NATO Biodosimetry Exercise
- Inter-Assay Comparison -

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Aim

Determine the suitability of the well established dicentric chromosome assay [DCA] and cytokinesis-block micronucleus assay [CBMN] and the emerging γ-H_2AX foci and gene expression assays for biodosimetry and radiation injury assessment.

Method

Lithium-heparinized whole blood from one healthy donor was irradiated (240 kVp, 13 mA, X-ray, dose rate 1 Gy/min, at ~37°C). Ten blind (and calibration) samples irradiated with single doses between 0-6.4 Gy were sent to participants to run their assays (table 1, fig. 1). Provided dose estimates were analyzed using a linear model, logistic regression analysis and report time was documented.

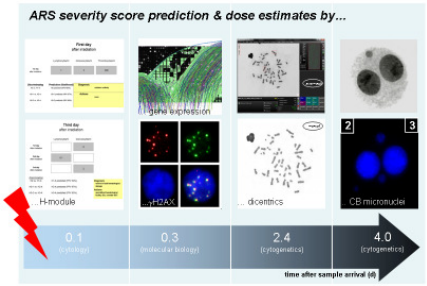

Figure 1: Description of assays compared within this exercise. Arrow indicates the earliest time required for report on dose estimates for each assay.

Institution	dicentric assay	CB micronucl. Assay	γH2AX DNA repair foci	gene expression	blood cell counts/ ARS degree
Ghent University, Department of Basic Medical Sciences, Research group 'Radiation and DNA repair', Ghent, Belgium		X			
Institut de Recherche Biomédical des Armées/ CRSSA, Grenoble, France	X			X	
Life Technologies, Company, Frankfurt, Germany				X	
Bundeswehr Institute of Radiobiology, Munich, Germany	X	X	X	X	X
Bundesamt für Strahlenschutz, Munich, Germany	X	X	X		
Qiagen, Company, Hilden				X	
Health Protection Agency, Centre for Radiation, Chemical and Environmental Hazards, Chilton, Didcot, Oxon, UK	X	X	X	X	
Basic Medical Sciences, Center for Applied Nanobioscience and Medicine, College of Medicine Phoenix, University of Arizona, USA				X	
DxTerity Diagnostics, Company, Rancho Dominguez, California, USA				X	
Sezione di Istologia e Biologia e Molecolare, Centro Studie Ricerche di Sanita'e Veterinaria, Roma, Italy	X	X	X	X	
Defence Scientist, Radiation Biology, CARDS, Ottawa ON, Canada	X	X			
	6	6	4	8	1

Table 1: Institutions involved in the exercise and contributing assays.

Results

Report time for dose estimates was 8-13 times earlier for molecular biology assays compared to cytogenetic assays (fig. 1). However, inter-laboratory variance of dose estimates (preliminary data) was smallest for DCA (about 2.3-5.6 times relative to all other assays) and increased in an assay-dependent manner as DCA < CBMN < gene expression < foci. (fig. 2). Variance of dose estimates with DCA as a reference category was statistically significantly higher in all other assays (p-value, range: 0.001-0.01) when comparing variance of dose estimates taken from all performer or restricting it to the 50% and the 25% percentile of reported variances of the dose estimates (table 2). However, these differences among assays became insignificant when using CBMN as the reference category. Binary categories of dose estimates could be discriminated with equal efficiency for all assays, but at doses ≥ 1.5 Gy a 10% decrease in efficiency was observed for the foci assay (table 3).

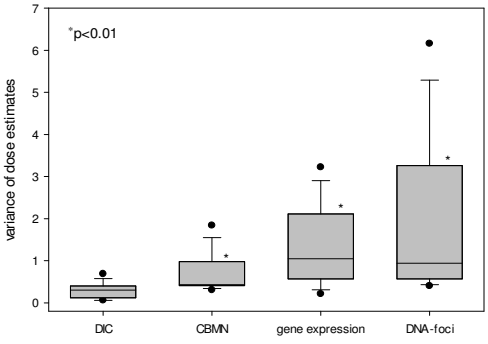

Figure 2: Preliminary data on variance of dose estimates per assay. DCA was used as the reference for statistical analysis (t-test or Mann Whitney)

	CBMN	Gene expression	DNA-Foci
all performer (reference: DIC, n=10, mean=0.29, median=0.38)			
n	11	8	8
test	MannWhitney	t-test	MannWhitney
median/mean	0.43	1.05	0.94
ratio to reference	1.40	3.60	3.10
p-value	0.008	0.005	0.002
50% percentile (reference: DIC, n=5, mean=0.14)			
n	6	4	4
test	t-test	t-test	t-test
mean	0.39	0.51	0.61
ratio to reference	2.80	3.60	4.40
p-value	<0.001	0.01	0.003
25% percentile (reference: DIC, n=3, mean=0.08, median=0.07)			
n	4	2	2
test	t-test	MannWhitney	t-test
median/mean	0.37	0.39	0.46
ratio to reference	4.60	5.40	5.75
p-value	<0.001	0.2	0.005

	DIC	Gene expression	DNA-Foci
all performer (reference: CBMN, n=11, median=0.43, mean=0.74)			
n	10	8	8
test	MannWhitney	t-test	MannWhitney
median/mean	0.30	1.36	0.94
ratio to reference	0.70	1.80	2.20
p-value	0.008	0.101	0.149
50% percentile (reference: CBMN, n=6, mean=0.39)			
n	5	4	4
test	t-test	t-test	t-test
median/mean	0.14	0.51	0.81
ratio to reference	0.40	1.30	1.80
p-value	<0.001	0.198	0.037
25% percentile (reference: CBMN, n=4, mean=0.37, median=0.38)			
n	3	2	2
test	t-test	MannWhitney	t-test
median/mean	0.09	0.38	0.46
ratio to reference	0.20	1.00	1.20
p-value	<0.001	1	0.172

Table 2: Preliminary statistical analysis of variance of dose estimates using DCA (left side) or CBMN assays (right side) as reference and examining all performer or 50% and 25% percentiles.

	# never	# ever	concordant (%)	ROC area
Never/ever radiation exposure				
Dicentric assay	8	71	94.7	0.958
CB micronucleus assay	11	98	94.1	0.944
DNA foci assay	7	63	95.2	0.968
gene expression	4	36	93.8	0.962
	# < 0.1 Gy	# > 0.1 Gy	concordant (%)	ROC area
≤ 0.1 Gy versus > 0.1 Gy radiation exposure				
Dicentric assay	63	16		1.000
CB micronucleus assay	22	86	98.5	0.995
DNA foci assay	14	56	98.4	0.964
gene expression	8	32		1.000
	# < 1.5 Gy	# ≥ 1.5 Gy	concordant (%)	ROC area
< 1.5 Gy versus ≥ 1.5 Gy radiation exposure				
Dicentric assay	32	47	99.9	0.999
CB micronucleus assay	44	86	99.50	0.995
DNA foci assay	26	42	88	0.886
gene expression	16	24	99	0.991
	# 2-4 Gy	# ≥ 4 Gy	concordant (%)	ROC area
2-4 Gy versus ≥ 4 Gy radiation exposure				
Dicentric assay	32	15	98.5	0.965
CB micronucleus assay	44	22	94.2	0.944
DNA foci assay	26	14	89.1	0.806
gene expression	8	16	94.5	0.949

Table 3: Comparison on discrimination ability of assays related to dose estimates aggregated into binary dose categories of clinical significance.

Conclusion

Dose estimates based on foci and gene expression assays are reported 8-13 times earlier compared to the DCA and CBMN assay, but estimates are 2.3-5.6 times more precise when running the DCA. This advantage in precision becomes negligible when discriminating dose estimates merged in binary dose categories of clinical relevance. All assays do show an upper limit below 6 Gy.

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