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Potentials of cytokinesis blocked micronucleus assay in radiation triage and biological dosimetry

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ABSTRACT

The measurement of micronucleus (MN) in the cytokinesis-block arrested binucleated cells has been extensively used as a biomarker in many radiation biology applications in specific biodosimetry. Following radiation casualties, medical management of exposed individuals begins with triage and biological dosimetry. The cytokinesis blocked micronucleus (CBMN) assay is the alternate for the gold standard dicentric chromosome assay in radiation dose assessment. In recent years, the CBMN assay has become well-validated and emerged as a method of choice for evaluating occupational and accidental exposures scenario. It is feasible due to its cost-effective, simple, and rapid dose assessment rather than a conventional chromosome aberration assay. PubMed search tool was used with keywords of MN, biodosimetry, radiotherapy and restricted to human samples. Since Fenech and Morely developed the assay, it has undergone many technical and technological reforms as a biomarker of various applications. In this review, we have abridged recent developments of the CBMN assay in radiation triage and biodosimetry, focusing on (a) the influence of variables on dose estimation, (b) the importance of baseline frequency and reported dose–response coefficient values among different laboratories, (c) inter-laboratory comparison and (d) its limitations and means to overcome them.

Contents

Abbreviations: BN, Binucleated; CBMN, Cytokinesis Blocked Micronucleus; CT, Computed Tomography; Cyto-B, Cytochalasin-B; DC, Dicentric; DSBs, Double-Strand Breaks; FBS, Fetal Bovine Serum; FISH, Fluorescence in-situ Hybridization; Gy, Gray; IAEA, International Atomic Energy Agency; ILC, Inter-Laboratory Comparison; ISO, International Organization for Standardization; MN, Micronucleus; mSv, milli Sievert; NBUDs, Nuclear Buds; NPBs, Nucleoplasmic Bridges; PBLs, Peripheral Blood Lymphocytes; PCC, Premature Chromosome Condensation; PHA, Phytohemagglutinin; RPMI, Roswell Park Memorial Institute.

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1. Background

Upon cellular exposure to ionizing radiation, traversal of electron track followed by energy deposition induces transient or permanent changes in the molecules within the cell. The changes in the exposed test system (cells/ molecules) are being used to monitor the exposure-related consequences, including dose estimation and management of the exposed. The earliest and most straightforward dose determination method following radiation exposure involves registering daily counts of different cell types circulating in the peripheral blood; the extent and duration of the decline and subsequent recovery have been shown to correlate well with dose.[1] Consequently, other indicators like changes in enzymes, chromosomes, proteins, metabolic intermediates and gene expression and their regulators such as microRNA (miRNA) have been developed. Amongst the spectrum of changes, cytogenetic indicators are widely used for estimating the dose in accidental and suspected overexposed individuals.[2] The radiation absorbed by the exposed cells can induce strand breaks on the chromosomes. While repair, the mis-repaired breaks can result in abnormal chromosome structures. Various types of abnormal chromosomes can be identified and related to the dose. Methods that are used to estimate the chromosomal changes are dicentric chromosomes (DC) assay,[3] premature chromosome condensation (PCC) assay[4] and cytokinesis blocked micronucleus (CBMN) assay.[5] Of those three assay methods, though PCC assay can be performed even in the un-cultured cells (time-saving) rather than the DC assay, both assays require technical expertise and analysis requires longer time. Thus, CBMN assay (Fig. 1) is a widespread option for measuring radiation absorbed dose during radiation triage.

The micronucleus (MN) are extra-nuclear bodies that originate from acentric chromosomes or whole chromosomes; the fragments that lag at the anaphase of dividing cells and are not included in the nucleus appear as a small extra nucleus in cells that have completed (Table 1) one cell division.[6] Though considerably smaller in size, they are enveloped by the nuclear membrane and resemble the structure of the daughter nuclei. Acentric fragments are arising from double-stranded DNA breaks that are unrepaired before entry into anaphase. During anaphase, the malsegregation of chromosomes is usually triggered by mitotic spindle failure, centromeric DNA hypomethylation, kinetochore damage and defects in the cell cycle control system. Aside from MN, the CBMN cytome assay permit the detection of other indicators such as nucleoplasmic bridges (NBBs), nuclear buds (NBUDs), the proportion of dividing cells (cytostasis parameter), and cells undergoing apoptosis and necrosis (parameters of cytotoxicity).[7] The well-established and standardized CBMN assay in peripheral blood lymphocytes (PBLs) remains a valuable biodosimetry technique for ionizing radiation exposure and a viable alternative to the DC assay[8] because it permits the analysis of larger samples in lesser time. Despite those merits, the MN assay falls short of the DC assay's sensitivity and specificity. Regardless, owing to the advantages like time, simplicity, and associated sensitivity due to more cell count, the MN is considered an attractive assay in many biodosimetry laboratories.

2. Need for biodosimetry

Individual exposure to ionizing radiation is inevitable in day-to-day life due to its background everywhere.[9] However, the probability of additional higher exposure levels is not absent among the radiation workers as part of their occupation. At workplaces, those workers wear personnel monitoring dosimeter, allowing qualified experts to assess each worker's dose and apply the envisaged measures, a mandate and regulatory requirement. In contrast, neither is it a compulsion nor in practice that the general public would monitor with a personal monitoring device for radiation exposures. Many unfortunate incidents like nuclear terrorism or natural calamities can result in large radiation exposure to the general public.[10] Segregation of population based on the quantum of radiation exposure and or quantification of absorbed

Table 1
Base-line MN frequency observed in various populations.

S.No	Country	No. of Donors	Age range (yrs)	Cells scored	No. of MN	MN Frequency (±SE)	Reference
1.	Great Britain	14	20–45	9000	119	0.013 (±0.001)	37
2.	Germany	12	22–37	17,141	339	0.020 (±0.001)	34
3.	India	25	NA*	25,000	265	0.011 (±0.001)	138
4.	Portugal	1	29	3792	24	0.006 (±0.001)	35
5.	UK	8	23–57	28,161	332	0.012 (±0.001)	139
6.	Turkey	3	24–28	11,964	10	0.001 (±0.0002)	11
7.	France	47	25–30	42,033	470	0.011 (±0.001)	19
8.	India	21	28–46	21,000	255	0.012 (±0.001)	25

SE-Standard error.
*NA – Not Available.

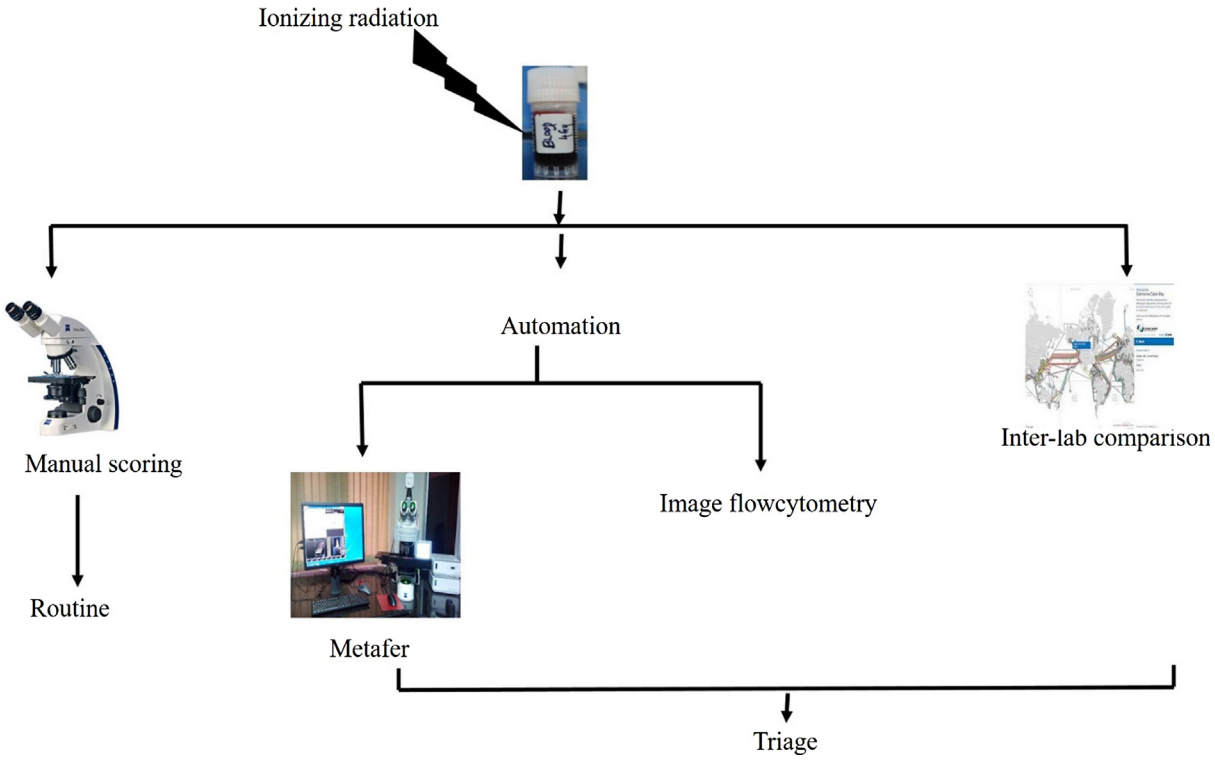

Fig. 1. Schematic representation of CBMN assay usage in the current scenario.

dose in exposed individuals is paramount to medical management. In the absence of physical dosimeters, biological indicators have been adopted to quantify the absorbed dose and confirm the suspected over-exposures. It has been extensively reported that MN frequency obtained from blood lymphocytes of exposed individuals can be adopted for those applications.[2,11–14] Using the CBMN assay to provide information on genotoxic, cytotoxic, and cytostatic effects suggests that the assay's predictive value could be improved, which merits further exploration. This systematic review emphasizes recent developments, significant limits, and steps that must be considered to achieve universal adoption of CBMN assay for dose assessment during radiation emergencies.

3. Methodology

The “PubMed” database (National Library of Medicine, National Institutes of Health, Bethesda, MD, USA) was used to search for the literature for this systematic review. The search approach was based on terms such as “MN,” “applications of MN and Radiation,” “MN and radiotherapy,” “MN scoring software,” “baseline frequency of MN,” “MN dose–response,“ “MN inter-laboratory comparisons,” and restricted to studies using human peripheral blood samples. Based on the obtained results, the assay's triage and routine biodosimetry applications and associated recent advancements have been classified according to their use and relevance to different radiation exposure scenarios.

3.1. Factors that influence reliable dose estimation

Biological dosimetry is useful for triaging casualties in the immediate aftermath of radiation emergencies and suspected radiation over-exposures. Its main goal is to give a rapid dose assessment to aid in medical management and treatment decisions to handle a larger exposed population. MN frequency has shown a reliable biomarker in human populations, exposed to ionizing radiation therapeutically, occupationally, unintentionally or naturally. The majority of the studies found that exposed populations had considerably greater MN rates than controls.[15] MN frequency obtained from an exposed person is adopted to calculate the absorbed radiation dose, which is subsequently extrapolated from the lab's standard *in vitro* reference dose–response curve using available software.[16] Although the methodology for the CBMN is well established[8] time of sample collection,[2] transport of the collected sample to the laboratory,[17] reagents used to culture and process the samples,[18] culture methodology,[19] scoring method[20] and scorers skill[21–22] had been shown to influence the yield of MN and then dose estimation in addition to the well-established factors like dose, dose-rate, types of radionuclides and radio-sensitivity. Due to economic, logistical, and infrastructure-related constraints, blood cultures are the reference method for dose estimation during unintentional ionizing radiation exposure. Thus achieving reliable and accurate dose estimation from blood cultures.

3.2. Cell culture reagents

To prepare the binucleated cells from the blood samples, widely used chemicals are culture medium, growth factors from serum and mitogens to stimulate the cells into cycling. It is well-known that composition/nutrient sources differ among culture media, particularly ascorbate, folic acid, and vitamin B12. This was because few researchers used modified McCoy's culture media,[23] whereas most of the laboratories throughout the world used RPMI 1640 medium.[24] The results demonstrated that the culture medium did not affect base-line micronucleated cell frequency when isolated lymphocytes were used for culture separately in the two types of media on the same day. It would be acceptable for the standard technique to be based on culture using RPMI 1640 medium to retain consistency with previous data. It would be essential to confirm if the results acquired are statistically identical.[23–24] Similar to that culture medium, the concentration of foetal bovine serum (FBS) used as growth factors in the culture varies among the studies, like 10 %, 20 % of total culture volume.[19,25]

Though there has been no coordinated examination of the effects of different methodological features on the outcome of MN frequency, many inter-lab comparison studies have been published that recommend modifications to the standard techniques.[26–28] The findings acquired using McCoy's medium ($R = 0.4$, $p < 0.01$) were significantly correlated with the data obtained using RPMI 1640.[23]

3.3. Temperature

Blood samples occasionally need to be transported or stored and preserved, and it's important to know how this impacts the sensitivity of the lymphocytes used in biomonitoring. Although blood samples can be kept at a reduced temperature (4 °C), it results in a loss of cell viability.[29] It has been reported that the yield of MN depends on temperature and storage time. Before, during, and after irradiations, the sample can be subjected/processed at a temperature ranging from 4 to 37 °C. It has been reported that the yield of DC depends on temperature, time[30]; and storage of exposed blood at refrigerated conditions or 20 °C (48 h) leads to the accumulation of damages.[31] Irradiated blood kept at 22 °C or 5 °C, showed no changes in MN yield and also been reported that storage of blood at 4 °C and 20 °C for 48hr with phytohemaggultinin (PHA) did not alter cell viability and proliferation[32] as well as reduction in viability.[2] Cell viability is observed maximum of 100 % immediately upon sampling, then reduces upon storage at a low temperature with and without mitogenic stimulation.[32] Except for the samples maintained at 4 °C and 20 °C in the presence of PHA (98.0 ± 0.70 % and 99.0 ± 1.15 %), showed a decline in cell viability of samples stored at 37 °C with and without PHA after 72 h shows a significant decrease compared to samples stored at 4 °C or 20 °C with and without PHA (82.0 ± 2.82 % and 75.0 ± 1.75 % compared to 90.0 ± 3.40 % to 96.5 ± 0.70 %, respectively). Cell viability values observed after 96 h of storage in samples kept at 4 °C with or without PHA (96.8 ± 1.15 % and 96.5 ± 0.71 %, respectively) demonstrated high percentages of cell viability.[32] In comparison to other samples (58.0 ± 2.12 % to 69.0 ± 2.08 %), the optimal storage condition for sustaining cell viability was at 4 °C in the presence of PHA (86.0 ± 3.05 %). To prevent apoptosis and maintain cell proliferation, keeping at 4 °C for about 96 h in the presence of PHA is ideal. It was also recommended to stimulate lymphocytes with PHA immediately after venous blood collection and keep them below 20 °C to halt lymphocytes from transforming and progressing through the cell cycle until they are incubated at 37 °C.[2]

3.4. Culture time

Human peripheral lymphocytes, a representative cell population synchronized in G0 stage, desynchronize progressively on mitogenic stimulation, with varied dynamics for each individual. It is reported[33] that a mean cycle time of roughly 12 h for peripheral lymphocytes in RPMI 1640 medium follows the first mitosis after stimulation. Evidence suggesting the medium and temperature impact the length of the cell cycle in culture.[18] Cultures were carried out for various durations, followed by adding different concentrations of Cytochalasin-B (cyto-B) to determine the optimum concentration and harvest time at which cyto-B is most efficient in yielding binucleate cells.[19]

3.5. Chemicals/Reagents

The cyto-B is a mycotoxin used to arrest the cells at the cytokinesis stage had been shown as an essential factor in determining the percentage of binucleated cells. Both the concentration and cell harvest duration followed by the addition of cyto-B were reported to vary among the studies; concentration of cyto-B varied between 3 to 6 μg/ml and cultures were harvested 24 to 28hr after the cyto-B addition.[5,34–35] Surrallés et al.,[36] have reported that 6 μg/ml concentration of cyto-B is more in halting cytokinesis than 3 μg/ml; however, it resulted in a decreased frequency of MN. In contrast, found no differences in MN frequency between the two concentrations.[37] When dealing with the cytokinesis block approach, the findings of monitoring proliferation in various individuals show that procedures with defined durations for applying cyto-B may be risky. Individual donor's responses to PHA stimulation vary in promptness; therefore this is a factor to consider.[34] The formation of MN is increased by prolonged culture of lymphocytes with PHA at varied time intervals.[38–39] An *in vitro* study reported delayed mitogenic stimulation decreases DNA damage or prolonged peripheral blood storage without mitogenic stimulation could lead to interphase cell death.[17] Prolonged culture time with PHA, delayed mitogenic stimulation and late arising first division metaphase followed by exposure of human PBL in G0 influenced chromosomal aberrations and MN frequency.[38–40]

3.6. Scoring of aberrations

Guidelines to adopt the number of cells to analyze and interpret various aberrations are described in detail.[2] However, identifying aberrations is an objective phenomenon that can be influenced by many parameters such as quality of slides, stain used, scorer ability, and experience to classify the aberration.[21] Many inter-laboratory exercises have been conducted to maintain consistency and minimize such variations.[21,26–27] To determine the extent of residual variation when laboratories scored cells from the same cultures using the same set of standard scoring criteria, an inter-laboratory slide-scoring exercise was performed among 34 laboratories from 21 countries with 51 slide scorers involved. This study shows that even under these optimized conditions, there is a variation in the MN frequency obtained by individual laboratories and scorers. It has been proved that there is an intra- and inter-lab and scorer variation in the scoring of MN.

3.7. Background frequency of MN and gender

The first step in biological dosimetry using any biomarker is constructing a reference dose–response curve. Generally, a linear-quadratic dose–response has been reported in the majority of the studies for CA and MN aberration frequency when it was scored from blood samples exposed to low LET radiations. It was emphasized that the number of cells and samples used to obtain background aberration frequency could influence the co-efficient of the reference dose–response curve. The existing literature consistently shows that the MN frequencies in a selected age group for healthy subjects vary between 1.4 fold and 2.3 fold depending on age and gender. Thus the minimum and maximum values for MN frequency in any particular age group differed, with the most significant differences occurring in the 40–59 year age range[23] in a population ($n = 62$) consisting of healthy adults of both genders. Another study further supported the results in which a significant gender effect on baseline MN favors females. At the same time, yields of radiation-induced MN did not differ significantly between genders.[41] Thus, age and gender are the most important demographic variables affecting the MN index; frequency is higher in females than males by 1.2 to 1.6, depending on the age group.[23,42] The baseline MN frequency observed in various populations around the globe is mentioned in Table 1. It's also critical to develop standardized protocols that allow more reliable data comparisons between laboratories.

3.8. Lifestyle factors

Unhealthy lifestyle factors contribute to cardiovascular disease, diabetes, obesity[43] and cancer[44]; as MN biomarker has been prospectively connected with these lifestyle diseases, lifestyle choices that cause these diseases may be linked to MN frequency in PBL. The lifestyle practices include exercise, consumption of alcohol, smoking, working hours, sleeping hours, balanced nutrition and mental stress.[45]

Physical activity has been shown to influence MN in PBL in six trials.[45–50] In a study involving 208 healthy adult male Japanese hard-metal workers aged 19–59 years, Huang et al.,[45] observed the impact of lifestyle practices. It generally ranges from a reduction in MN in fit participants following moderate activity or a triathlon event[45,50] to null effects,[47,49] and even a rise in MN in healthy volunteers following acute exhausting exercise.[46,48] Many demographic studies have looked into the effect of tobacco smoking on the frequency of MN in human lymphocytes. A total of 5710 participants were included in the database, including 3501 nonsmokers, 1409 current smokers, and 800 past smokers from occupational and environmental surveys. When compared to non-smokers, the overall outcome of the re-analysis indicated a slight non-significant decrease in MN frequencies in current and previous smokers.[51] Although when the interaction with occupational exposure is considered, heavy smokers were the only group exhibiting a substantial increase in genotoxic damage as determined by the CBMN assay in lymphocytes. Alcohol use is associated with greater MN frequency in PBL, according to studies[52–54] that have specifically and thoroughly explored the link between consumption of alcohol and MN formation. Using centromere probes, one study investigated the type of MN generated by alcoholism. It determined that the increase in MN frequency in alcoholics compared to controls is primarily due to MN originating from complete chromosomes.[55] The micronutrient concentrations required as cofactors in DNA synthesis and repair are strongly influenced by the meals and supplements consumed and the amount consumed. The relationship between MN and nutrition was initially found in erythrocytes in studies of anemia induced by a folate and vitamin B12 deficiency.[56] The MN frequency in PBL is strongly interconnected with either dietary consumption levels or plasma concentrations of vitamin B12, folate, riboflavin, pantothenate, biotin, vitamin E, beta-carotene, retinol and calcium, according to *in vivo* investigations in humans.[57–59] As concluding remarks from these study groups, mental stress and drinking alcohol had no effect on MN frequency, whereas, smoking, nutritional deficiencies, a lack of regular exercise, poor sleep, and extra work all contributed in the increased MN frequency. These data suggests that bad lifestyle practices increase MN frequency in human lymphocytes considerably.

3.9. Suitability of MN as a biological indicator for accidental exposure

The CBMN approach has been proposed for use in a mass screening role for quick biological dosimetry to corroborate the initial triage (sorting) of vast numbers of people who may have been exposed following a severe radiation event. However, less evidence is available for its use for such a purpose has been found. The speed with which data can be acquired is thought to benefit. Individualized, early and definitive assessments of radiation doses are required to give medical aid in the days following a disaster in potential radiation exposure scenarios involving huge loss of life. Because of its simplicity and rapid quantification, MN has shown to be a promising perspective tool for triage in the medical management of a nuclear disaster. However, due to a spontaneous MN frequency of 0.002–0.036/cell, its sensitivity is just 0.25 Gy[2]. CBMN assay was employed in the Chernobyl nuclear disaster[60] and the Istanbul bombing.[61].

Nonetheless, during operation, a fault at the entrance into the depleted uranium-shielding device of a ^{192}Ir source exposed an industrial radiography worker. MN frequency was not increased above the laboratory's control value of MN background frequency of unexposed individuals due to the absorbed dose falling below the lower detection limit of 0.3 Gy photon-equivalent whole-body exposure[62]. In triage mode, biological dosimetry must respond as rapidly as feasible. A preliminary dose estimate is sufficient since it divides victims into three groups for medical follow-up (less than 1 Gray (Gy), 1–2 Gy, and more than 2 Gy).[63] To meet the triage requirements, other solutions are being developed that involve automation in scoring and sharing the workload with other laboratories approaches have been proposed. The available results confirm the efficacy of the automated CBMN assay for fast population triage in a multicentric setting, in the case of large radiation accidents[64–68].

3.10. MN as a biological indicator for occupational radiation exposure

Technicians and physicians who operate with X-ray equipment, industries, and mines personnel are regularly exposed to low levels of radiation. MN is not radiation specific because various clastogenic agents can cause it in the form of acentric chromosome fragments and aneugenic agents in entire chromosomes. As a result, the CBMN assay is frequently employed in general toxicological testing[6]. The CBMN assay has proven to be a reliable, wholly established, and standardized technique in radiation biology since ionizing radiation is a robust clastogenic agent and hence a significant inducer of MN. The number of radiation-induced MN is closely connected with radiation dose and is dependent on the radiation quality.[69–72] MN yields in PBLs of diverse groups of patients treated with fractionated partial body radiotherapy, such as cervical cancer, prostate cancer, or Hodgkin's disease, were examined to determine the applicability of the CBMN assay for biological dosimetry. The doses obtained using MN analysis are similar to the averaged whole-body doses calculated using radiation treatment plans.[73–77] Studies performed in thyroid cancer patients undergoing radioiodine treatment further demonstrated that the CBMN assay is sensitive enough to detect low average whole-body doses from internal exposure scenarios.[78–80] The CBMN assay was used in specific radiation disaster investigations to determine protracted exposure owing to populations in the area of the Chernobyl nuclear power station[81] and the Semipalatinsk nuclear test site ingesting long-lived radionuclides.[82] The estimated internal absorbed dosage was significantly linked with MN frequencies measured in many residents.

Radiation employees, such as nuclear power plant personnel and hospital staff, are the subjects of large-scale biomonitoring studies, have shown that the CBMN assay, particularly when combined with fluorescence in-situ hybridization (FISH) staining for centromeres, can detect radiation-induced chromosomal damage to the population level for accumulated doses received during their occupation exceeding 50 milliSivert (mSv). These biomonitoring investigations, which looked at a broad group of radiation workers (between 70 and 270 participants) who were exposed to accumulated doses ranging from 10 to 248 mSv, revealed a clear relationship between MN development and the accumulated dose[83–86]. In nuclear power plant workers, the biomonitoring investigation revealed 0.03 MN/1000 BN cells/mSv[87] and 0.025 MN/ 1000 BN cells/mSv[84] of exposure. On the other hand, medical radiation has become a significant source of radiation exposure for the general public. It has also been shown that dosages received during radiology imaging and interventional procedures induce considerable DNA damage in patients' cells[88] and healthcare personnel[89]. The usage of MN not only ends with biomonitoring investigations in industries and cancer patients; it also extends its application to estimating absorbed dose during diagnostic radiological procedures[90–91]. The biological effects of modest dosages received during diagnostic imaging are under investigation; immediate and long-term effects are unknown. The CBMN assay has found a substantial increase in MN in PBLs from patients with plain or contrast CT scans[90]. Infants who got a second computed tomography (CT) scan after 48 h had a higher MN, implying that earlier CT imaging improved cellular responses to successive CT scans[92]. Medical radiation professionals exposed to low-dose ionizing radiation PBLs were monitored for cytogenetic changes and revealed a significant increase in MN.[93]

Table 2
α and β coefficients obtained with MN dose–response curve for different types of radiations in various laboratories.

S.No	Type of Radiation	Dose range (Gy) and Dose rate (Gy/min)	Scoring method	α ± SE x 10^{-2} Gy^{-1}	β ± SE x 10^{-2} Gy^{-1}	Reference
1.	^{60} Co-gamma	0.6	Manual	9.5 ± 1.7	4.8 ± 0.6	140
2.	^{137}Cs	0–2 & 0.9	Flow cytometry	1.84	0.91	108
3.	^{60} Co	0–4 & 0.5	Manual	1.9 ± 1.1	5.7 ± 0.45	138
4.	^{60}Co	0–5 & 0.5	Manual	9.3 ± 0.9	13.3 ± 1.4	35
5.	^{60}Co	0–4 & 0.5	Manual	5.24 ± 0.98	3.15 ± 0.35	101
6.	^{60}Co	0–4 & 0.43	Manual	4.29 ± 0.74	1.87 ± 0.23	
7.	^{60}Co	0–6 & 0.09	Manual	7.5 ± 2.23	1.76 ± 0.55	
8.	^{60}Co	0–4 & 0.7	Manual	1.48 ± 0.55	3.33 ± 0.22	
9.	^{60}Co	0–5 & 0.45	Manual	0.44 ± 0.2	1.16 ± 0.1	11
10.	^{60}Co (conventional method)	0–4 & 0.75	Manual	6.39 ± 1.2	3.22 ± 0.41	141
11.	^{60}Co (synchronized method)	0–4 & 0.75	Manual	14.70 ± 0.96	2.48 ± 0.28	141
12.	^{60}Co	0–4 & 0.5	Manual	7.06 ± 1.30	2.79 ± 0.43	19
13.	^{60}Co	0–4 & 0.75	Manual	6.39 ± 1.29	3.23 ± 0.42	25
14.	^{60}Co	0.5	Manual	7.1	2.8	142
15.	^{60}Co	0–5 & 2.05	Manual	7.5 ± 0.2	2.8 ± 0.06	143
16.	^{60}Co		Manual	7.5 ± 1.1	1.6 ± 0.3	144
17.	^{60}Co		Automated	6.14 ± 1.11	1.6 ± 0.42	26
18.	^{60}Co		Semi automated	4.56 ± 0.90	3.22 ± 0.37	
19.	^{60}Co		Manual	7.67 ± 1.8	4.18 ± 0.73	
20.	^{60}Co		Automated	1.84 ± 0.13	2.67 ± 0.07	
21.	^{137}Cs	0–8 & 6.0	Automated	92.28	−0.22	145
22.	^{137}Cs		Manual	7.18 ± 4.07	4.73 ± 9.63	26
23.	X rays (250kVp)	0–5& 1.0	Manual	11.7 ± 0.6	0.8 ± 1.6	37
24.	X rays	0–4	Manual	13.9 ± 4.4	3.68 ± 1.8	146
25.	X rays (220kVp)	0–4 & 0.5	Manual	9.77 ± 0.95	4.78 ± 0.33	147
26.	X rays	0–4	Manual	4.09 ± 2.23	4.33 ± 0.6	148
27.	X rays (240kVp)	1.0	Manual	0.120	0.023	34
28.	X rays (250kVp)	0–4 & 0.5	Manual	6.3 ± 1.8	4.3 ± 0.7	138
29.	X rays (250kVp)	0–5& 2.0	Manual	4.95 ± 1.6	4.61 ± 0.6	139
30.	X rays	0–4 & 1.0	Manual	5.96 ± 1.10	3.92 ± 0.36	149
31.	X-rays		Automated	4.94	4.1	150
32.	X ray (240kVp)	1.0	Automated	3.7 ± 1.14	3.6 ± 0.44	26
33.	X ray (240kVp)	1.0	Semi automated	5.47 ± 0.96	1.66 ± 0.02	
34.	X ray (240kVp)	1.0	Automated	5.35 ± 0.66	1.54 ± 0.12	
35.	X ray (240kVp)	1.0	Automated	5.89 ± 1.16	−–	
36.	X ray (240kVp)		Manual	9.23 ± 1.59	6.01 ± 0.58	
37.	X rays (250kVp)	0–4 & 1.3	Imaging flow cytometry	0.06	0.98	151
38.	2.27 MeV β particles	0–2	Manual	2.48 ± 1.3	3.8 ± 1.0	139
39.	Electrons		Manual	8.4 ± 1.6	2.1 ± 0.3	144
40.	X-rays (225kVp)		Manual	8.2 ± 0.9	2.3 ± 0.6	152
	X-rays (3 MV)		Manual	7.4 ± 3.9	1.8 ± 1.3	
	X-rays (6 MV)		Manual	6.0 ± 1.4	1.8 ± 0.5	

α − Linear coefficient; β − Quadratic coefficient; SE − Standard error.

3.11. Recent advances and developments in biodosimetry using CBMN assay

Potential scenarios of radiation exposure resulting in mass casualties require individual, early, and definitive radiation dose assessment to provide medical aid within days of a disaster. The essential steps in triage medical management are preliminary dose assessment and segregation of exposed and non-exposed people. Furthermore, first responders must be checked on a regular basis to verify that the dose levels they are exposed to during evacuation are safe. Alternative ways are being developed to satisfy the need, as traditional cytogenetic procedures by manual scoring are time-consuming; current improvements in biodosimetry include task sharing among expert groups, automation of analytical processes, and early indicators to ionizing radiation. Table 2 shows α and β coefficients obtained with MN dose–response curve for different types of radiations in various laboratories irrespective of scoring methods.

3.12. Need for the network for biodosimetry

In recent years, the arrangements to undertake triage in radiation mass casualty scenarios have been a key new development. A biological dosimetry laboratory's response to a rapid rise in cases is discussed, including using assays in a triage mode, speeding up analysis with computer-assisted microscopy, and networking with other labs. International recommendations on quality assurance, quality control, and participation in inter-laboratory comparison (ILC) exercises have coincided with this enhanced provision for collaborative emergency response work.[2,94] Biodosimetry laboratories can only handle a certain number of victims at a time. Sharing the workload among the experts is an attractive option for handling a considerable sample size. Thus, in the event of a mass-casualty radiation disaster, when the management of hundreds of victims is required, national and/or international networking is required. On the other hand, networking must be founded on the ability to produce consistent outcomes.[95–96] This means that to

Table 3
Comparison of different automated platforms available for robustness in MN analysis during triage.

Platform	Advantages	Disadvantages
Microscopy analysis of CBMN cells stained with Giemsa	• Slides can be stored for longer time and can be reanalysed for verification	• At times artefacts due to non-specific staining can be counted as MN
Fluorescence microscopy analysis of CBMN cells stained with DAPI/PI	• Better sensitivity than giemsa stained preparations	• Fluorescence microscope is expensive • Slides can't be used for reanalysis or verification
Flow cytometry	• Cells measured in fluid content • Rapid analysis	• Not suitable for biodosimetry
Image flow cytometry	• Cells measured in fluid content • Robust analysis • High-resolution image	• Necessitates expert knowledge • Rigorous optimization is required • Cannot re-locatable • Sensitivity > 1 Gy
MetaSystems	• Slide based analysis • Manual correction is possible • Easily re-locatable • Suitable for Giemsa and fluorescent dyes • More than 100 slides per day • No expertise required	• Sensitivity > 0.25 Gy
IMSTAR Pathfinder	• Based on unique algorithms • It enables the MN scoring in mono- bi- or poly nucleated-cells	• Necessitates expert knowledge • Not yet used for biodosimetry purpose
iCyte® automated imaging cytometer, CompuCyte	• It works by combining of flow and image cytometry • 10–20 slides per day	• Not yet commercialized
Rapid Automated Biodosimetry Tool (RABiT)	• Completely automated (right from sampling to analysis) • High-throughput • Increases scoring efficiency and handling capacity	• Not yet commercialized • Cost factor

get consistent dose estimate/results from the participating laboratories, all the labs should adopt a similar culture methodology, reagents used to culture cells, sample processing and scoring methods.[97] International accreditation bodies like the International Organization for Standardization (ISO) have contributed immensely to this goal by providing standard performance criteria (pertaining to quality assurance and quality control) for cytogenetic service laboratories undertaking biodosimetry[98]. It is now widely agreed that networking should include regular international ILC exercises exhibiting various scenarios, ensuring a faster reaction and higher dose estimate reliability.[99]

ILC also aids in the harmonisation of culture conditions, scoring standards, and statistical analysis techniques. If laboratories build up networks to respond to a mass casualty event, this harmonisation is required. The number of potentially exposed individuals to be analyzed the response capabilities of the local responders[100]. The mutual assistance of several laboratories is required to increase the number of samples to be processed and achieve faster results availability. In 2007, the WHO initiated "BioDoseNet," a network of more than 30 laboratories worldwide and implemented revised regulations pertaining to human health, including the field of radio-nuclear incidents.[97] The approach for increasing throughput is the development of networks among laboratories available on a global scale. Several networks have been known to improve the rate of dose estimation, such as the Latin American Biological Dosimetry Network[101], the National Biological Dosimetry Response Plan (NBDRP) in Canada[102], the Chromosome Network in Japan[103], the European Network, Realizing the European Network of Biodosimetry (RENEB)[104], NATO biodosimetry group[26], India[105] and in China[106] for the DCA; as it requires more time and remains the sensitive assay. However, compared to DCA, ILC using CBMN assay is limited[21,26,28]. The goal of ILCs is to determine problems that participants have and develop solutions, such as harmonization, training, and dose estimation activities. Garcia et al.,[101] reported that 11 of the 15 estimates of dose based on DC and 9 of the 12 based on MN fell within ± 30 % of the correct dose. The outcome of the ILCs showed that MN is suitable for dose estimation during radiation triage. However, it has been reported that differences in scoring criteria, analysis of frequencies, dose rate and the radiation source for dose estimation and lack of homogeneity in calculating the uncertainties were observed as limits and pitfalls of ILCs.[107]

3.13. Automation

In addition to the ILC exercise among the participant laboratories to handle larger samples, rapid technological advancement has been made to score the MN with increased efficiency and faster automation. Several automated platforms, Flow cytometry,[108] automated microscope,[109] imaging flow cytometry,[110] IMSTAR Pathfinder™,[111] iCyte® automated imaging cytometer[112] and Rapid Automated Biodosimetry Tool (RABiT)[113] have been established to execute the CBMN assay and analysis. These platforms have advantages and limitations in robust, high-throughput, sensitivity, specificity, and visual confirmation of MN (Table 3).

3.14. Flow cytometry-based micronucleus (FCMN) assay

Developing an FCMN assay to measure the MN frequency can improve reproducibility and reduce turn-around time compared with the traditional microscopy-based analysis. Flow cytometry is a technology that simultaneously measures and analyses multiple physical characteristics of single particles, usually cells, as they flow in a fluid stream through a beam of light; properties measured include a particle's relative size, granularity, or internal complexity and fluorescence intensity. These characteristics are determined using an optical-to-electronic coupling system that records how the cell or particle scatters incident laser light and emits fluorescence. Any suspended particle or cell from 0.2–150 μm in size is suitable for analysis. Therefore, FCMN was considered as a high throughput and potential for triage. Speed is an inherent advantage of flow cytometry; however each particle present in the suspension produces a signal and is registered separately, making it difficult or sometimes impossible to discriminate between MN from the nonspecific background.[108]

Moreover, DNA content in MN lower than 2 % of the G1-phase cells could not be discriminated from debris. An improvement has been made to separate debris from the MN according to their different physical properties to increase sensitivity and reproducibility and to measure the number and size distribution of MN.[108] Even though this technique agreed well with microscopy, difficulties were observed in differentiating true MN from other DNA positive events.[114–115] The existing modern flow cytometry approach retains the capability to

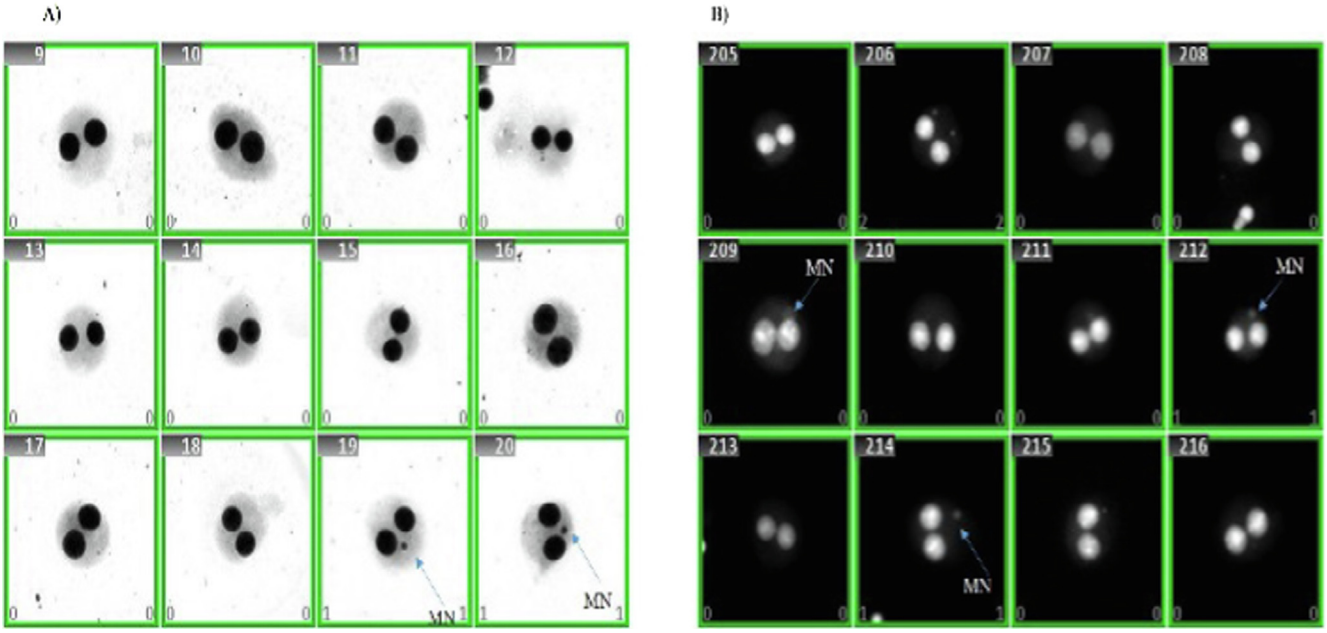

Fig. 2. Images of cytochalasin-B blocked binucleated cells from human peripheral blood lymphocytes obtained using Metafer microscope. (A) Giemsa-stained slides −binucleated cells with cytoplasm (B) propidium iodide stained slides − binucleated cells.

acquire data from thousands of cells in a limited time and permits automatic analysis of all data. The rapidity of this technique and the requirement for nominal operator intervention are attractive features for toxicology testing. Avlasevich et al.,[116] and Byrce et al.,[117] had made substantial developments to the procedure by combining fluorescent dyes to overcome the problems encountered when distinguishing cellular debris, MN and free chromosomes from mitotic cells. Research engaging various well-known clastogens and aneugens have confirmed that statistically significant increases in MN frequency can be detected compared with controls using *In Vitro* MicroFlow Kit (Litron Laboratories, Rochester, NY).[116–117] Inter- and intra-individual variations of spontaneous and radiation-induced MN frequencies, the minimum detection limit has been reported as above the dose of 1 Gy.[117–119] However, the lack of cellular visualization (Mono, bi and poly nucleated cells) is the major drawback of conventional flow cytometry methods. It is difficult to confirm that MN identified are genuine. An additional disadvantage is that cell membranes need to be lysed to release both the main nuclei and MN from a cell, leading to debris such as individual apoptotic bodies. Regardless of the contemporary developments in the approach, it is difficult to make a decisive difference between MN and DNA positive debris.[112] To overcome this, Rodrigues[120] developed a microscopic-based method called an imaging flow cytometer, which can combine the high-throughput nature of traditional flow cytometry with microscopy's high-resolution imaging. The IDEAS® software was used to make a rapid and automated MN assay based on high throughput picture capture and feature-based image analysis using imaging flow cytometry with the ImageStream®. The Amnis® AI software was used to construct a deep-learning algorithm based on convolutional neural networks to score imaging flow cytometry data in both the cytokinesis-blocked and unblocked versions of the MN test to overcome the complexity and rigidity of feature-based image analysis.[110] However, optimization and validation are required for the next step in the development of automated MN assay.

3.15. Automated microscope for MN scoring

Despite the potential for rapid scoring of MN with flow cytometry, difficulty in discriminating MN from artifacts arising from either cell processing or as a consequence of apoptosis and necrosis leads to a false-positive interpretation[119] and compromise in the sensitivity; furthermore, difficulty in analyzing nucleoplasmic bridges (NPBs) and nuclear buds (NUBDs), sample preservation and re-analysis are added limitations.[22] Automated image cytometry is preferred because improved computer algorithms allow more advanced image analysis on a cell-by-cell basis with higher sensitivity. Tates et al.,[121] reported that 63 % of the BN cells and 57 % of the MN within these BN cells and exposure could be detected automatically. Castelain et al.,[122] were able to detect 67 % of the BN cells and 33 % of the MN in these BN cells automatically with the help of the Magiscan Image Analysis System; thus though the speed was increased using automated scoring, it is capable of detecting 1/2th of the BN cells and 3/4th of the MN in these cells. It was attributed to that relatively high inaccuracy in the classification of the BN cells. Of late, systems like MetaSystems Metafer MNScore,[109] IMSTAR Pathfinder™ Screentox Auto-MN[111] and Compucyte iCyte® Laser Scanning cytometer,[112] which are commercially available to increase the scoring speed of MN with better accuracy in identifying the MN and BN cells.

3.16. MetaSystems

MetaSystems developed and commercialized a scoring system with a software module (Metaphase finder platform Metafer4), which allows automatic scoring of MN in BN cells.[109] This image analysis software detects the BN cells based on the similarity of the daughter nuclei [Fig. 2]. However, one should keep it in mind that the MetaSystems software employed to analyze the MN does not use the cytoplasmic boundary to identify BN cells, critical criteria adopted in manual scoring; further, if required, the cytoplasmic boundary should be visualized using phase-contrast microscopy[123] to verify the accuracy of BN cell detection. The first commercially available and widely used system is the Metafer MNScore (MetaSystems) platform in cancer research,[124] biomonitoring studies in the field of air pollution,[125] cell lines in *in vitro* studies[126] and radiation biodosimetry.[14] The comparison of manual and automated scoring was 23.6 % different, with an efficiency of 24.9 % in the obtained MN frequencies. Slides stained with fluorescent dyes are better for automated scoring than Giemsa-stain.[14] This MN technique allows reliable dose reconstruction after

high radiation doses with a method adapted for automated high-speed sample processing systems.[127] The manually scored slides can eliminate false positives, quantify cytotoxicity, and conduct mechanistic research. The classifier setting should be used when performing MN scoring on the semi-automated Metafer system to account for chemical or cell line-specific morphological alterations and avoid false findings (positive and negative). These semi-automated and completely automated platforms can thus be employed for dose–response analysis since they can score a much larger number of cells, allowing for more statistical power. Future studies should focus on addressing these problems and assessing inter-laboratory repeatability for these methodologies to be more extensively employed for MN scoring and dose–response analyses.

3.17. IMSTAR PathfinderTM

The IMSTAR Pathfinder™ Screentox Auto-MN, an automated imaging system for the CBMN assay, is based on advanced unique algorithms in successive steps; first, the cytoplasm of each cell within the whole sample, accurate recognition of the number and morphology characteristics of nuclei and MN within every cell; thus it enable the MN scoring in mono- bi- or poly nucleated-cells, the measurement of the cytokinesis block proliferation index (CBPI), as well as the validation by an expert/technician.[111]

3.18. iCyte® automated imaging cytometer, CompuCyte

Laser scanning cytometry (LSC) using iCyte® (CompuCyte Corporation, Westwood, MA, USA) is an alternative method suitable for automated MN scoring, which offers unique analytical capabilities that combine those of flow and image cytometry.[128] This system has been validated to score propidium iodide (PI)-stained MN in BN cells in cell lines,[129] PBL or bone marrow erythrocytes and buccal cells. The number of slides that can be analyzed per day is relatively low in the range of 10–20 per day and for Giemsa stain, this automated method is not validated. Of late, many types of equipment with automation platforms to score MN are developed and used by individual laboratories. They are not commercialized; for example, Cellomics,[130] GE Healthcare,[131] ROBIAS (Robotic Image Analysis System),[132] RABiT (Rapid Automated Biodosimetry Tool).[133]

3.19. Rapid automated biodosimetry Tool (RABiT)

The RABiT system is designed as entirely automated, from the input of the capillary blood sample into the machine to the output of a dose estimate. Using RABiT, the blood pricked from the finger is sufficient to get all the data based on the complete automation of two well-characterized biodosimetric assays like the CBMN assay. The CBMN assay can be performed and are highly radiation-specific; the estimated blind doses were within 20 % of the actual dose in 97 % of the samples[113]. The obtained results using the high-throughput RABiT-IFC CBMN assay maintain the potential to increase scoring and handling capacity for triage biodosimetry during a large-scale radiological/nuclear event.[134] Several studies have confirmed the reliability of the automated CBMN assay for high throughput population triage.[113,135–136] Recently, a quick CBMN harvest protocol has been proposed for isolated peripheral blood mononuclear cells. It is a viable alternative to cytocentrifugation, as many scorable binucleated cells were obtained with routine biodosimetry.[137] In summary, however, the advancements mentioned earlier might be helpful after the validation and standardization of the proposed technique.

4. Conclusion

The methodology described here can improve triage radiation biodosimetry response capacity using CBMN assay. The traditional CBMN assay (microscopic analysis of CBMN cells) has been thoroughly validated and standardized. The alternative approaches, such as centromere staining, NPB scoring, and MN scoring automation, have recently been optimized and are still being developed, making the CBMN assay more sensitive and specific for radiation dose estimations and a particular interest in large-scale screening applications. Comparable results can be obtained and the high throughput of automated MN scoring can be increased to allow a rapid response to large-scale radiation accidents by establishing and creating an international network of trained laboratories using similar equipment for MN automation and the same classifiers standardized fixation protocols, and so on.

To optimize the usage in retrospective biodosimetry and investigate cases of extended and partial-body exposure, the CBMN assay has to be refined further. Only a limited amount of data is available on MN's disappearance, and more research and validation are required. For more complex exposure scenarios, appropriate calibration curves must also be constructed. At last, a test system that combines the high-throughput, high-content, and multiplexing capabilities of flow cytometry with the image analysis benefits of re-validation and data storage would be a significant step toward creating a genuinely twenty-first-century method.

5. Consent for publication

Not Applicable.

6. Ethics approval and consent to participate

Not Applicable.

7. Author's contributions

TG wrote the first draft of the manuscript and edited by PV. All authors discussed, edited and approved the final version of the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Vorobiev AI. Acute radiation disease and biological dosimetry in 1993. *Stem Cells*. 2009;15:269–274. https://doi.org/10.1002/stem.5530150736.
2. IAEA 2011 2011. https://www.iaea.org/publications/8735/cytogenetic-dosimetry-applications-in-preparedness-for-and-response-to-radiation-emergencies (accessed April 29, 2022).
3. Ohtaki K, Shimba H, Awa AA, Sofuni T. Comparison of type and frequency of chromosome aberrations by conventional and G-staining methods in Hiroshima atomic bomb survivors. *J Radiat Res*. 1982;23:441–449. https://doi.org/10.1269/jrr.23.441.

4. Hittelman WN, Rao PN. Premature chromosome condensation. I. Visualization of x-ray-induced chromosome damage in interphase cells. *Mutat Res*. 1974;23:251–258. https://doi.org/10.1016/0027-5107(74)90145-6.
5. Fenech M, Morley A. Solutions to the kinetic problem in the micronucleus assay. *Cytobios*. 1985;43:233–246.
6. Luzhna L, Kathiria P, Kovalchuk O. Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond. *Front Genet*. 2013;4. https://doi.org/10.3389/fgene.2013.00131.
7. Fenech M. Cytokinesis-block micronucleus assay evolves into a "cytome" assay of chromosomal instability, mitotic dysfunction and cell death. *Mutat Res*. 2006;600:58–66. https://doi.org/10.1016/j.mrfmmm.2006.05.028.
8. ISO 2014. ISO 17099:2014. ISO n.d. https://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/05/91/59141.html (accessed April 29, 2022).
9. UNSCEAR 1982 report n.d. https://www.unscear.org/unscear/en/publications/1982.html (accessed April 29, 2022).
10. Williams M, Armstrong L, Sizemore DC. *Biologic, Chemical, and Radiation Terrorism Review*. StatPearls, Treasure Island (FL): StatPearls Publishing; 2022.
11. Bender MA, Gooch PC. Persistent chromosome aberrations in irradiated human subjects. *Radiat Res*. 1962;16:44–53.
12. Koksal G, Pala FS, Dalci DO. In vitro dose-response curve for chromosome aberrations induced in human lymphocytes by 60Co gamma-radiation. *Mutat Res*. 1995;329:57–61. https://doi.org/10.1016/0027-5107(95)00019-f.
13. Bertho JM, Roy L. A rapid multiparametric method for victim triage in cases of accidental protracted irradiation or delayed analysis. *Br J Radiol*. 2009;82:764–770. https://doi.org/10.1259/bjr/49063618.
14. Tamizh Selvan G, Chaudhury NK, Venkatachalam P. Comparison of results of the manual and automated scoring of micronucleus frequencies in (60)Co-irradiated peripheral blood lymphocytes for triage dosimetry. *Appl Radiat Isot*. 2015;97:70–77. https://doi.org/10.1016/j.apradiso.2014.12.018.
15. Sari-Minodier I, Orsière T, Bellon L, Pompili J, Sapin C, Botta A. Cytogenetic monitoring of industrial radiographers using the micronucleus assay. *Mutat Res*. 2002;521:37–46. https://doi.org/10.1016/s1383-5718(02)00213-9.
16. Ainsbury EA, Lloyd DC. Dose estimation software for radiation biodosimetry. *Health Phys*. 2010;98:290–295. https://doi.org/10.1097/01.HP.0000346305.84577.b4.
17. Selvan GT, Bhavani M, Vijayalakshmi J, Solomon FDP, Chaudhury NK, Venkatachalam P. Delayed Mitogenic Stimulation Decreases DNA Damage Assessed by Micronucleus Assay in Human Peripheral Blood Lymphocytes after ^{60}CO Irradiation. Dose-Response 2014;12:dose-response.1. doi: 10.2203/dose-response.13-060.Selvan.
18. Purrott RJ, Lloyd DC, Vulpis N. Chromosome dosimetry: the influence of culture media on the proliferation of irradiated and unirradiated human lymphocytes. *Radiat Prot Dosim*. 1981;1:203–208.
19. Paillole N, Voisin P. Is micronuclei yield variability a problem for overexposure dose assessment to ionizing radiation? *Mutat Res*. 1998;413:47–56. https://doi.org/10.1016/s1383-5718(98)00015-1.
20. Brown JK, Williams A, Withers HR, et al. Sources of variability in the determination of micronuclei in irradiated peripheral blood lymphocytes. *Mutat Res*. 1997;389:123–128. https://doi.org/10.1016/s1383-5718(96)00127-1.
21. Fenech M, Bonassi S, Turner J, et al. Intra- and inter-laboratory variation in the scoring of micronuclei and nucleoplasmic bridges in binucleated human lymphocytes. *Mutation Research/genetic Toxicol Environ Mutagen*. 2003;534:45–64. https://doi.org/10.1016/S1383-5718(02)00248-6.
22. Fenech M, Kirsch-Volders M, Rossnerova A, et al. HUMN project initiative and review of validation, quality control and prospects for further development of automated micronucleus assays using image cytometry systems. *Int J Hyg Environ Health*. 2013;216:541–552. https://doi.org/10.1016/j.ijheh.2013.01.008.
23. Fenech M. Important variables that influence base-line micronucleus frequency in cytokinesis-blocked lymphocytes-a biomarker for DNA damage in human populations. *Mutat Res*. 1998;404:155–165. https://doi.org/10.1016/s0027-5107(98)00109-2.
24. Surrallés J, Natarajan AT. Human lymphocytes micronucleus assay in Europe. An International Survey. *Mutat Res*. 1997;392:165–174. https://doi.org/10.1016/s0165-1218(97)00054-2.
25. Venkatachalam P, Solomon FD, Prabhu BK, Mohankumar MN, Gajendiran N, Jeevanram RK. Estimation of dose in cancer patients treated with fractionated radiotherapy using translocation, dicentrics and micronuclei frequency in peripheral blood lymphocytes. *Mutat Res*. 1999;429:1–12. https://doi.org/10.1016/s0027-5107(99)00096-2.
26. Romm H, Barnard S, Boulay-Greene H, et al. Laboratory Intercomparison of the Cytokinesis-Block Micronucleus Assay. *Radiat Res*. 2013;180:120–128. https://doi.org/10.1667/RR3234.1.
27. Wilkins RC, Beaton-Green LA, Lachapelle S, et al. Evaluation of the annual Canadian biodosimetry network intercomparisons. *Int J Radiat Biol*. 2015;91:443–451. https://doi.org/10.3109/09553002.2015.1012305.
28. Depuydt J, Baeyens A, Barnard S, et al. RENEB intercomparison exercises analyzing micronuclei (Cytokinesis-block Micronucleus Assay). *Int J Radiat Biol*. 2017;93:36–47. https://doi.org/10.1080/09553002.2016.1206231.
29. Granath F, Darroudi F, Auvinen A, et al. Retrospective dose estimates in Estonian Chernobyl clean-up workers by means of FISH. *Mutat Res*. 1996;369:7–12. https://doi.org/10.1016/s0165-1218(96)90042-7.
30. Virsik-Peuckert RP, Harder D. Temperature and the formation of radiation-induced chromosome aberrations. II. The temperature dependence of lesion repair and lesion interaction. *Int J Radiat Biol Relat Stud Phys Chem Med*. 1986;49:673–681. https://doi.org/10.1080/09553008514552921.
31. Moroni MM, Krasnopolsky K, Subramanian U, Martin PR, Doherty KM, S PG, et al. *Corresponding Author: 2008.
32. Belloni P, Meschini R, Palitti F. Effects of storage conditions of human whole blood on the viability of lymphocytes. *Int J Radiat Biol*. 2008;84:613–619. https://doi.org/10.1080/09553000802203630.
33. Gonsebatt ME, Mutchinick O. Human lymphocyte proliferation kinetics in Hanks' BSS supplemented with autologous plasma and in synthetic medium. *Mutat Res*. 1990;243:255–258. https://doi.org/10.1016/0165-7992(90)90140-f.
34. Gantenberg HW, Wuttke K, Streffer C, Müller WU. Micronuclei in human lymphocytes irradiated in vitro or in vivo. *Radiat Res*. 1991;128:276–281.
35. Silva MJ, Carothers A, Dias A, Luis JH, Piper J, Boavida MG. Dose dependence of radiation-induced micronuclei in cytokinesis-blocked human lymphocytes. *Mutat Res*. 1994;322:117–128. https://doi.org/10.1016/0165-1218(94)00019-0.
36. Surrallés J, Carbonell E, Marcos R, Degrassi F, Antoccia A, Tanzarella C. A collaborative study on the improvement of the micronucleus test in cultured human lymphocytes. *Mutagenesis*. 1992;7:407–410. https://doi.org/10.1093/mutage/7.6.407.
37. Prosser JS, Moquet JE, Lloyd DC, Edwards AA. Radiation induction of micronuclei in human lymphocytes. *Mutat Res*. 1988;199:37–45. https://doi.org/10.1016/0027-5107(88)90228-x.
38. Hoffmann GR, Sayer AM, Littlefield LG. Higher frequency of chromosome aberrations in late-arising first-division metaphases than in early-arising metaphases after exposure of human lymphocytes to X-rays in G0. *Int J Radiat Biol*. 2002;78:765–772. https://doi.org/10.1080/09553000210152962.
39. Krishnaja AP, Sharma NK. Differential radiation effects in smokers–culture time dependence of the yield of gamma ray-induced chromosome damage in first division metaphases. *Int J Radiat Biol*. 2006;82:363–377. https://doi.org/10.1080/09553000600774097.
40. Holmberg K, Meijer AE, Harms-Ringdahl M, Lambert B. Chromosomal instability in human lymphocytes after low dose rate gamma-irradiation and delayed mitogen stimulation. *Int J Radiat Biol*. 1998;73:21–34. https://doi.org/10.1080/095530098142671.
41. Joksic G, Petrovic S, Ilic Z. Age-related changes in radiation-induced micronuclei among healthy adults. *Braz J Med Biol Res*. 2004;37:1111–1117. https://doi.org/10.1590/S0100-879X2004000800002.
42. Bolognesi C, Abbondandolo A, Barale R, et al. Age-related increase of baseline frequencies of sister chromatid exchanges, chromosome aberrations, and micronuclei in human lymphocytes. *Cancer Epidemiol Biomarkers Prev*. 1997;6:249–256.
43. Andreassi MG, Barale R, Iozzo P, Picano E. The association of micronucleus frequency with obesity, diabetes and cardiovascular disease. *Mutagenesis*. 2011;26:77–83. https://doi.org/10.1093/mutage/geq077.
44. Metcalfe C, Davey Smith G, Macleod J, Hart C. The role of self-reported stress in the development of breast cancer and prostate cancer: a prospective cohort study of employed males and females with 30 years of follow-up. *Eur J Cancer*. 2007;43:1060–1065. https://doi.org/10.1016/j.ejca.2007.01.027.
45. Huang P, Huang B, Weng H, Nakayama K, Morimoto K. Effects of lifestyle on micronuclei frequency in human lymphocytes in Japanese hard-metal workers. *Prev Med*. 2009;48:383–388. https://doi.org/10.1016/j.ypmed.2008.12.023.
46. Schiffl C, Zieres C, Zankl H. Exhaustive physical exercise increases frequency of micronuclei. *Mutat Res*. 1997;389:243–246. https://doi.org/10.1016/s1383-5718(96)00154-4.
47. Hartmann A, Pfuhler S, Dennog C, Germadnik D, Pilger A, Speit G. Exercise-Induced DNA Effects in Human Leukocytes Are Not Accompanied by Increased Formation of 8-Hydroxy-2′-Deoxyguanosine or Induction of Micronuclei. *Free Radic Biol Med*. 1998;24:245–251. https://doi.org/10.1016/S0891-5849(97)00249-9.
48. Umegaki K, Higuchi M, Inoue K, Esashi T. Influence of one bout of intensive running on lymphocyte micronucleus frequencies in endurance-trained and untrained men. *Int J Sports Med*. 1998;19:581–585. https://doi.org/10.1055/s-2007-971963.
49. Pittaluga M, Parisi P, Sabatini S, et al. Cellular and biochemical parameters of exercise-induced oxidative stress: relationship with training levels. *Free Radic Res*. 2006;40:607–614. https://doi.org/10.1080/10715760600623015.
50. Reichhold S, Neubauer O, Ehrlich V, Knasmüller S, Wagner K-H. No acute and persistent DNA damage after an Ironman triathlon. *Cancer Epidemiol Biomarkers Prev*. 2008;17:1913–1919. https://doi.org/10.1158/1055-9965.EPI-08-0293.
51. Bonassi S, Neri M, Lando C, et al. Effect of smoking habit on the frequency of micronuclei in human lymphocytes: results from the Human MicroNucleus project. *Mutat Res*. 2003;543:155–166. https://doi.org/10.1016/s1383-5742(03)00013-9.
52. Ishikawa H, Yamamoto H, Tian Y, Kawano M, Yamauchi T, Yokoyama K. Effects of ALDH2 gene polymorphisms and alcohol-drinking behavior on micronuclei frequency in non-smokers. *Mutat Res*. 2003;541:71–80. https://doi.org/10.1016/s1383-5718(03)00179-7.
53. Ishikawa H, Miyatsu Y, Kurihara K, Yokoyama K. Gene-environmental interactions between alcohol-drinking behavior and ALDH2 and CYP2E1 polymorphisms and their impact on micronuclei frequency in human lymphocytes. *Mutat Res*. 2006;594:1–9. https://doi.org/10.1016/j.mrfmmm.2005.07.005.
54. Ishikawa H, Ishikawa T, Yamamoto H, Fukao A, Yokoyama K. Genotoxic effects of alcohol in human peripheral lymphocytes modulated by ADH1B and ALDH2 gene polymorphisms. *Mutat Res*. 2007;615:134–142. https://doi.org/10.1016/j.mrfmmm.2006.11.026.
55. Maffei F, Forti GC, Castelli E, Stefanini GF, Mattioli S, Hrelia P. Biomarkers to assess the genetic damage induced by alcohol abuse in human lymphocytes. *Mutat Res*. 2002;514:49–58. https://doi.org/10.1016/s1383-5718(01)00318-7.

56. Dawson DW, Bury HP. The significance of Howell-Jolly bodies and giant metamyelocytes in marrow smears. *J Clin Pathol*. 1961;14:374–380. https://doi.org/10.1136/jcp.14.4.374.
57. Fenech M, Rinaldi J. The relationship between micronuclei in human lymphocytes and plasma levels of vitamin C, vitamin E, vitamin B12 and folic acid. *Carcinogenesis*. 1994;15:1405–1411. https://doi.org/10.1093/carcin/15.7.1405.
58. Titenko-Holland N, Jacob RA, Shang N, Balaraman A, Smith MT. Micronuclei in lymphocytes and exfoliated buccal cells of postmenopausal women with dietary changes in folate. *Mutation Res./Genet. Toxicol. Environ. Mutagen.*. 1998;417:101–114. https://doi.org/10.1016/S1383-5718(98)00104-1.
59. Schneider M, Diemer K, Engelhart K, Zankl H, Trommer WE, Biesalski HK. Protective effects of vitamins C and E on the number of micronuclei in lymphocytes in smokers and their role in ascorbate free radical formation in plasma. *Free Radic Res*. 2001;34:209–219. https://doi.org/10.1080/10715760100300201.
60. Mikhalevich LS, De Zwart FA, Perepetskaya GA, Chebotareva NV, Mikhalevich EA, Tates AD. Radiation effects in lymphocytes of children living in a Chernobyl contaminated region of Belarus. *Int J Radiat Biol*. 2000;76:1377–1385. https://doi.org/10.1080/09553000050151655.
61. Internationale Atomenergie-Organisation, Internationale Atomenergie-Organisation, editors. Cytogenetic analysis for radiation dose assessment: a manual. Vienna: IAEA; 2001.
62. Beinke C, Ben-Shlomo A, Abend M, Port M. A case report: cytogenetic dosimetry after accidental radiation exposure during (192)Ir industrial radiography testing. *Radiat Res*. 2015;184:66–72. https://doi.org/10.1667/RR14013.1.
63. Herate C, Sabatier L. Retrospective biodosimetry techniques: Focus on cytogenetics assays for individuals exposed to ionizing radiation. *Mutation Res/Rev Mutat Res*. 2020;783. https://doi.org/10.1016/j.mrrev.2019.108287 108287.
64. Willems P, August L, Slabbert J, et al. Automated micronucleus (MN) scoring for population triage in case of large scale radiation events. *Int J Radiat Biol*. 2010;86:2–11. https://doi.org/10.3109/09553000903264481.
65. Ainsbury EA, Al-Hafidh J, Bajinskis A, et al. Inter- and intra-laboratory comparison of a multibiodosimetric approach to triage in a simulated, large scale radiation emergency. *Int J Radiat Biol*. 2014;90:193–202. https://doi.org/10.3109/09553002.2014.868616.
66. Lee Y, Jin YW, Seong KM, Wilkins RC, Jang S. Improving radiation dosimetry with an automated micronucleus scoring system: correction of automated scoring errors. *Radiat Environ Biophys*. 2023;62:349–356. https://doi.org/10.1007/s00411-023-01030-7.
67. Bertucci A, Wilkins RC, Lachapelle S, Turner HC, Brenner DJ, Garty G. Comparison of isolated lymphocyte and whole blood-Based CBMN assays for radiation triage. *Cytogenet Genome Res*. 2023;1–11. https://doi.org/10.1159/000533488.
68. Ting Goh VS, Fujishima Y, Nakayama R, et al. Manual scoring with shortened 48 h cytokinesis-block micronucleus assay feasible for triage in the event of a mass-casualty radiation accident. *Radiat Res*. 2023;199. https://doi.org/10.1667/RADE-22-00191.1.
69. Verhaegen F, Vral A. Sensitivity of micronucleus induction in human lymphocytes to low-LET radiation qualities: RBE and correlation of RBE and LET. *Radiat Res*. 1994;139:208–213.
70. Vral A, Verhaegen F, Thierens H, De Ridder L. Micronuclei induced by fast neutrons versus 60Co gamma-rays in human peripheral blood lymphocytes. *Int J Radiat Biol*. 1994;65:321–328. https://doi.org/10.1080/09553009414550381.
71. Wuttke K, Müller WU, Streffer C. The sensitivity of the in vitro cytokinesis-blocked micronucleus assay in lymphocytes for different and combined radiation qualities. *Strahlenther Onkol*. 1998;174:262–268. https://doi.org/10.1007/BF03038719.
72. Gersey B, Sodolak J, Hada M, et al. Micronuclei induction in human fibroblasts exposed in vitro to Los Alamos high-energy neutrons. *Adv Space Res*. 2007;40:1754–1757. https://doi.org/10.1016/j.asr.2007.03.018.
73. Fenech M, Denham J, Francis W, Morley A. Micronuclei in cytokinesis-blocked lymphocytes of cancer patients following fractionated partial-body radiotherapy. *Int J Radiat Biol*. 1990;57:373–383. https://doi.org/10.1080/09553009014552471.
74. Thierens H, Vral A, Van Eijkeren M, Speleman F, De Ridder L. Micronucleus induction in peripheral blood lymphocytes of patients under radiotherapy treatment for cervical cancer or Hodgkin's disease. *Int J Radiat Biol*. 1995;67:529–539. https://doi.org/10.1080/09553009514550641.
75. le Roux J, Slabbert J, Smit B, Blekkenhorst G. Assessment of the micronucleus assay as a biological dosimeter using cytokinesis-blocked lymphocytes from cancer patients receiving fractionated partial body-radiotherapy. *Strahlenther Onkol*. 1998;174:75–81. https://doi.org/10.1007/BF03038479.
76. Lee TK, O'Brien KF, Naves JL, et al. Micronuclei in lymphocytes of prostate cancer patients undergoing radiation therapy. *Mutat Res*. 2000;469:63–70. https://doi.org/10.1016/s1383-5718(00)00072-3.
77. Silva-Barbosa I, Pereira-Magnata S, Amaral A, Sotero G, Melo HC. Dose assessment by quantification of chromosome aberrations and micronuclei in peripheral blood lymphocytes from patients exposed to gamma radiation. *Genet Mol Biol*. 2005;28:452–457. https://doi.org/10.1590/S1415-47572005000300021.
78. Livingston GK, Foster AE, Elson HR. Effect of in vivo exposure to iodine-131 on the frequency and persistence of micronuclei in human lymphocytes. *J Toxicol Environ Health*. 1993;40:367–375. https://doi.org/10.1080/15287399309531802.
79. Monsieurs MA, Bacher K, Brans B, et al. Patient dosimetry for 131I-lipiodol therapy. *Eur J Nucl Med Mol Imaging*. 2003;30:554–561. https://doi.org/10.1007/s00259-002-1108-7.
80. Iarmarcovai G, Ceppi M, Botta A, Orsière T, Bonassi S. Micronuclei frequency in peripheral blood lymphocytes of cancer patients: a meta-analysis. *Mutat Res*. 2008;659:274–283. https://doi.org/10.1016/j.mrrev.2008.05.006.
81. Livingston GK, Jensen RH, Silberstein EB, et al. Radiobiological evaluation of immigrants from the vicinity of Chernobyl. *Int J Radiat Biol*. 1997;72:703–713. https://doi.org/10.1080/095530097142861.
82. Tanaka K, Iida S, Takeichi N, et al. Unstable-type chromosome aberrations in lymphocytes from individuals living near semipalatinsk nuclear test site. *JRR*. 2006;47:A159–A164. https://doi.org/10.1269/jrr.47.A159.
83. Thierens H, Vral A, De Ridder L. A cytogenetic study of radiological workers: effect of age, smoking and radiation burden on the micronucleus frequency. *Mutat Res/Environ Mutagen Related Subject*. 1996;360:75–82. https://doi.org/10.1016/0165-1161(95)00058-5.
84. Thierens H, Vral A, Barbé M, Aousalah B, De Ridder L. A cytogenetic study of nuclear power plant workers using the micronucleus-centromere assay. *Mutat Res*. 1999;445:105–111. https://doi.org/10.1016/s1383-5718(99)00134-5.
85. Thierens H, Vral A, Morthier R, Aousalah B, De Ridder L. Cytogenetic monitoring of hospital workers occupationally exposed to ionizing radiation using the micronucleus centromere assay. *Mutagenesis*. 2000;15:245–249. https://doi.org/10.1093/mutage/15.3.245.
86. Thierens H. Inter-laboratory comparison of cytogenetic endpoints for the biomonitoring of radiological workers. *Int J Radiat Biol*. 1999;75:23–34. https://doi.org/10.1080/095530099140771.
87. Cytogenetic monitoring of Workers from a Nuclear Power Plant n.d. https://www.nnk.gov.hu/cejoem/Volume3/Vol3No1/ce971-4.html (accessed April 29, 2022).
88. Reddy A, Rebekha A, Modi P, et al. Radiation-induced DNA damage in patients exposed to X-rays during cerebral angiography. *Int J Low Radiat*. 2010;7:10–19. https://doi.org/10.1504/IJLR.2010.032767.
89. Basheerudeen SAS, Murtaza S, Raavi V, et al. Assessment of early and late DNA damages in interventional radiologists exposed to protracted low dose and dose rate of X-radiation. *Int J Low Radiat*. 2016;10:198–209. https://doi.org/10.1504/IJLR.2016.081452.
90. Kanagaraj K, Abdul Syed Basheerudeen S, Tamizh Selvan G, Jose MT, Ozhimuthu A, Panneer Selvam S, et al. Assessment of dose and DNA damages in individuals exposed to low dose and low dose rate ionizing radiations during computed tomography imaging. Mutat Res Genet Toxicol Environ Mutagen 2015;789–790:1–6. doi: 10.1016/j.mrgentox.2015.05.008.
91. Basheerudeen SAS, Kanagaraj K, Jose MT, et al. Entrance surface dose and induced DNA damage in blood lymphocytes of patients exposed to low-dose and low-dose-rate X-irradiation during diagnostic and therapeutic interventional radiology procedures. *Mutat Res/genetic Toxicol Environ Mutagenes*. 2017;818:1–6. https://doi.org/10.1016/j.mrgentox.2017.04.001.
92. Khattab M, Walker DM, Albertini RJ, et al. Frequencies of micronucleated reticulocytes, a dosimeter of DNA double-strand breaks, in infants receiving computed tomography or cardiac catheterization. *Mutat Res/genetic Toxicol Environ Mutagenes*. 2017;820:8–18. https://doi.org/10.1016/j.mrgentox.2017.05.006.
93. Tian X-L, Lu X, Cai T-J, Lyu Y-M, Tian M, Liu Q-J. Cytogenetic monitoring of peripheral blood lymphocytes from medical radiation professionals occupationally exposed to low-dose ionizing radiation. *Mutat Res/genet Toxicol Environ Mutagenes*. 2021;867. https://doi.org/10.1016/j.mrgentox.2021.503370 503370.
94. 14:00-17:00. ISO 13528:2015. ISO n.d. https://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/05/61/56125.html (accessed April 29, 2022).
95. Kulka U, Ainsbury L, Atkinson M, et al. Realising the European network of biodosimetry: RENEB-status quo. *Radiat Prot Dosimetry*. 2015;164:42–45. https://doi.org/10.1093/rpd/ncu266.
96. Voisin P. Standards in biological dosimetry: A requirement to perform an appropriate dose assessment. *Mutat Res Genet Toxicol Environ Mutagen*. 2015;793:115–122. https://doi.org/10.1016/j.mrgentox.2015.06.012.
97. Christie DH, Chu MC, Carr Z. Global networking for biodosimetry laboratory capacity surge in radiation emergencies. *Health Phys*. 2010;98:168–171. https://doi.org/10.1097/HP.0b013e3181abaad4.
98. Roy L, Buard V, Delbos M, et al. International intercomparison for criticality dosimetry: the case of biological dosimetry. *Radiat Prot Dosimetry*. 2004;110:471–476. https://doi.org/10.1093/rpd/nch349.
99. Wojcik A, Lloyd D, Romm H, Roy L. Biological dosimetry for triage of casualties in a large-scale radiological emergency:capacity of the EU member states. *Radiat Prot Dosimetry*. 2010;138:397–401. https://doi.org/10.1093/rpd/ncp279.
100. Di Giorgio M, Barquinero JF, Vallerga MB, et al. Biological dosimetry intercomparison exercise: an evaluation of triage and routine mode results by robust methods. *Radiat Res*. 2011;175:638–649. https://doi.org/10.1667/RR2425.1.
101. Garcia OF, Ramalho AT, Di Giorgio M, et al. Intercomparison in cytogenetic dosimetry among five laboratories from Latin America. *Mutat Res*. 1995;327:33–39. https://doi.org/10.1016/0027-5107(94)00066-e.
102. Miller SM, Ferrarotto CL, Vlahovich S, Wilkins RC, Boreham DR, Dolling J-A. Canadian cytogenetic emergency network (CEN) for biological dosimetry following radiological/nuclear accidents. *Int J Radiat Biol*. 2007;83:471–477. https://doi.org/10.1080/09553000701370860.
103. Yoshida MA, Hayata I, Tateno H, et al. The Chromosome Network for biodosimetry in Japan. *Radiat Meas*. 2007;6–7:1125–1127. https://doi.org/10.1016/j.radmeas.2007.05.047.

104. Kulka U, Ainsbury L, Atkinson M, et al. Realising the European network of biodosimetry (RENEB). *Radiat Prot Dosim*. 2012;151:621–625. https://doi.org/10.1093/rpd/ncs157.
105. Bhavani M, Tamizh Selvan G, Kaur H, et al. Dicentric chromosome aberration analysis using giemsa and centromere specific fluorescence in-situ hybridization for biological dosimetry: an inter- and intra-laboratory comparison in Indian laboratories. *Appl Radiat Isot*. 2014;92:85–90. https://doi.org/10.1016/j.apradiso.2014.06.004.
106. Pan Y, Ruan J, Gao G, Wu L, Piao C, Liu J. Laboratory Intercomparison of Cytogenetic Dosimetry Among 38 Laboratories in China. Dose-Response 2019;17:155932581983347. doi: 10.1177/1559325819833473.
107. Gregoire E, Barquinero JF, Gruel G, et al. RENEB Inter-Laboratory comparison 2017: limits and pitfalls of ILCs. *Int J Radiat Biol*. 2021;97:888–905. https://doi.org/10.1080/09553002.2021.1928782.
108. Schreiber GA, Beisker W, Braselmann H, Bauchinger M, Bögl KW, Nüsse M. An automated flow cytometric micronucleus assay for human lymphocytes. *Int J Radiat Biol*. 1992;62:695–709. https://doi.org/10.1080/09553009214552651.
109. Schunck C, Johannes T, Varga D, Lörch T, Plesch A. New developments in automated cytogenetic imaging: unattended scoring of dicentric chromosomes, micronuclei, single cell gel electrophoresis, and fluorescence signals. *Cytogenet Genome Res*. 2004;104:383–389. https://doi.org/10.1159/000077520.
110. Rodrigues MA, Probst CE, Zayats A, et al. The in vitro micronucleus assay using imaging flow cytometry and deep learning. *Npj Syst Biol Appl*. 2021;7:20. https://doi.org/10.1038/s41540-021-00179-5.
111. Decordier I, Papine A, Plas G, et al. Automated image analysis of cytokinesis-blocked micronuclei: an adapted protocol and a validated scoring procedure for biomonitoring. *Mutagenesis*. 2008;24:85–93. https://doi.org/10.1093/mutage/gen057.
112. Darzynkiewicz Z, Smolewski P, Holden E, et al. Laser scanning cytometry for automation of the micronucleus assay. *Mutagenesis*. 2011;26:153–161. https://doi.org/10.1093/mutage/geq069.
113. Repin M, Pampou S, Garty G, Brenner DJ. RABiT-II: A fully-automated micronucleus assay system with shortened time to result. *Radiat Res*. 2019;191:232. https://doi.org/10.1667/RR15215.1.
114. Wessels JM, Nüsse M. Flow cytometric detection of micronuclei by combined staining of DNA and membranes. *Cytometry*. 1995;19:201–208. https://doi.org/10.1002/cyto.990190303.
115. Roman D, Locher F, Suter W, Cordier A, Bobadilla M. Evaluation of a new procedure for the flow cytometric analysis of in vitro, chemically induced micronuclei in V79 cells. *Environ Mol Mutagen*. 1998;32:387–396.
116. Avlasevich S, Bryce S, De Boeck M, et al. Flow cytometric analysis of micronuclei in mammalian cell cultures: past, present and future. *Mutagenesis*. 2011;26:147–152. https://doi.org/10.1093/mutage/geq058.
117. Bryce SM, Avlasevich SL, Bemis JC, et al. Interlaboratory evaluation of a flow cytometric, high content in vitro micronucleus assay. *Mutat Res/genet Toxicol Environ Mutagenes*. 2008;650:181–195. https://doi.org/10.1016/j.mrgentox.2007.11.006.
118. Nusse M, Kramer J. Flow cytometric analysis of micronuclei found in cells after irradiation. *Cytometry*. 1984;5:20–25. https://doi.org/10.1002/cyto.990050105.
119. Laingam S, Froscio SM, Humpage AR. Flow-cytometric analysis of in vitro micronucleus formation: comparative studies with WIL2-NS human lymphoblastoid and L5178Y mouse lymphoma cell lines. *Mutat Res/genetic Toxicol Environ Mutagen*. 2008;656:19–26. https://doi.org/10.1016/j.mrgentox.2008.06.015.
120. Rodrigues MA. Automation of the in vitro micronucleus assay using the Imagestream ® imaging flow cytometer. In Vitro MN Assay by Imaging Flow Cytometry. *Cytometry*. 2018;93:706–726. https://doi.org/10.1002/cyto.a.23493.
121. Tates AD, van Welie MT, Ploem JS. The present state of the automated micronucleus test for lymphocytes. *Int J Radiat Biol*. 1990;58:813–825. https://doi.org/10.1080/09553009014552191.
122. Castelain Ph, Van Hummelen P, Deleener A, Kirsch-Volders M. Automated detection of cytochalasin-B blocked binucleated lymphocytes for scoring micronuclei. *Mutagenesis*. 1993;8:285–293. https://doi.org/10.1093/mutage/8.4.285.
123. Eastmond DA, Tucker JD. Identification of aneuploidy-inducing agents using cytokinesis-blocked human lymphocytes and an antikinetochore antibody. *Environ Mol Mutagen*. 1989;13:34–43. https://doi.org/10.1002/em.2850130104.
124. Varga D, Michel I, Patino-Garcia B, Paiss T, Vogel W, Maier C. Radiosensitivity detected by the micronucleus test is not generally increased in sporadic prostate cancer patients. *Cytogenet Genome Res*. 2005;111:41–45. https://doi.org/10.1159/000085668.
125. Rossnerova A, Spatova M, Rossner P, Solansky I, Sram RJ. The impact of air pollution on the levels of micronuclei measured by automated image analysis. *Mutat Res/fundament Molecul Mechan Mutagenes*. 2009;669:42–47. https://doi.org/10.1016/j.mrfmmm.2009.04.008.
126. Doherty AT, Hayes J, Fellows M, Kirk S, O'Donovan M. A rapid, semi-automated method for scoring micronuclei in mononucleated mouse lymphoma cells. *Mutat Res/genetic Toxicol Environ Mutagenes*. 2011;726:36–41. https://doi.org/10.1016/j.mrgentox.2011.08.002.
127. Pujol-Canadell M, Perrier JR, Cunha L, et al. Cytogenetically-based biodosimetry after high doses of radiation. *PLoS One*. 2020;15:e0228350.
128. Henriksen M. Quantitative imaging cytometry: instrumentation of choice for automated cellular and tissue analysis. *Nat Methods*. 2010;7:i–ii. https://doi.org/10.1038/nmeth.f.302.
129. Smolewski P, Ruan Q, Vellon L, Darzynkiewicz Z. Micronuclei assay by laser scanning cytometry. *Cytometry*. 2001;45:19–26. https://doi.org/10.1002/1097-0320(20010901)45:1<19::aid-cyto1140>3.0.co;2-g.
130. Diaz D, Scott A, Carmichael P, Shi W, Costales C. Evaluation of an automated in vitro micronucleus assay in CHO-K1 cells. *Mutat Res*. 2007;630:1–13. https://doi.org/10.1016/j.mrgentox.2007.02.006.
131. Shibai-Ogata A, Kakinuma C, Hioki T, Kasahara T. Evaluation of high-throughput screening for in vitro micronucleus test using fluorescence-based cell imaging. *Mutagenesis*. 2011;26:709–719. https://doi.org/10.1093/mutage/ger037.
132. Frieauff W, Martus HJ, Suter W, Elhajouji A. Automatic analysis of the micronucleus test in primary human lymphocytes using image analysis. *Mutagenesis*. 2013;28:15–23. https://doi.org/10.1093/mutage/ges047.
133. Garty G, Chen Y, Salerno A, et al. The rabit: a rapid automated biodosimetry tool for radiological triage. *Health Phys*. 2010;98:209–217. https://doi.org/10.1097/HP.0b013e3181ab3cb6.
134. Wang Q, Rodrigues MA, Repin M, et al. Automated triage radiation biodosimetry: integrating imaging flow cytometry with high-throughput robotics to perform the cytokinesis-block micronucleus assay. *Radiat Res*. 2019;191:342. https://doi.org/10.1667/RR15243.1.
135. Rodrigues MA, Beaton-Green LA, Wilkins RC. Validation of the cytokinesis-block micronucleus assay using imaging flow cytometry for high throughput radiation biodosimetry. *Health Phys*. 2016;110:29–36. https://doi.org/10.1097/HP.0000000000000371.
136. Rodrigues MA, Beaton-Green LA, Wilkins RC, Fenech MF. The potential for complete automated scoring of the cytokinesis block micronucleus cytome assay using imaging flow cytometry. *Mutat Res Genet Toxicol Environ Mutagen*. 2018;836:53–64. https://doi.org/10.1016/j.mrgentox.2018.05.003.
137. Goh VST, Nakayama R, Blakely WF, et al. Improved harvest and fixation methodology for isolated human peripheral blood mononuclear cells in cytokinesis-block micronucleus assay. *Int J Radiat Biol*. 2021;97:194–207. https://doi.org/10.1080/09553002.2021.1844338.
138. Sreedevi and Rao 1994. Assay of Micronuclei in Peripheral Blood Lymphocytes as a Biological Indicator of Radiation Dose | Radiation Protection Dosimetry | Oxford Academic n.d. https://academic.oup.com/rpd/article-abstract/51/1/41/1602795?redirectedFrom=fulltext (accessed May 2, 2022).
139. Mill AJ, Wells J, Hall SC, Butler A. Micronucleus induction in human lymphocytes: comparative effects of X rays, alpha particles, beta particles and neutrons and implications for biological dosimetry. *Radiat Res*. 1996;145:575–585.
140. Mitchell JC, Norman A. The induction of micronuclei in human lymphocytes by low doses of radiation. *Int J Radiat Biol Relat Stud Phys Chem Med*. 1987;52:527–535. https://doi.org/10.1080/09553008714552031.
141. Paul SFD, Venkatachalam P, Jeevanram RK. A comparative study of synchronised and conventional culture methods on the micronucleus dose–response curve. *Mutat Res/Genet Toxicol Environ Mutagenes*. 1997;391:91–98. https://doi.org/10.1016/S0165-1218(97)00038-4.
142. Voisin P, Benderitter M, Claraz M, et al. The cytogenetic dosimetry of recent accidental overexposure. *Cell Mol Biol (noisy-Le-Grand)*. 2001;47:557–564.
143. Pala FS, Alkaya F, Tabakçioğlu K, et al. The Effects of micronuclei with whole chromosome on biological dose estimation. *Turk J Biol*. 2008;32:283–290.
144. Acharya S, Sanjeev G, Bhat NN, Siddappa K, Narayana Y. The effect of electron and gamma irradiation on the induction of micronuclei in cytokinesis-blocked human blood lymphocytes. *Radiat Environ Biophys*. 2009;48:197–203. https://doi.org/10.1007/s00411-008-0209-5.
145. Bolognesi et al. 2011 MH& S. Micronucleus test for radiation biodosimetry in mass casualty events: Evaluation of visual and automated scoring accessed May 2. *MetaSystems N.d.*. 2022 https://metasystems-international.com/en/publications/bolognesi11/.
146. Kormos C, Köteles GJ. Micronuclei in X-irradiated human lymphocytes. *Mutat Res*. 1988;199:31–35. https://doi.org/10.1016/0027-5107(88)90227-8.
147. Littlefield LG, Sayer AM, Frome EL. Comparisons of dose-response parameters for radiation-induced acentric fragments and micronuclei observed in cytokinesis-arrested lymphocytes. *Mutagenesis*. 1989;4:265–270. https://doi.org/10.1093/mutage/4.4.265.
148. Thierens H, Vral A, de Ridder L. Biological dosimetry using the micronucleus assay for lymphocytes: interindividual differences in dose response. *Health Phys*. 1991;61:623–630. https://doi.org/10.1097/00004032-199111000-00005.
149. Venkatachalam.P. Estimation of long term radiation exposure using translocation frequency in chromosoms. University; 1993.
150. Thierens H, Vral A. The micronucleus assay in radiation accidents. *Ann Ist Super Sanita*. 2009;45:260–264.
151. Rodrigues MA, Beaton-Green LA, Kutzner BC, Wilkins RC. Automated analysis of the cytokinesis-block micronucleus assay for radiation biodosimetry using imaging flow cytometry. *Radiat Environ Biophys*. 2014;53:273–282. https://doi.org/10.1007/s00411-014-0525-x.
152. Tamizh Selvan G, Kanagaraj K, Venkatachalam P. The relative biological effectiveness of high-energy clinical 3 and 6 MV X-rays for micronucleus induction in human lymphocytes. *Int J Radiat Biol*. 2021;97:687–694. https://doi.org/10.1080/09553002.2021.1906972.