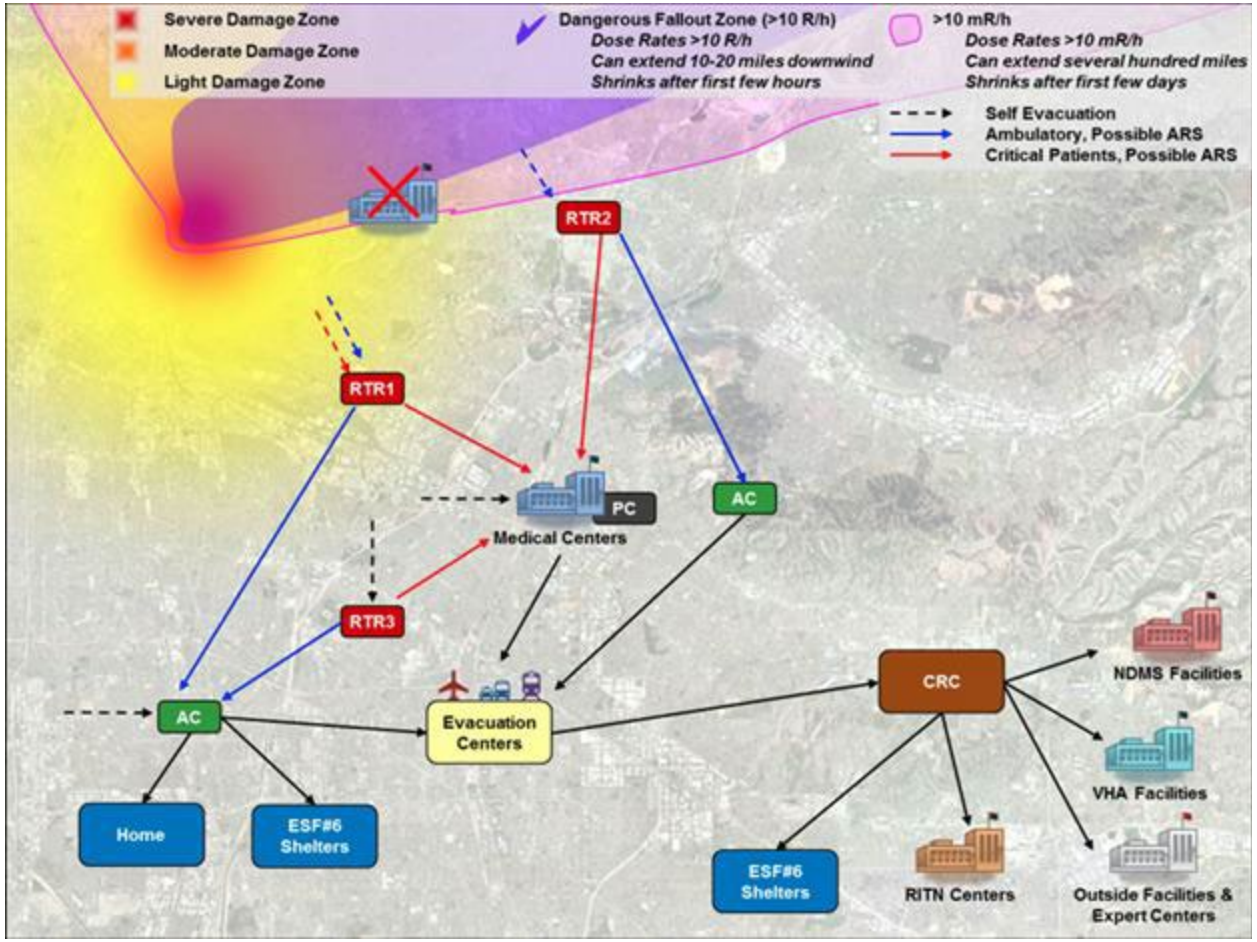
Analytical and Clinical Validation of a Cytogenetic Biodosimetry System

Richard Kowalski, Ph.D
15 Sept 2024

Biodosimetry Needs

- FDA authorized biodosimetry tests are needed to support the response to a mass-casualty nuclear incident
  - Developed and validated in compliance with medical device regulations (21 CFR 820)
- Capable of providing clinically actionable results at scale of 100,000's to 1M+ people
  - Not research tools
- Pre-deployed to provide rapid response and exercised regularly – ready to go when needed

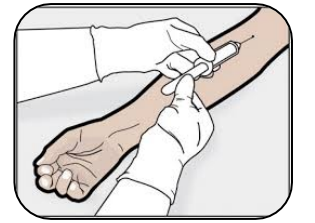
The RTR Functional Response System (Radiation TRiage, TReatment, and TRansport)[1]



1) Coleman CN, Weinstock DM, Casagrande R, et al. Triage and treatment tools for use in a scarce resources-crisis standards of care setting after a nuclear detonation. Disaster Med Public Health Prep. 2011;5(SUPPL 1). doi:10.1001/DMP.2011.22

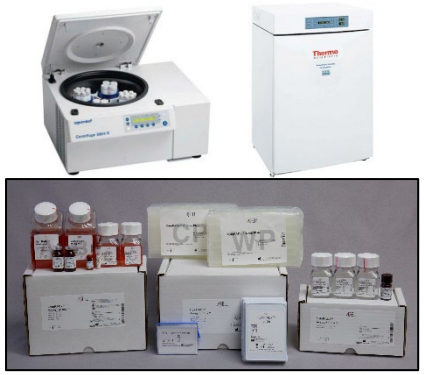
CytoRADx™ Quantitative Biodosimeter

- Provides quantitative estimates of absorbed dose to inform patient treatment
  - Customized version of Cytokinesis-Block Micronucleus (CBMN) assay
  - Uses MetaSystems' Metafer automated microscopes and existing commercial equipment in CLIA reference labs
- Features & Benefits:
  - Direct whole blood input – no lymphocyte isolation required
  - Customized reagent formulations, cGMP manufactured and packaged in an easy-to-use, 3-box kit – shelf life of >1 year
  - High throughput – cell culture in microtiter plates with multichannel pipettes; rapid, automated slide scanning
  - Integrated dose calculation – no lab-specific calibration curves required
  - Robust performance across a wide range of demographics, common diseases and likely comorbidities



1. Whole blood collected in commercial heparin tubes; shipped to CytoRADx labs

ThermoFisher SCIENTIFIC



2. Samples processed using CytoRADx assay, creating slides

3. Slides scanned and analyzed with RADxScan; dose estimates obtained

MetaSystems

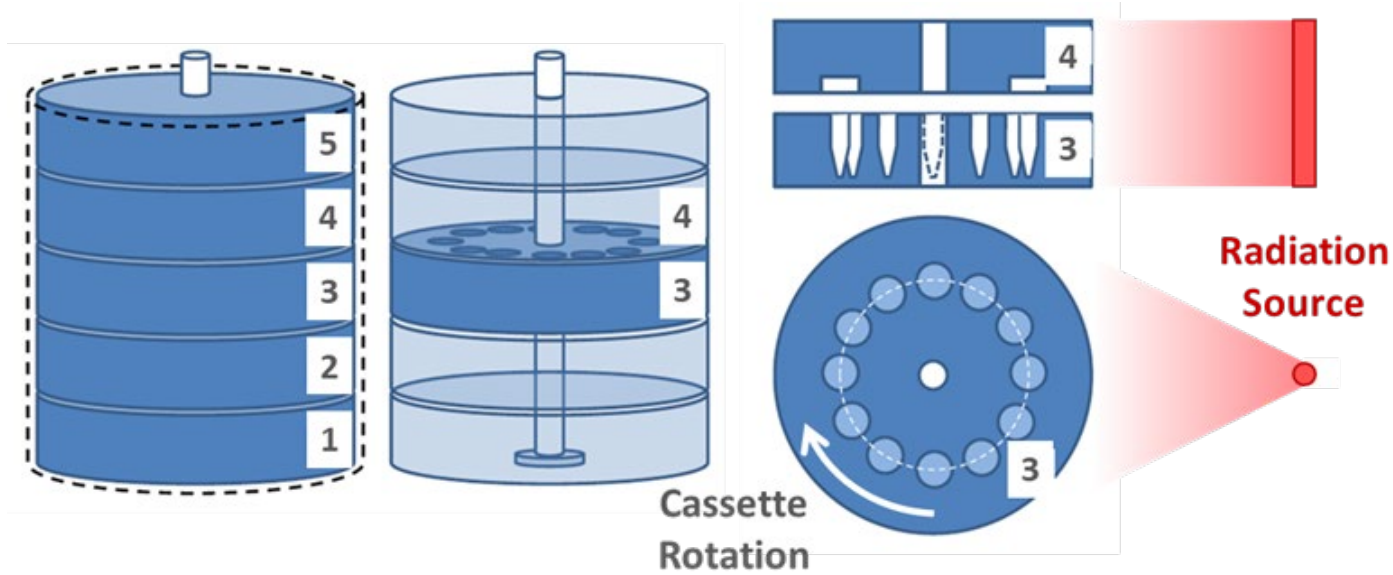
4. Results available to clinicians, informing treatment

Validation Studies for IVDs

- Validation of an IVD medical device includes a wide range of required studies to demonstrate safety and effectiveness
  - Typically include Accuracy, Range, LOD/B/Q, Precision, Reproducibility, Shelf Life, Interferents, Reference Interval, Confounding Populations, etc.
- For biodosimetry, study designs need to be adapted based on limited access to intended use population and other inherent limitations of the particular biodosimetry test
- CytoRADx validation included testing of samples from over 1200 subjects and over 10,000 individual test results
- Results from select validation studies of CytoRADx presented in following slides

Ex Vivo Irradiations

- Use of contrived samples necessary for CytoRADx device validation (can't irradiate healthy people)
- All *ex vivo* irradiations were conducted by Wake Forest University Health Sciences (WFUHS) under direction of Dr. J. Dan Bourland
- A Gammacell® 3000 Elan Irradiator was used for irradiations
  - ^{137}Cs, 0.662 MeV γ–rays at a dose rate of ~3.4 Gy/min
- The system is independently verified according to ISO/IEC 17025:2017 using Lithium Fluoride Thermoluminescent dosimeters
- Each irradiation run was QC'd using radiochromic films

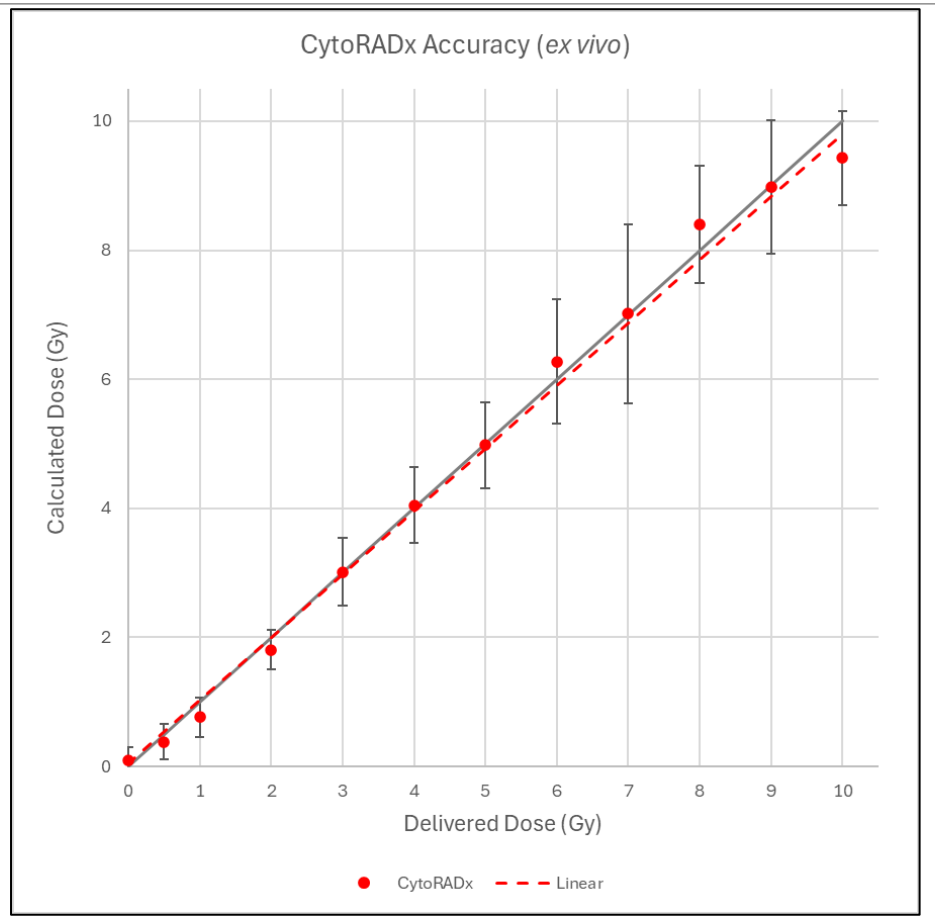


Visual schematic of Gammacell 3000 Elan irradiator configuration for ex vivo irradiation of blood samples. The cassette assembly consists of 5 layers of ABS material: one layer with conical bores matched to the blood tubes (labeled 3), one layer with cutouts for the tube caps (labeled 4), and 3 solid layers (labeled 1, 2, and 5). Blood tubes are loaded into layer 3, layers 4 and 5 are stacked on top, and the assembled cassette is locked in the irradiator and rotated continuously whenever the radiation source is exposed.

Capaccio, C., et al. (2021). CytoRADx: A High-Throughput, Standardized Biodosimetry Diagnostic System Based on the Cytokinesis-Block Micronucleus Assay. Radiation Research, 196(5), 523–534. https://doi.org/10.1667/RADE-20-00030.1

Accuracy and Range

- Samples acquired from at least 49 healthy adults and shipped overnight to WFUHS at room temperature
- Upon receipt, WFUHS irradiated samples at 0, 0.5, and 1-10 Gy in steps of 1 Gy
- Irradiated samples were then shipped overnight to ASELL and tested with CytoRADx (one replicate per dose level per subject)
- Results demonstrate accurate, linear response across a range of dose (0 to 10 Gy)
  - Linear fit: calc dose = 0.976 * delivered dose + 0.047 Gy
  - R^2-value of 0.942



CytoRADx accuracy results from at least 49 subjects per dose level. Subjects were tested in singlets at a given dose. Markers are mean results per dose; error bars are one standard deviation. The red dashed and solid grey lines are the linear regression and one-to-one concordance lines, respectively.

Reproducibility

Between Subjects, Sites, Operators and Instruments

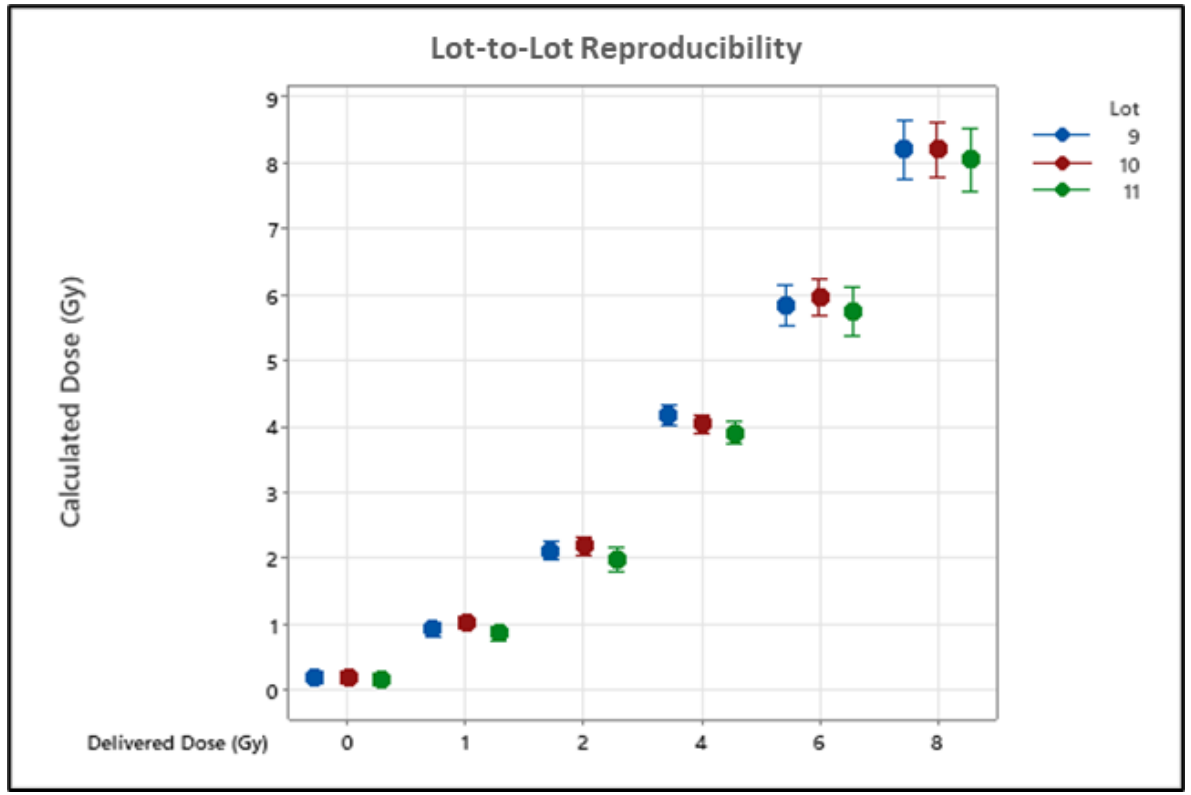
- Blood samples acquired from 15 healthy adults, shipped overnight, and irradiated *ex vivo* at one of 5 dose levels by WFUHS
- Irradiated blood samples were then shipped overnight from WFUHS to each of three CytoRADx testing labs (NeoGenomics in FL, NeoGenomics in TX, and ASELL in MD)
- Mean of triplicate samples per subjects at each dose level shown with the standard deviation and %CV for each reproducibility assessment
- %CVs for a cell-based assay are excellent

Dose	Mean (Gy)	Between-Subjects		Between-Sites		Between-Operators		Between-Instrument	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
0 Gy	0.15	0.226	N/A	0.060	N/A	0.014	N/A	0.010	N/A
1 Gy	1.09	0.172	15.7%	0.260	23.8%	0.085	7.8%	0.022	2.1%
2 Gy	2.30	0.184	8.0%	0.388	16.9%	0.312	13.6%	0.067	2.9%
4 Gy	4.46	0.285	6.4%	0.356	8.0%	0.111	2.5%	0.000	0.0%
6 Gy	6.21	0.587	9.4%	0.652	10.5%	0.152	2.4%	0.033	0.5%

Summary table of reproducibility results. Mean consists of results obtained from each of three sites. Standard deviations (SD) and variance (%CV) are based on the results per dose level per category.

Reproducibility

Lot-to-Lot



CytoRADx Lot-to-Lot comparison study results. Samples from 12 subjects were collected, shipped overnight to WFUHS, irradiated ex vivo at 0, 1, 2, 4, 6, or 8 Gy, shipped overnight, and tested by ASELL. Data points are mean values and error bars are the 95% CIs.

Lot-to-Lot Reproducibility

- 3 unique lots of CytoRADx assay kits were manufactured at 2-month intervals
  - All 3 lots were tested at the same time with samples irradiated *ex vivo* from the same 12 subjects
- Results show consistent performance for all lots across dose levels (0, 1, 2, 4, 6, and 8 Gy)

Interference Testing

Endogenous and Exogenous

- Whole blood samples from 6 healthy adults per compound were irradiated by WFUHS at dose levels of 0, 2 and 6 Gy
- Irradiated samples were shipped to ASELL, supplemented with potentially interfering substances, and tested with the CytoRADx System
  - The concentrations of interferents were based on CLSI guidance or between 1 – 3x C_{max} in serum
- None of the compounds affected the CytoRADx results compared to controls

Endogenous Interferents

Interferent (conc. in blood)	Dose (Gy)	p-value	Result
Albumin (60 mg/mL)	0	<0.001	Equivalent
	2	0.002	Equivalent
	6	0.094	Equivalent
Human IgG (18 mg/mL)	0	0.002	Equivalent
	2	0.001	Equivalent
	6	0.013	Equivalent
Conjugated Bilirubin (5 µg/mL)	0	<0.001	Equivalent
	2	<0.001	Equivalent
	6	0.009	Equivalent
Unconjugated Bilirubin (20 µg/mL)	0	<0.01	Equivalent
	2	<0.01	Equivalent
	6	<0.01	Equivalent
Heparin (3.3 U/mL)	0	0.004	Equivalent
	2	<0.001	Equivalent
	6	0.015	Equivalent
Hemoglobin (525 µg/mL)	0	<0.001	Equivalent
	2	<0.001	Equivalent
	6	0.034	Equivalent
Intralipid (10 mg/mL)	0	<0.001	Equivalent
	2	<0.001	Equivalent
	6	0.034	Equivalent

Exogenous Interferents

Interferent (conc. in blood)	Dose (Gy)	p-value	Result
Acetaminophen (156 µg/mL)	0	0.002	Equivalent
	2	<0.001	Equivalent
	6	<0.001	Equivalent
Acetylsalicylic Acid (30µg/mL)	0	<0.001	Equivalent
	2	<0.001	Equivalent
	6	0.002	Equivalent
Ibuprofen (220 µg/mL)	0	<0.001	Equivalent
	2	<0.001	Equivalent
	6	0.037	Equivalent
Naproxen (100 µg/mL)	0	0.001	Equivalent
	2	0.001	Equivalent
	6	<0.001	Equivalent
Amoxicillin clavulanic acid (54 µg/mL + 2.5 µg/mL)	0	<0.001	Equivalent
	2	<0.001	Equivalent
	6	0.016	Equivalent
Loperamide (10 ng/mL)	0	<0.001	Equivalent
	2	<0.001	Equivalent
	6	<0.001	Equivalent
Ondansetron (340 ng/mL)	0	<0.001	Equivalent
	2	<0.001	Equivalent
	6	0.003	Equivalent

Clinical Validation

Reference Interval

- Human reference interval study performed with 245 subjects representing general population of United States
  - Subjects enrolled across 5 sites geographically distributed across the US
  - Samples tested at NeoGenomics (high complexity CLIA-certified reference lab)
- Results showed a narrow reference interval of [0, 0.3] Gy for the entire population
  - Consistent with published limits of the CBMN Assay that range from 0.18 Gy[A] to 1 Gy[B]
- No subgroups showed a significant difference in reference interval, with a maximum interval of [0, 0.4] Gy for all stratifications evaluated
  - Age, Race, Sex, and Ethnicity shown at right

Population		95% Reference Interval	Number (%) of Subjects	Percentage in US Population(2)
Total Study		**(0, 0.3) Gy**	**245 (100%)**	**N/A**
Age	2 – 4 years of age	(0, 0) Gy	8 (3.3%)	3.6%
	5 – 17 years of age	(0, 0) Gy	46 (18.8%)	16.6%
	18 – 65 years of age	(0, 0.2) Gy	158 (64.5%)	62.4%
	Over 65 years of age	(0, 0.4) Gy	33 (13.5%)	15.2%
Race	White / Caucasian	(0, 0.3) Gy	198 (80.8%)	75.8%
	Black or African American	(0, 0.3) Gy	31 (12.7%)	13.6%
	Asian	(0, 0.2) Gy	13 (5.3%)	6.1%
	American Indian or Alaskan Native	(0, 0) Gy(1)	2 (0.8%)	1.3%
	Native Hawaiian or Other Pacific Islander	(0, 0) Gy(1)	1 (0.4%)	0.3%
Sex	Male	(0, 0.3) Gy	120 (49.0%)	49.1%
	Female	(0, 0.4) Gy	125 (51.0%)	50.9%
Ethnicity	Hispanic or Latino	(0, 0.3) Gy	45 (18.4%)	18.9%
	Not Hispanic or Latino	(0, 0.3) Gy	200 (81.6%)	81.1%

(1) Sample size for this subgroup was not sufficient to estimate the reference interval, so values of (0, 0) were assigned to the reference interval.
(2) Based on 2020 US Census values.

CytoRADx reference interval study results from 245 subjects. Samples were collected across 5 geographically distributed collection sites, shipped overnight, and tested by NeoGenomics in Ft Myers, FL (a high complexity CLIA-certified lab representative of the intended use).

A: IAEA Guidance ISO 17099:2014(E). Radiological protection-Performance criteria for laboratories using the cytokinesis block micronucleus (CBMN) assay in peripheral blood lymphocytes for biological dosimetry
B: McNamee, J. P., et al. (2009). Validation of the cytokinesis-block micronucleus (CBMN) assay for use as a triage biological dosimetry tool. *Radiation Protection Dosimetry*, *135*(4), 232–242.

Clinical Validation

Social Demographics

Category	Population	95% Reference Interval	Number of Subjects (Percentage in Study)
Smoking	Never smoked	(0, 0.3) Gy	196 (80.0%)
	Former smoker	(0, 0.4) Gy	26 (10.6%)
	Current smoker	(0, 0.2) Gy	23 (9.4%)
Alcohol	Non-drinker of alcohol	(0, 0.35) Gy	144 (58.8%)
	Former Drinker of alcohol	(0, 0.4) Gy	23 (9.39%)
	Drinker of alcohol	(0, 0.25) Gy	78 (31.8%)
Caffeine	Non-drinker of caffeine	(0, 0.2) Gy	77 (31.4%)
	Former Drinker of caffeine	(0, 0.4) Gy	9 (3.67%)
	Drinker of caffeine	(0, 0.3) Gy	159 (64.9%)

- eCRFs from the 245 subjects enrolled for the Reference Interval study were used to assess whether prevalent social demographics may impact CytoRADx results
- While smoking has been shown to increase the baseline micronucleus frequency in various studies, our results show no clinically significant difference in dose calculation when compared to the overall reference interval of [0, 0.3] Gy
  - Similarly, alcohol and caffeine usage were not different from the overall reference interval
- Taken together, the Reference Interval study and social demographics analysis showed that none of the assessed populations materially impacted CytoRADx results

Special Populations

Potential Confounding Pre-existing or Comorbid Medical Conditions

- Conditions that may affect proliferation of lymphocytes, thereby confounding CytoRADx results, were tested
  - Autoimmune diseases, diabetes, influenza infection, pregnancy, and age
- Subjects meeting IRB approved inclusion and exclusion criteria were prospectively enrolled at multiple US medical centers
  - At least 40 subjects were tested per cohort
- Whole blood samples were shipped to WFUHS and gamma-irradiated at 0, 2 and 6 Gy
- Irradiated samples were then sent to NeoGenomics for CytoRADx testing
- All special populations were equivalent to healthy adult results (tested in parallel)
  - Minor exception of subjects with NAT positive influenza tested with 6 Gy *ex vivo* irradiation

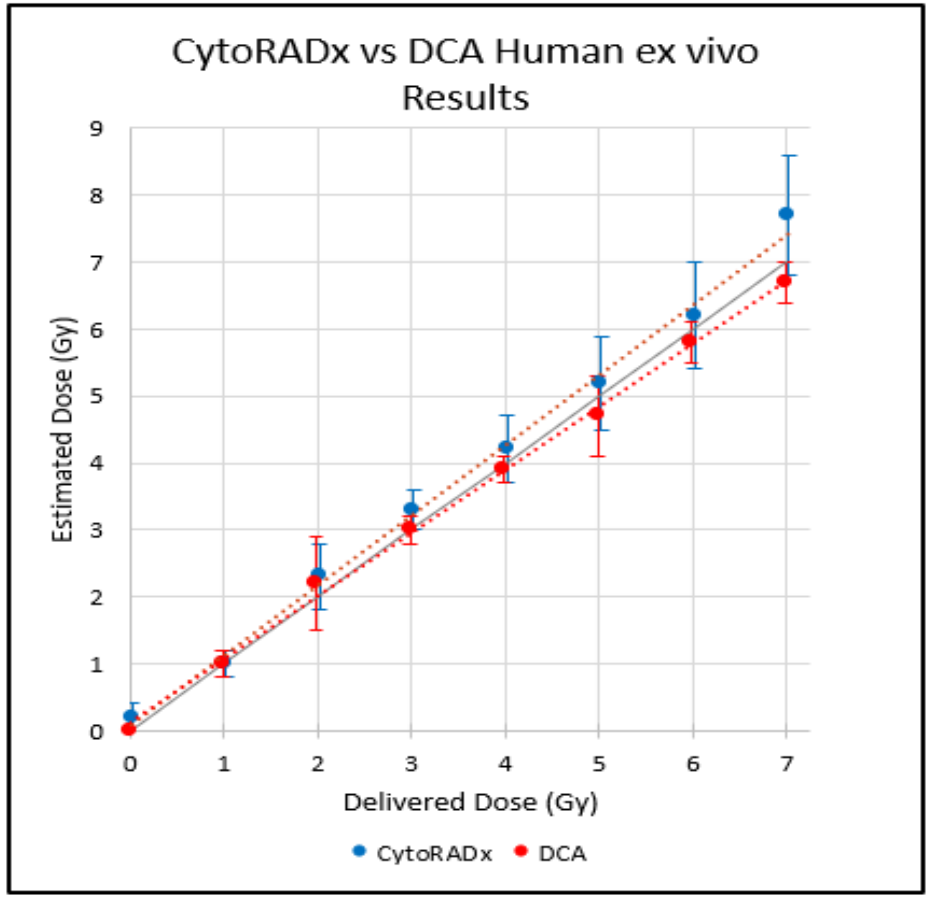
Cohort	Delivered Dose (Gy)	90% CI for Difference (Gy)	p-values (≤ limit/≥ limit) for the 90% CI	Result
Geriatric	0	(-0.04, 0.09)	0.000/0.000	Equivalent
	2	(-0.28, 0.03)	0.000/0.000	Equivalent
	6	(0.00, 0.71)	0.000/0.005	Equivalent
Adolescent	0	(-0.13, 0.00)	0.000/0.000	Equivalent
	2	(-0.33, 0.00)	0.000/0.000	Equivalent
	6	(-0.86, 0.00)	0.016/0.000	Equivalent
Pediatric	0	(-0.13, 0.00)	0.000/0.000	Equivalent
	2	(-0.21, 0.00)	0.000/0.000	Equivalent
	6	(-0.97, 0.00)	0.072/0.000	Equivalent
Influenza	0	(0.00, 0.18)	0.000/0.000	Equivalent
	2	(-0.13, 0.16)	0.000/0.000	Equivalent
	6	(0.00, 1.05)	0.000/0.140	Not equivalent
Autoimmune	0	(0.00, 0.15)	0.000/0.000	Equivalent
	2	(-0.32, 0.00)	0.000/0.000	Equivalent
	6	(-0.26, 0.26)	0.000/0.000	Equivalent
Diabetes	0	(0.00, 0.13)	0.000/0.000	Equivalent
	2	(-0.24, 0.00)	0.000/0.000	Equivalent
	6	(-0.04, 0.44)	0.000/0.000	Equivalent
Pregnant	0	(-0.07, 0.04)	0.000/0.000	Equivalent
	2	(-0.29, 0.00)	0.000/0.000	Equivalent
	6	(-0.67, 0.00)	0.002/0.000	Equivalent

Summary table of special populations tested by CytoRADx. Confidence intervals at 90% along with associated p-values at 90% are shown. Equivalent results indicate no significant difference from healthy adults run in parallel

“Gold-Standard” Comparison

Ex vivo irradiated human blood samples

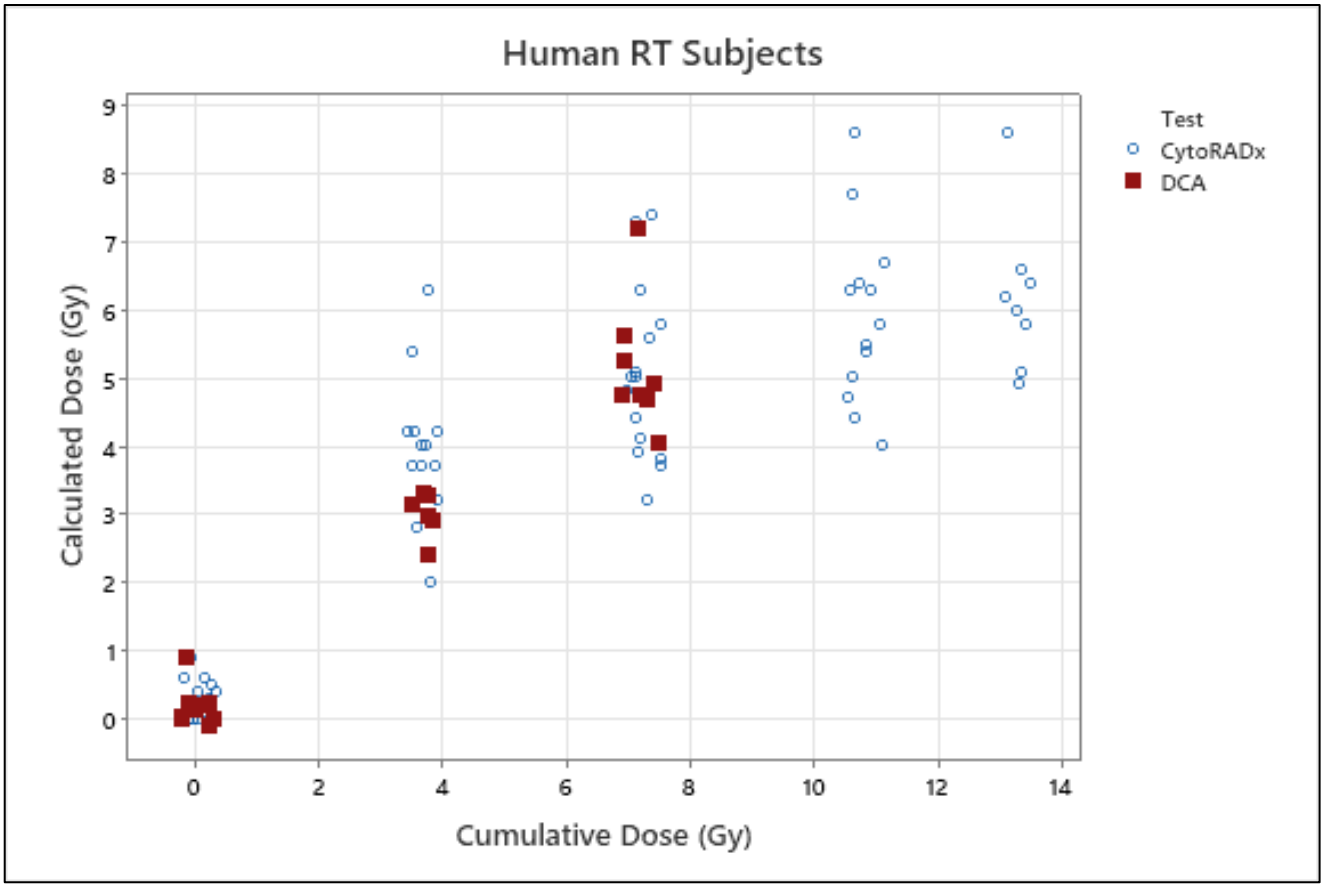
- Whole blood samples from 20 healthy adults were irradiated *ex vivo* at WFUHS at doses of 0, 1, 2, 3, 4, 5, 6, and 7 Gy
- Each sample was split and shipped overnight to ASELL and REAC/TS
  - CytoRADx testing performed by ASELL
  - DCA testing performed by REAC/TS (CLIA-certified)
- Strong agreement between the two cytogenetic methods with similar performance levels
  - CytoRADx: calc dose = 1.05 * delivered dose + 0.07 Gy
    - r-sq 99.6%
  - DCA: calc dose = 0.95 * delivered dose + 0.12 Gy
    - r-sq 99.8%



CytoRADx vs DCA results from 20 subjects irradiated ex vivo. Results are single culture with up to 7 slides per sample for DCA versus one replicate for CytoRADx. Markers are mean results per dose, error bars are one standard deviation, red lines are the linear regressions, and the grey line is the one-to-one concordance.

“Gold-Standard” Comparison

Samples from in vivo irradiated subjects



Dot plot of cytogenetic results from TBI RT subjects. Open blue symbols are CytoRADx results and solid red symbols are DCA results at 0, 3.6, 7.2, 10.8 and 13.2 Gy cumulative dose (jitter added to x-axis to improve clarity). Each fractionated cumulative dose level shows the results from testing of the same 10 RT subjects.

- 10 human subjects receiving myeloablative radiation treatment for various hematologic disorders were enrolled at City of Hope by Dr. Jeffrey Wong
  - Subjects received 1.2 Gy x 3 fractions of total body irradiation per day for 3 days and 1.2 Gy x 2 fractions on Day 4
- Blood samples were drawn daily during treatment and shipped overnight at room temperature to ASELL
  - Samples represent 0, 3.6, 7.2, 10.8 and 13.2 Gy cumulative dose
- For comparison testing to DCA, samples were split at ASELL and a portion sent to REAC/TS
  - CytoRADx testing was initiated within 72 hours of blood draw
  - Batch DCA testing was conducted at REAC/TS using PHA stabilized blood sent from ASELL
- Both cytogenetic assays showed good agreement through 7.2 Gy
  - At higher doses, too few metaphase spreads were available for estimating dose by DCA

Summary

- There is an unmet need for FDA-authorized Biodosimetry diagnostic devices for use in the aftermath of Rad/Nuc disaster capable of testing hundreds of thousands to millions of people within the first weeks after exposure
- CytoRADx is a ***quantitative biodosimetry system*** that uses a cytogenetic assay (modified CBMN) and automated imager to calculate an absorbed dose to inform patient treatment
- CytoRADx analytical validation studies show that the analytical range encompasses clinically relevant thresholds for medical management of radiation exposure, that the test is reproducible, and that many potential endogenous and exogenous interferents do not affect assay performance
- CytoRADx clinical validation studies show that the Reference Interval is consistent across demographics with values within expected limits for the CBMN assay, and that special human populations tested do not impact CytoRADx results
- The CytoRADx System provides comparable results to the “gold-standard” DCA
- The CytoRADx System is currently being evaluated by the FDA for pre-Emergency Use Authorization

Acknowledgements

BARDA

MetaSystems

City of Hope

Wake Forest University Health Sciences

REAC/TS

NeoGenomics

ThermoFisher Scientific

Emmes

ICON

Pharmalex

Mike Porter

Jay Perrier

Chris Capaccio

Ryan Mahnke

Jackie Chan

Emma Greene

Deaven Cyprien

Lili Fanua

David Waid

Michael Zdanis

Brad DesLauriers

Dave Winer

Matt Snyder

Ken Damer

Dave Tilles

This project has been funded in whole or in part with Federal funds from the Department of Health and Human Services; Administration for Strategic Preparedness and Response; Biomedical Advanced Research and Development Authority, under Contract HHSO100201700022C.

Turning Science Into Solutions