	A-B-E	A-D-E	B-C-F	C-D-F	A-B-C-F	A-C-D-F	B-C-E-F	A-B-C-E-F	
D	A-E	B-F	D-F	A-B-F	A-D-F	B-D-E	C-E-F	A-B-D-E	A-C-E-F	B-D-E-F	A-B-D-E-F	
E	A-F	C-D	E-F	A-C-D	A-E-F	B-D-F	D-E-F	A-B-D-F	A-D-E-F	C-D-E-F	A-C-D-E-F	
F											B-C-D-E-F	

Results and conclusions: Among expert scorers, Quickscan was completed in 2.5–6 min for no/low (0–1 Gy), 3.5–8 min for intermediate (>1–2 Gy) and 2–10 min for high (>2 Gy) doses. On the other hand, triage scoring was completed in 6–13 min for no/low, 8–21 min for intermediate and 11–23 min for high doses. Though Quickscan was expectedly faster than triage scoring, several scorers estimated 0.5–1 Gy for the 0 Gy sample. Furthermore, the estimated dose error of ± 0.5 Gy did not greatly improve with more scorers. In conclusion, single scorers are sufficient for triage dose estimation. It may be more worthwhile to improve scoring efficiency (i.e. score more cells per sample within the expected time frame) instead of increasing the number of scorers for enhanced dose estimation accuracy.

Keywords Triage; dicentric chromosome assay; dose estimation; multiple scorers

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Transition of EAST H-ARS Tools using In-house Non-code App Solutions

Kurada L., Ph.D.[1]*, Blakely W.F., Ph.D.[2], Bolduc D.L., Ph.D.[2]

[1]Henry M. Jackson Foundation, Rockville, MD; [2]Armed Forces Radiobiology Research Institute, Uniformed Services University of the Health Sciences, Bethesda, MD.
*Presenting Author: lalitha.kurada.ctr@usuhs.edu

The Armed Forces Radiobiology Research Institute (AFRRI) Radiation Biodosimetry Research Group aims to develop biodosimetry tools that support military operations in radiation-risk environments. Exposure and Symptom Triage (EAST) tools are designed to aid first responders in the triage of patients involved in mass-casualty radiological incidents. The use of these tools is recommended for use after primary trauma triage and life-saving treatments have been addressed to assist in the decision-making process for administering FDA-approved drugs for hematopoietic acute radiation syndrome (H-ARS). These treatments must be administered early (24-48 hours) following exposure. EAST tools utilize a multi-parameter biological and dosimetry-based approach. This includes physical dosimetry, dose estimates based on location, clinical signs and symptoms, lymphocyte counts (available via point-of-care devices), and selective proteomic biomarkers (such as flt-3 ligand) that serve as early-response bioindicators of ARS severity. Ongoing efforts are underway to transition these worksheets into Progressive Web Apps (PWAs) accessible for military use. To implement the EAST H-ARS tool, we explored an in-house, no-code development approach using Drupal 11. This open-source platform was selected for its security compliance, modular design, and ability to generate PWA compatible with both web and mobile devices.

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Keywords: biodosimetry, EAST tools, H-ARS, bioindicators, Drupal.

Application of Electron Spin Resonance (ESR) Dating to a Fossil Tooth from Chuí Creek, Southern Brazil

Baffa, O.[1]*, Lopes, R. P.[2], Menezes, M. A. B. C.[3], Kinoshita, A.[4]

[1]Department of Physics, São Paulo University, USP,14040-901 Ribeirão Preto, SP, Brazil.
[2]Department of Geography, Federal University of Pelotas, UFPEL,96010-900, Pelotas, RS, Brazil
[3]Laboratory for Neutron Activation Analysis, Division for Analysis and Environment, Nuclear Technology Development Center, Brazilian Commission for Nuclear Energy (CDTN/CNEN), 31270-901, Belo Horizonte, MG, Brazil
[4]Pro-Rectory of Research and Graduate Studies, University of Western São Paulo, UNOESTE, 19067-175, Presidente Prudente, SP, Brazil
*Presenting Author: baffa@usp.br

ESR dating was applied in this work to date a fossil tooth found in Chuí Creek, Rio Grande do Sul State, Southern Brazil. The fossiliferous deposits in this coastal plain have been known since the late XIX century; however, a better understanding of its biostratigraphic and chronostratigraphic context is of great relevance in order to elucidate its evolution and the impact of changes in sea level [1].The fossil tooth was collected in -33.584576°S and -53.338620°W, approximately 2m deep and at an approximate altitude of 12 m. The experiment consisted of preparing the material by cleaning it in running water and then treating it with 1:10 HCl in an ultrasonic bath for 2 min to extract a thin layer. In this way, the enamel thickness was reduced by 200 μm in order to exclude the effects of alpha particle irradiation. Next, the enamel was manually crushed into ~75 μm particles and divided into aliquots of approximately 50 mg, which were irradiated with Gamma radiation from a ^{137}Cs source, with doses of 50 to 2 kGy. The spectra of these aliquots were recorded, and the amplitude of the typical CO_2^- radical signal was used to construct the dose-response curve (DRC). The points were adjusted using Ikeya's equation, Single Saturating Exponential Curve to obtain D_e, the equivalent dose. The conversion of D_e to age was performed using the ROSY software. For this purpose, the concentrations of U, Th, and K in enamel, dentin, and sediment were determined through neutron activation analysis, k0 method. After converting D_e to age, the age results obtained were 28.8 ka (EU, Early Uptake), 44.9 ka (LU Linear Uptake) and 39.6 ka (CU Combination). The result of the CU model is usually adopted, considering the absorption of U by the Linear model in enamel and Early in dentin, considering the differences in porosity and hardness of the materials. This result is consistent with dating of sediments obtained in these deposits by OSL [2] and other fossil teeth [1] collected nearby. The application of ESR dating to the fossil tooth from Chuí Creek has provided reliable chronological data, reinforcing the method's effectiveness for establishing the age of Quaternary fossil remains in complex depositional environments.

Keywords: Chuí Creek; fossil tooth; ESR dating.
Financial support: Grants FAPESP 2025/26818-7 & 2013/07699-0, CNPq 408417/2024-2

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Mitigating Effect of Piperazine Derivatives after Gamma Irradiation

Chmil, V.[1]*, Živná, N.[2,3], Milanová, M.[1], Filipová, A.[1], Pejchal, J.[2], Muthná, D.[4], Havelek, R.[4], Řezáčová, M.[4], Marek, J.[3,5], Tichý, A.[1,3]

[1]Department of Radiobiology, Military Faculty of Medicine, University of Defence in Brno, Trebesska 1575, 500 05 Hradec Kralove, Czech Republic
[2]Department of Toxicology and Military Pharmacy, Military Faculty of Medicine, University of Defence in Brno, Trebesska 1575, 500 05 Hradec Kralove, Czech Republic
[3]Biomedical Research Centre, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic
[4]Department of Medical Biochemistry, Faculty of Medicine in Hradec Kralove, Charles University, Simkova 870, 500 03 Hradec Kralove, Czech Republic
[5]Department of Epidemiology, Military Faculty of Medicine, University of Defence in Brno, Trebesska 1575, 500 05 Hradec Kralove, Czech Republic
*Presenting Author: vojtech.chmil@unob.cz

Ionizing radiation induces severe biological damage, including DNA double-strand breaks, chromosomal aberrations, and increased cancer risk. While radioprotective agents are typically administered prior to exposure, the development of effective radiomitigators—compounds capable of reducing radiation damage after exposure—remains a critical challenge.

In this study, we investigated the mitigation potential of two selected 1-(2-hydroxyethyl)piperazine derivatives following gamma irradiation. Whole blood samples were exposed to ionizing radiation, and the compounds were subsequently administered at 1, 2, and 4 hours post-irradiation. The extent of radiation-induced DNA damage was evaluated using the dicentric chromosome assay.

Both compounds exhibited comparable mitigation effects. Administration at 1 hour post-irradiation led to a significant reduction in dicentric chromosome frequency, indicating effective attenuation of radiation-induced chromosomal damage. However, the mitigating effect was markedly reduced when the compounds were applied 2 hours after irradiation and was no longer observable at 4 hours post-exposure.

These findings demonstrate that the tested piperazine derivatives possess time-dependent radiomitigating activity, with the highest efficacy observed shortly after irradiation. This study highlights their potential as post-exposure therapeutic agents, while also emphasizing the importance of early intervention for effective mitigation of radiation-induced damage.

Keywords Piperazine derivatives; Radiomitigation; Gamma irradiation; Dicentric chromosome assay; DNA damage

Evaluation of Interleukin Supplementation for Optimizing Dicentric Chromosome Assay Performance in Cryopreserved Whole Blood

Milanová, M.[1]*, Chmil, V.[1], Gachová, A.[1], Tichy, A.[1], Smetanová, A.[2]

[1]Department of Radiobiology, Military Faculty of Medicine, University of Defence, Hradec Králové, Czech Republic
[2]Department of Biology and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic
*Presenting Author: marcela.milanova@unob.cz

The dicentric chromosome assay (DCA) is widely recognized as the gold standard for biological dosimetry due to its high specificity and dose-dependent response to ionizing radiation [1]. Despite its robustness, the method remains limited by the availability of sufficient numbers of high-quality metaphases and its limited throughput, particularly in large-scale radiation emergency scenarios [2]. These limitations are further exacerbated when cryopreserved blood samples are used, as cryopreservation may negatively affect lymphocyte viability and proliferative capacity.

This study aims to evaluate the impact of interleukin (IL) supplementation on lymphocyte culture performance in cryopreserved whole blood. Peripheral blood samples from healthy donors were cryopreserved using standard protocols, thawed under controlled conditions, and cultured with phytohemagglutinin stimulation. Cultures were supplemented with IL-2, IL-7, and IL-15 [3].

The effect of IL supplementation is assessed based on metaphase yield, number of scorable metaphases, and proportion of analyzable spreads as indicators of culture performance, minimizing bias from colcemid-induced accumulation. In parallel, the potential impact of IL on biodosimetric response is assessed by analyzing dicentric chromosome frequency.

The results of this study will provide insight into whether cytokine supplementation can improve the robustness and efficiency of DCA in cryopreserved samples, or whether it introduces variability that may affect downstream biodosimetric interpretation. The findings are expected to contribute to the optimization and harmonization of biodosimetry workflows, particularly in the context of high-throughput analysis required for radiation emergency response.

Keywords Biodosimetry; Dicentrics; Cryopreserved blood; Metaphase yield; Interleukins

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Impact of Low-Dose Ionizing Radiation on Lens Protein Expression and Cataractogenesis in Chornobyl Voles

Grzegorczyk, P.[1]*, Seymour, C.[1], Williams, D.[2], Wood, M.[3], Garshchak, S.[4], Barnett, C.[5], Mothersill, C.[1], Taiyab, A.[1]

[1]McMaster University, Hamilton Ontario, Canada, [2]University of Cambridge, UK, [3]University of Salford, UK, [4]Chornobyl Center for Nuclear Safety, Ukraine, [5]UK Centre for Ecology & Hydrology.
*Presenting Author: grzegorp@mcmaster.ca

Radiation-induced cataracts are a well-recognized outcome of exposure to ionizing radiation (IR), and have historically been assumed to require a minimum dose to occur. This dose threshold has decreased over time and was most recently lowered to 0.5 Gray by the International Commission on Radiological Protection (ICRP) in 2011 [1]. However, there are significant gaps in our understanding of how chronic low doses of IR contribute to cataract formation, and both the magnitude and existence of a dose threshold remain uncertain [2]. The objective of this study is to explore the minimum dose required to induce detectable changes in the lenses of voles from the Chornobyl Exclusion Zone (CEZ), which is the 30-kilometer area surrounding the site of the 1986 reactor accident. We hypothesize that low doses of ionizing radiation are sufficient to induce early changes in key lens proteins that could potentially develop into cataracts.

Voles were collected from the CEZ in 2018 and grouped by total dose rate into control (<1 μGy/hr), low (3–13 μGy/hr), medium (20–23 μGy/hr), and high (300–400 μGy/hr) exposure categories. Eyes were fixed in formalin, sectioned, and stained with hematoxylin and eosin to analyze morphology. Immunohistochemistry was used to evaluate the expression of proteins involved in lens transparency, stress response, and homeostasis, particularly αA-crystallin, αB-crystallin, and Aquaporin 0 (AQP0). Preliminary analysis showed progressive alterations in lens morphology and protein expression consistent with early changes preceding cataract formation (n = 6 eyes per exposure group). Hematoxylin and eosin staining revealed nuclei of differentiating lens fibers extending past the bow region with increasing doses of radiation. αA- and αB-crystallin both showed dose-dependent changes within the equatorial region, with meridional rows shifting from a perpendicular orientation to more parallel and diffuse patterns at medium to high low-dose exposures. We also observed differences in the localization of AQP0, where higher doses showed increasingly disorganized patterns and loss of peripheral AQP0 compared to controls. Future work will involve analysis of mouse lenses exposed to similar doses to examine differences between environmental and experimental radiation effects.

Keywords Radiation exposure; Low dose; Lens; Cataract.

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Emerging Roles of Multiparametric Biodosimetry and Artificial Intelligence in Advancing Radiation Dose Assessment for Triage

Balajee, A.S.

Cytogenetic Biodosimetry Laboratory, Radiation Emergency Assistance Center/Training Site, Oak Ridge Institute for Science and Education, Oak Ridge Associated Universities, Oak Ridge, TN 37830, USA.
Adayabalam.balajee@orau.org

Biodosimetry plays a crucial role in radiation emergency preparedness/readiness response by enabling efficient allocation of medical resources through prioritization of individuals needing urgent care to improve survival outcomes. As currently used biodosimetry tools greatly differ in sensitivity and specificity, a single biodosimetry assay may not be sufficient for various exposure scenarios that differ in radiation quality (low or high LET, single or mixed beam), radiation dose, dose rates (low or high) and exposure modes (acute or chronic, whole, or partial body). Complex mass casualty incidents may require a tiered multiparametric biodosimetry (MPBD) approach to improve dose prediction accuracy and scalability of radiation dose measurements. A tiered MPBD approach can be developed through careful combination of clinical signs/symptoms with biological/physical dosimetry assays to achieve the first line of rapid screening (24-48 hrs. after the incident), exposure dose categorization for medical treatment (minimal exposure <1 Gy, moderate exposure 1-2 Gy, severe exposure 2-6 Gy and lethal exposure > 6 Gy) and definitive dose assessment for predicting/monitoring long-time health risks. A strategic tiered MPBD approach integrated with Artificial Intelligence (AI) based tools will be presented for mediating an effective medical triage in the aftermath of small and large-scale radiological/nuclear incidents.

Keywords: Multiparametric biodosimetry, artificial intelligence, biomarkers, tiered approach and triage

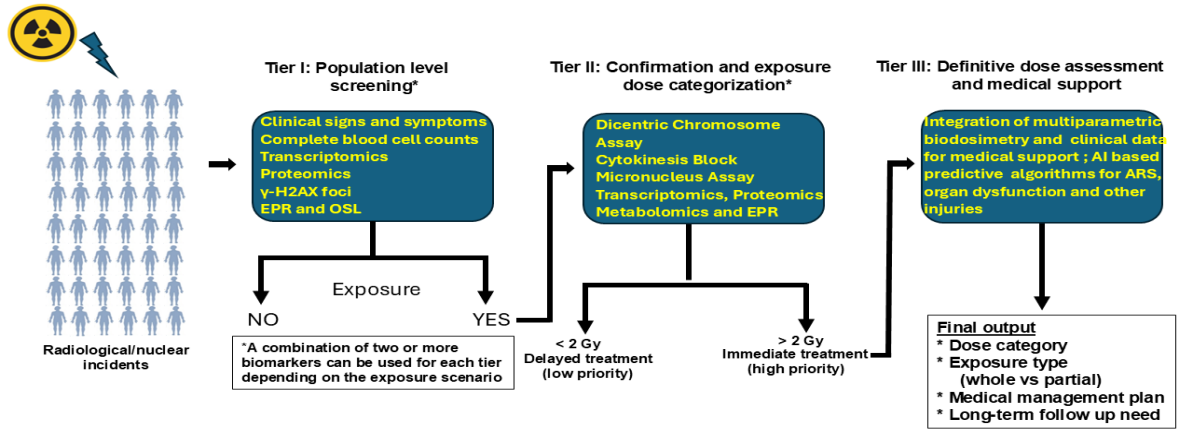

Fig.1. A schematic diagram for a tiered multiparametric approach with integration of AI tools for advancing dose estimation and triage.

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Application of Artificial Intelligence to Metaphase Finder and Automated Chromosome Aberration Finding (Second Report)

Furukawa, A.

Department of Accelerator and Medical Physics, Institute for Quantum Medical Science,
National Institutes for Quantum Science and Technology
furukawa.akira@qst.go.jp

Biological dosimetry is used to estimate individual absorbed radiation dose by quantifying an appropriate biological marker. The most popular gold-standard marker is the appearance of dicentric chromosomes in metaphase. The metaphase finder is a tool for automation of biological dosimetry that finds metaphase cells on glass slides. The author and a software company have designed a new system and are now preparing to produce the system commercially. This system was capable of not only normal chromosomes but also PCC cells. The metaphase finder consists of an automated microscope, an auto-focus system, an X-Y stage, a camera, and a computer. To enhance the accuracy of the system, an artificial intelligence (AI) with deep learning was tested. The pre-selection of metaphases using mathematical morphology before the AI process was enabled the AI classification of true metaphases or not. The author reported that the prototype of AI implemented metaphase finder was combined with the microscope system by file sharing and image transfer program, and that the metaphase finder system's accuracy was compared with previous non-AI system, using the same sample [1].

The next project is to make an automated chromosome aberration finding and counting system with AI. We have already made an automated chromosome aberration finding and counting system with conventional image analysis and a fuzzy logic [2]. For the first time, we tried to simply segment images of chromosomes from the metaphase cell image and apply them to the AI. We have checked whether this AI system for metaphase finder can detect the images including dicentric chromosomes.

This work was supported by JSPS KAKENHI Grant Number JP25K15446.

Keywords metaphase finder, biodosimetry, chromosome aberration

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Development of an Artificial Neural Network-based Dose Reconstruction and Conversion Model for Predicting Bone and Blood Doses in External Exposure Scenarios

Kim, H.*, Choi, Y., Kim, M.C.

Korea Atomic Energy Research Institute, Daejeon, Republic of Korea
*Presenting Author: kht84@kaeri.re.kr

Rapid and reliable dose assessment is essential for medical triage and retrospective dosimetry following radiological or nuclear emergencies. In particular, electron paramagnetic resonance (EPR) and biological dosimetry can provide valuable dose information using nail, bone, tooth, and blood samples. However, in certain exposure scenarios, additional computational approaches are required to convert measured sample-based doses into organ-specific dose quantities relevant to risk assessment and medical decision-making [1,2]. Therefore, this study aimed to develop an artificial neural network (ANN)-based dose reconstruction and conversion model for predicting skeletal-region and blood doses in external photon exposure scenarios.

To generate the dataset, Monte Carlo simulations were performed using Geant4 with the ICRP adult male mesh-type reference computational phantom. Three radionuclides frequently involved in small-scale external exposure accidents, Ir-192, Cs-137, and Co-60, were considered as photon sources. Point sources were randomly positioned around the human phantom to represent a wide range of external exposure geometries. A total of 1.39 million simulation datasets were generated and divided into training, validation, and test sets at a ratio of 8:1:1. The target quantities included 22 skeletal and cartilage-related regions—upper and lower humeri, ulnae and radii, wrists and hand bones, upper and lower femora, tibiae, fibulae and patellae, ankles and foot bones, clavicles, pelvis, ribs, scapulae, sacrum, sternum, cervical spine, thoracic spine, lumbar spine, cranium, mandible, teeth, costal cartilage, and intervertebral disc cartilage—as well as blood dose. Input parameters describing source energy and source-to-phantom geometry were used to train ANN-based regression models, and the predicted doses were compared with Monte Carlo reference values.

The developed model enabled rapid estimation of skeletal-region and blood doses for arbitrary source positions without requiring case-by-case Monte Carlo simulations. Prediction performance varied depending on the anatomical region, with larger skeletal structures generally showing more stable dose estimates than smaller or highly shielded regions. These results suggest that ANN-based dose reconstruction can provide a flexible and computationally efficient method for converting EPR and biodosimetry-related dose information into organ-specific dose quantities in complex external exposure scenarios.

Keywords Dose reconstruction, ANN model, External exposure, Organ dose estimation

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MATHELDE: Machine Learning Approach for Tooth Enamel Dose Estimation

Romeo, M.[1,2], de Farias Soares, A.[1,2], D'Oca, M.C.[1,2,3], Corvaia E.[1], Cottone G.[1,2], Marrale, M.[1,2]*

[1]Department of Physics and Chemistry "Emilio Segrè", University of Palermo, Palermo, Italy.
[2]National Institute for Nuclear Physics (INFN), Catania Division, Catania, Italy.
[3]Advanced Technologies Network Centre, University of Palermo, Palermo, Italy.
*Presenting Author: maurizio.marrale@unipa.it

Retrospective electron paramagnetic resonance (EPR) dosimetry of tooth enamel is a well-established method for individual dose reconstruction after radiation exposure, but its performance depends strongly on acquisition and processing parameters [1,2]. Conventional protocols may require several hours per sample, which constrains throughput in emergency settings.

In large-scale radiological or nuclear emergencies, rapid retrospective dosimetry is critical for triage, particularly for identifying individuals who may have received doses at or above 2 Gy [2,3]. To cope this need, the approach here presented (named MATHELDE) proposes an artificial intelligence–assisted strategy for tooth enamel dose estimation using accelerated EPR acquisition. A dedicated dataset is generated from a large number of tooth enamel samples irradiated at known doses in the range of 100 mGy to 3 Gy. Fast acquisition protocols, providing an approximately tenfold reduction in measurement time relative to standard procedures, are used to build the training dataset. A classification model is then developed to discriminate samples above and below the clinically relevant threshold of 2 Gy.

In a second phase, the model is externally validated on samples irradiated at unknown doses. Classification results obtained from fast-acquisition spectra are compared with those derived from standard acquisition protocols to evaluate accuracy and precision, and the effective time gain achieved by the proposed workflow is quantified. This study aims to demonstrate the feasibility of integrating machine learning with accelerated EPR acquisition to enable rapid and reliable triage in radiological emergency scenarios.

Keywords: EPR dosimetry; tooth enamel; artificial intelligence.

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Evaluation of Multi-Dosimeter Approaches for Posture- and Geometry-Dependent Dose Assessment

Kim, M.C.*, Kim, H., Kim, S., Choi, Y.

Korea Atomic Energy Research Institute, Daejeon, Republic of Korea
*Presenting Author: minchaekim@kaeri.re.kr

Inhomogeneous radiation fields, such as those encountered during steam generator maintenance in nuclear power plants, can produce large spatial dose gradients across the body. Under these conditions, a single personal dosimeter worn on the chest may not adequately represent whole-body exposure, particularly when workers adopt non-standard postures such as bending, kneeling, or squatting. Multi-dosimeter monitoring, using additional dosimeters on the back together with the chest dosimeter, has therefore been recommended for high-dose or highly non-uniform radiation work [1]. However, the applicability of existing multi-dosimeter algorithms to realistic combinations of working posture and exposure geometry has not been fully evaluated.

This study aims to evaluate existing multi-dosimeter approaches for estimating worker dose in posture- and geometry-dependent exposure scenarios. An adult male mesh-type computational phantom was used to represent realistic worker anatomy, and 19 representative working postures [2] were defined based on combinations of torso bending angle, leg configuration, and arm position. The considered exposure geometries included anterior-posterior, posterior-anterior, left-lateral, right-lateral, rotational, isotropic, bottom-to-top, and top-to-bottom irradiation conditions. Photon fields from Ir-192, Cs-137, and Co-60 were considered to account for energy-dependent effects. Personal dosimeters were modeled at three body locations: chest, head, and back. Organ absorbed doses, whole-body dose, and dosimeter responses were calculated using Monte Carlo simulations (Geant4 v10.7.3).

Existing multi-dosimeter algorithms, including weighting-based approaches previously applied in Korean nuclear power plants [3], were evaluated for each posture and exposure geometry. In parallel, a posture- and geometry-specific single-dosimeter dose conversion method using the chest dosimeter response [2] was compared.

Keywords Multi-dosimeter, Non-uniform radiation field, Working posture, Monte Carlo Method

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AI-Driven Triage Classification at the 1 Gy Threshold Using Fortuitous OSL Materials and Portable Dosimetry

Della Monaca, S.[1], Maltar-Strmečki, N.[2]*, Quattrini, M.C.[1], Bortolin, E.[1]

[1]Core Facilities, Istituto Superiore di Sanità, viale Regina Elena 299, Rome, Italy
[2]Ruđer Bošković Institute, Zagreb, Croatia
*Presenting Author: nstrm@irb.hr

In large-scale radiological emergencies, rapidly identifying of individuals potentially exposed above clinically relevant thresholds is a critical operational priority. International guidelines define 1 Gy as a key decision level for early medical triage [1], shifting the focus from accurate dose reconstruction to fast and reliable classification. Within the framework of the NATO Science for Peace and Security project BioPhyMeTRE [2], this work proposes a machine-learning–assisted approach for binary triage classification (<1 Gy vs ≥1 Gy) based on Optically Stimulated Luminescence (OSL) signals from commercial magnesium-based dietary supplements used as opportunistic dosimeters. A dataset of 355 samples, irradiated in the range 0–5 Gy, was analysed using five supervised algorithms: Logistic Regression, Support Vector Machine, Decision Tree, Random Forest, and XGBoost. The models were trained using stratified cross-validation and evaluated with class-specific metrics, with particular emphasis on recall for the ≥1 Gy class. Despite the intrinsic heterogeneity of the materials and the significant overlap of OSL signals around the triage threshold, all models achieved robust classification performance. Tree-based ensemble methods and Logistic Regression showed the best results, reaching accuracies up to 0.89 and recall values for the ≥1 Gy class up to 0.96, corresponding to low false-negative rates. These results demonstrate that machine-learning approaches can effectively handle the variability typical of opportunistic dosimeters and enable reliable threshold-based decision-making where simple signal thresholding fails. The framework was implemented in a lightweight, browser-based application [3] enabling real-time classification from minimal input (OSL signal and material type), supporting rapid field deployment without the need for complex infrastructure. This study provides proof of concept that AI-assisted classification can transform heterogeneous, non-specialized materials into effective triage tools. The approach is not material-specific and can be extended to other TL/OSL-responsive substrates, offering a scalable strategy to complement existing emergency dosimetry and improve early decision-making in radiological incidents.

Keywords radiological emergency; triage; OSL dosimetry; machine learning; fortuitous dosimeters

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The Future of Biological Dosimetry: Integrating Automation and Artificial Intelligence for High-Precision Radiation Dose Assessment

Di Giorgio, M.[1], Abdelaziz, R.F.[2], Zahin, N.[2], Tichy, A.[3,4], Brown. A.[5], Beinke, C.[6], Achel, D.G.[7], Garty, G.[8], Damer,K.[9], Ali, M.R.[10], Bhat, N.N.[11], Prasanna, P.G.S.[12], Wilkins, R.C.[13], Tashiro, S.[14], Korraa, S.S.[15], Barnard, S.G.R.[16], Tamaki, T.[2], Blakely, W.F.[17], Belyakov, O.[2]*

[1]Autoridad Regulatoria Nuclear, Argentina [2]Applied Radiation Biology and Radiotherapy Section, Division of Human Health, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, Vienna International Centre, Vienna, Austria [3]Department of Radiobiology, Military Faculty of Medicine, University of Defence, Hradec Kralove, Czech Republic [4]Biomedical Research Centre, University Hospital, Hradec Kralove, Czech Republic [5]MetaSystems Hard & Software GmbH, Germany [6]Bundeswehr Institute of Radiobiology, University of Ulm, Neuherbergstraβe 11, Munich, Germany [7]Applied Radiation Biology Centre, Radiological and Medical Sciences Research Institute, Ghana Atomic Energy Commission, Legon-Accra GE-257-0465, Ghana [8]Center for Radiological Research, Columbia University, New York, NY, USA [9]ASELL, LLC, Owings Mills, MD, USA [10]Medical Technology Division, Malaysian Nuclear Agency, Bangi, Kajang, Selangor, Malaysia [11]Radiological Physics and Advisory Division, Bhabha Atomic Research Centre, Mumbai, India [12]Division of Cancer Treatment and Diagnosis, National Cancer Institute, NIH, Bethesda, MD, USA [13]Consumer and Clinical Radiation Protection Bureau, Health Canada, Canada [14]Department of Cellular Biology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima City, Japan [15]National Research Centre for Radiation Research and Technology (NCCRT), Egyptian Atomic Energy Authority, Cairo, Egypt [16]UK Health Security Agency, Radiation, Chemical, Climate and Environmental Hazards, Chilton, United Kingdom [17]Scientific Research Department, Armed Forces Radiobiology Research Institute, Uniformed Services University of the Health Sciences, Bethesda, MD USA
*Presenting Author: o.belyakov@iaea.org

Biological dosimetry is a cornerstone of radiation protection, clinical monitoring, and emergency response. Traditional cytogenetic assays, particularly the dicentric chromosome assay (DCA), remain the gold standard for individual dose estimation but are limited by highly skilled labor-intensive protocols and several days needed to estimate dose and clinical outcome assessments. The integration of automation and artificial intelligence (AI) offers transformative potential to enhance accuracy, reproducibility, and scalability. This paper synthesizes outcomes from the IAEA Consultants' Meeting (Vienna, November 2025) and incorporates a draft project proposal for a four-year initiative under CRP E35013. The consolidated report of presentations is included as Material and Methods, with expanded detail on institutional contributions from MetaSystems, Hiroshima University (HICARE), Health Canada, Egypt, ASELL, AFRRI, BARC India, University of Ghana, Columbia University, UK Health Security Agency, Malaysia, ARN - Argentina, and the IAEA-Biological Dosimetry Model Laboratory (BDML). Cross-cutting themes and new conclusions are also integrated to provide a comprehensive roadmap for harmonization, validation, use of automation, and integration of AI-driven biological dosimetry worldwide.

Keywords : Biological dosimetry; Automation, Artificial Intelligence, Radiation protection.

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Towards the Development of a Novel System for Microdosimetry and Cellular Radiation Response

Pasricha, P.[1]*, Khilji, S.[2], McNairn, C.[1], Muir, B.R.[3], Subedi, S.[4], Jirasek, A.[5], Andrews J.[6], Cassol, E.[7], Chauhan, V[2], Murugkar, S.[1], Thomson, R.M.[1]

[1]Department of Physics, Carleton University, [2]Consumer and Clinical Radiation Protection Bureau, Health Canada, [3]Metrology Research Centre, National Research Council,[4]Department of Statistics, Carleton University, [5]Department of Physics, University of British Columbia, [6]Department of Statistics, University of British Columbia, [7]Department of Health Sciences, Carleton University.
*Presenting Author: prarthanapasricha@cmail.carleton.ca

Understanding how radiation deposits energy at the cellular scale and how this translates into biological response remains a central challenge in radiobiology. Here, we present a novel, integrated approach that combines Monte Carlo (MC) simulations, Raman spectroscopy (RS), and radiobiological assays to bridge this gap.
MC38 murine colon cancer cells are irradiated to doses 0-10 Gy and evaluated at 24- and 48-hours post-irradiation. Cellular responses were quantified using flow cytometry to assess oxidative stress, membrane integrity, and proliferation, and RS is used to assess radiation-induced biochemical changes within individual cell nuclei. In parallel, MC simulations replicating the experimental setup were used to characterize energy deposition at the cellular and subcellular level (Fig. 1). Results reveal that stochastic variations in specific energy deposition decrease with dose, (relative standard deviation ~6% at 0.25 Gy to ~2% at 2 Gy). Minimal cytotoxicity is observed at 24 hours, while pronounced dose-dependent effects emerge at 48 hours, including increased oxidative stress, reduced proliferation, and decreased viability for doses ≥5 Gy. RS studies indicate significant biochemical alterations, with reductions in nucleic acid-associated signals and concurrent increases in protein-associated features. Additionally, a novel cells-on-radiochromic-film (cells+RCF) platform is investigated through MC simulations. Strong agreement (<2% difference) between energy deposition in cell nuclei and the underlying film supports the feasibility of this system for cellular-scale dosimetry. Together, these findings demonstrate the potential of our integrated approach to quantitatively connect microscopic energy deposition with cellular responses.

Keywords: Microdosimetry; Radiation Response; Monte Carlo simulations; Raman Spectroscopy; Flow Cytometry.

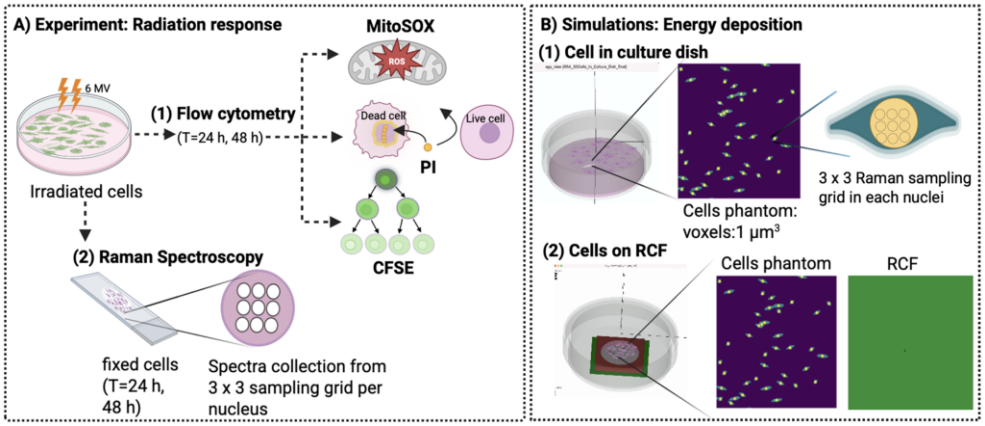

Figure 1: A schematic overview of methods used.

A Technical Advance in Automated Cytokinesis-Block Micronucleus Assay using AI-based Instance Segmentation

Fujishima, Y.[1]*, Goh, V.S.T.[2], Anderson, D.[1], Okimoto A.[1,3], Kamakura S.[1,3], Miura, T.[1]

[1]Institute of Radiation Emergency Medicine, Hirosaki University, Japan.
[2]Department of Radiobiology, Singapore Nuclear Research and Safety Institute, Singapore.
[3]Graduate School of Health Science, Hirosaki University, Japan.
*Presenting Author: yohei.fujishima@hirosaki-u.ac.jp

In radiation emergency medicine, accurate dose estimation for each exposed individual is crucial for predicting clinical conditions and guiding appropriate treatment. This is particularly critical during large-scale radiation incidents, where rapid and reliable triage is required [1]. However, cytogenetic dosimetry methods used for triage are bottlenecked by the time required for cell culture and the need for highly specialized expertise in scoring chromosomal damage. The objective of this study is to develop an automated analysis approach for the cytokinesis-block micronucleus (CBMN) assay using AI.

In our previous work, we demonstrated that AI-based object detection models can achieve high performance in detecting micronuclei (MN) (Figure 1A), and that dose-response relationships obtained using AI-based scoring were comparable to those from manual scoring [2]. However, to best of our knowledge, existing AI-based MN detection models, including our own, do not fully account for compliance with established scoring criteria [3].

To solve this problem, we introduce an instance segmentation technique, which enables precise identification, classification, and pixel-level delineation of individual objects. Previously we developed AI model for MN analysis (Figure 1A) [2], now we will try using YOLO26-based framework (Figure 1B) [4]. Then, the extent to which instance segmentation improves the precision and reliability of MN analysis compared with object detection alone will be evaluated. These results will be presented in the future.

Keywords Cytogenetic biodosimetry; Cytokinesis-block micronucleus assay; Image analysis; Artificial intelligence; Instance segmentation

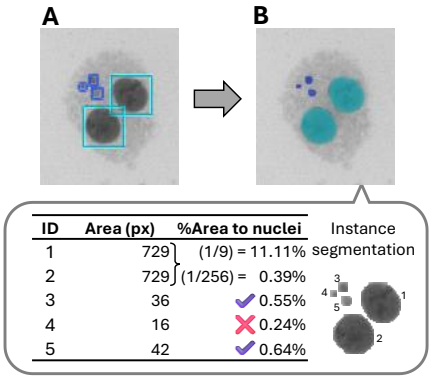

Figure 1. Instance segmentation of main nuclei and MNs for area analysis.

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An Oral Multi-Agent Countermeasure Against IR-Induced Injury That Preserves or Enhances Radiotherapy Efficacy

Oriol-Caballo, M.[1,2], Montoro, A.[3,4]*, López-Blanch, R.[2], Ortega-López, S.[2], Villaescusa, J.I.[3], Estrela, J.M.[1,2], Obrador. E.[1,2]

[1]Department of Physiology, Faculty of Medicine & Odontology, University of Valencia, Spain;
[2]Scientia BioTech,
[3]Service of Radiological Protection, La Fe University Hospital, Spain;
[4]Biomedical Imaging Research Group GIBI230, Health Research Institute (IISLaFe), University and Polytechnic La Fe Hospital, Spain.
*Presenting Author: montoro_ale@gva.es

High-dose ionizing radiation causes severe multi-organ injury, yet no broadly effective, orally available countermeasure has been validated. Here, we describe a fully oral, multi-component formulation combining bioavailable polyphenol derivatives (pterostilbene cocrystals and silybin-phosphatidylcholine), the NAD^+ precursor nicotinamide riboside, and the ACE inhibitor captopril. This combination provides robust systemic radioprotection, enabling long-term survival in 90% of mice exposed to a lethal (LD50/30) dose of X-rays. The formulation mitigates hematopoietic, intestinal, and neuromotor injury through complementary mechanisms involving enhanced DNA repair, reduced oxidative stress and inflammation, preservation of NAD^+ homeostasis and mitochondrial function, and activation of autophagy. Importantly, normal tissue protection does not compromise tumor control. Irradiation-induced tumor regression is preserved in triple-negative breast cancer, while glioblastoma radiosensitivity is significantly enhanced through sustained oxidative stress and inhibition of PARP1, HIF-1α, and VEGF signaling. These findings support a practical, orally deployable strategy to protect normal tissues while preserving or enhancing tumor radiosensitivity.

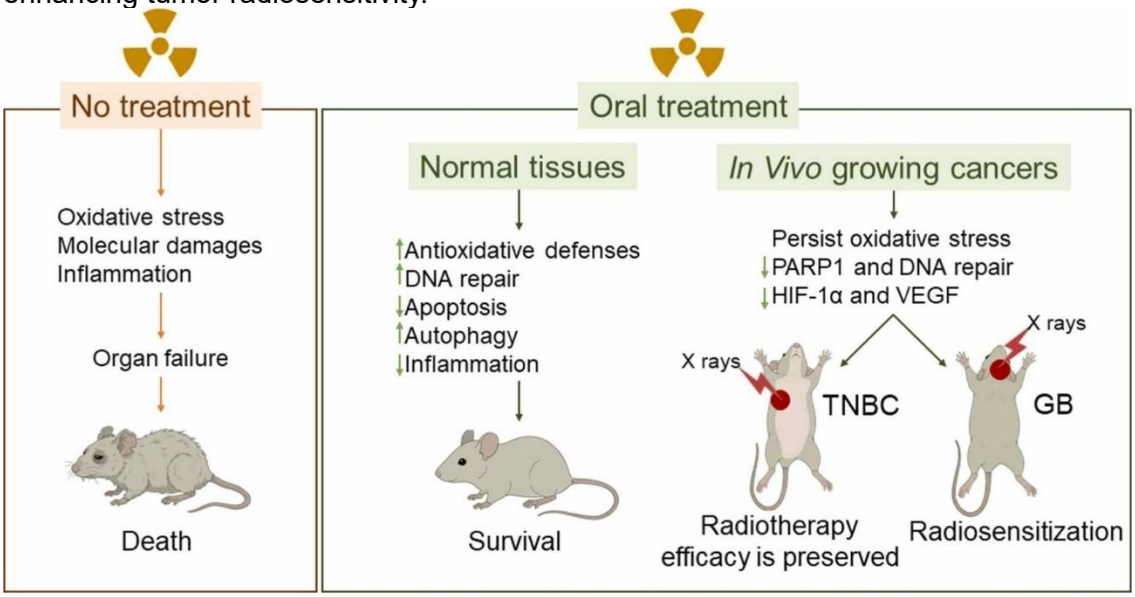

Figure 1. Graphical abstract

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