Performance of the automated dicentric and cytokinesis block micronucleus assays in a recent NATO exercise of established biodosimetry methods

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Aim

Examine the accuracy of scoring procedures such as manual versus semi-automatic and automatic scoring for well-established cytogenetic assays, namely, the dicentric chromosome assay (DCA) and the cytokinesis block micronucleus assay (CBMN). The automatic scoring allows a much higher throughput of both assays.

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 assay (table 1, figure 1). Cell scoring was done manually in triage mode or with new automated methods. Dose estimates provided by the participants were analyzed using a linear model, logistic regression analysis and report time was documented. Preliminary calculation of variances (squared difference between dose estimates and actual dose summed for 10 blind samples and divided by sample number, table 2) provides a measure for precision of each laboratory contribution.

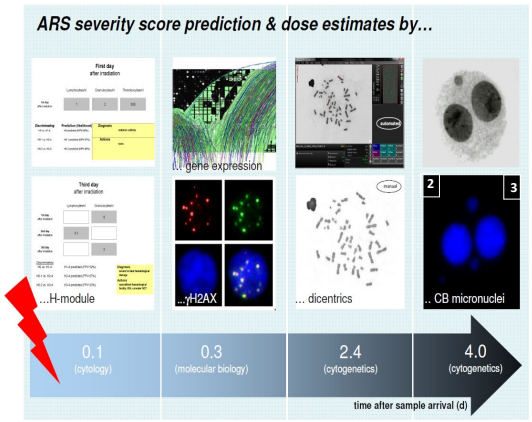

Figure 1: Overview of asssays compared within this exercise. The arrow indicates the time course of receiving the earliest dose estimates for each assay.

Laboratory	Dicentric Manually	Dicentric Automatic	CBMN Manually	CBMN Semi Automatic	CBMN Automatic
Lab 1	x	x	x	x	x
Lab 2	x			x	x
Lab 3					x
Lab 4	x		x		
Lab 5	x				x
Lab 6	x	x	x		
Lab 7	x	x			

Table 1: Contributed assays of the institutions involved in the exercise.

Results

Report time for dose estimates of cytogenetic assays was 2.4 - 4 days after receipt of blood samples, which was mainly due to cell culture time. It is the first intercomparison, where automated methods were applied simultaneously with conventional scoring. The dose estimates for various contributions of different laboratories are given in figure 2. The order of corresponding precision (variance, preliminary results) in table 2 show some variability in performance, but it gets obvious that the DCA assay is superior to the CBMN assay, and that the automated methods provide results comparable to the manual scoring procedure. We also merged dose into binary categories of clinical significance (logistic regression, table 3). Dose estimates fell into these categories with equal efficiency for both assays, irrespective of the scoring procedure, except that a 10% decrease in concordance was observed for the automated CBMN assay at # >4 Gy.

assay	procedure (cells)	radiation quality	Lab	*variance sq*
DIC	Automatic	X-ray	Lab 1	0.05
DIC	Manual	X-ray	Lab 4	0.07
DIC	Automatic	X-ray	Lab 6	0.12
DIC	Manual	X-ray	Lab 2	0.16
DIC	Manual	X-ray	Lab 5	0.30
CBMN	Manual (200)	X-ray	Lab 6	0.30
DIC	Manual	Co 60	Lab 1	0.31
CBMN	Automatic	X-ray	Lab 2	0.36
DIC	Manual	X-ray	Lab 6	0.38
DIC	Automatic	X-ray	Lab 7	0.40
CBMN	Manual (2000)	X-ray	Lab 6	0.41
CBMN	Semi-Automatic	X-ray	Lab 2	0.41
CBMN	Manual	Co 60	Lab 1	0.41
CBMN	Semi-Automatic	Co 60	Lab 1	0.43
DIC	Manual	Co 60	Lab 7	0.47
DIC	Manual	X-ray	Lab 7	0.68
CBMN	Automatic	Co 60	Lab 1	0.69
CBMN	Automatic	X-ray	Lab 1	0.94
CBMN	Manual	Cs 137	Lab 4	0.99
CBMN	Automatic	Co 60	Lab 3	1.36
CBMN	Automatic	X-ray	Lab 5	1.83

Table 2: The variance of the individual assays of the labs are given in ascending order (the lower the better).

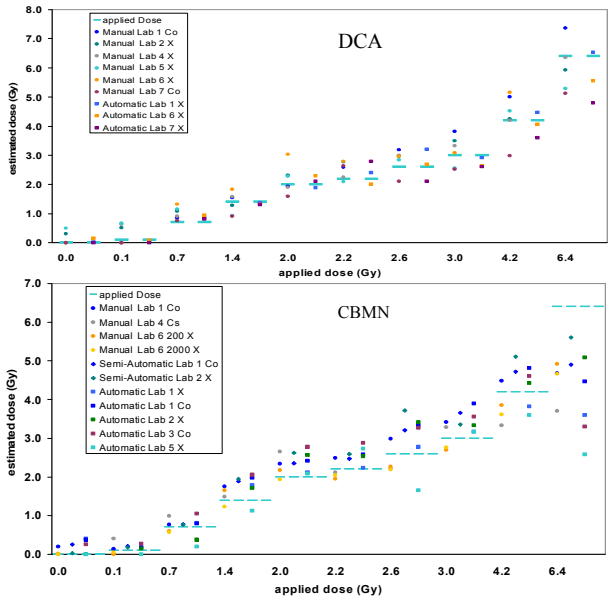

Figure 2: Comparison on different scoring procedures applied for the DCA and CBMN assay.

Never/Ever radiation exposure CBMN			% concordant	DCA			% concordant
	# never	# ever			# never	# ever	
automatic	5	45	91.1	automatic	3	27	93.8
semi automatic	2	18	94.4				
manual	4	36	95.8	manual	7	62	93.5
	# < 0.1 Gy	# > 0.1 Gy			# < 0.1 Gy	# > 0.1 Gy	
automatic	10	40	98.8	automatic	6	24	complete separation
semi automatic	4	16	complete separation				
manual	8	32	complete separation	manual	14	55	99.7
	# < 1.5 Gy	# > 1.5 Gy			# < 1.5 Gy	# > 1.5 Gy	
automatic	20	30	99.3	automatic	12	18	complete separation
semi automatic	8	12	complete separation				
manual	16	24	complete separation	manual	28	41	99.7
	# 2-4 Gy	# > 4 Gy			# 2-4 Gy	# > 4 Gy	
Automatic	20	10	89.5	automatic	12	6	complete separation
semi automatic	8	4	complete separation				
Manual	16	8	99.2	manual	28	13	95.1

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

Conclusion

The automated cytogenetic DCA and CBMN assays are almost as accurate as manual scoring in triage mode. This is also true when merging dose estimates into binary dose categories of clinical significance. Hence, our data support the use of high-throughput automated methods as a screening tool for dose estimation.