The imaging flow cytometry cytokinesis-block micronucleus assay for radiation biodosimetry

Ruth C. Wilkins¹, M.A. Rodrigues², L.A. Beaton-Green¹, C. Probst³, P. Morrissey³

¹ Consumer and Clinical Radiation Protection Bureau, Health Canada, Ottawa, Ontario, Canada
² Department of Physics, Carleton University, Ottawa, Ontario, Canada
³ Amnis- A Part of EMD Millipore, Seattle, Washington, USA

Study Goal: The purpose of this study was to develop and validate an automated version of the cytokinesis-block micronucleus (CBMN) assay using imaging flow cytometry for use as a high throughput method for triage biological dosimetry for ionizing radiation. Furthermore, recent modifications to the analysis software were evaluated for improved performance.

Abstract: The cytokinesis-block micronucleus (CBMN) assay is an accepted method for biodosimetry where the dose of radiation to potentially exposed individuals is estimated from the frequency of micronuclei (MN) in binucleated lymphocyte cells (BNCs). The assay is traditionally performed using a microscope-based scoring procedure which can be labour intensive, time consuming and subject to variability of interpretation between scorers. This study investigated the feasibility of adapting the CBMN assay to an imaging flow cytometry method using the ImageStream®X (ISX) imaging flow cytometer.

A protocol to perform the CBMN assay on the ISX along with a data analysis template using the Image Data Exploration and Analysis Software (IDEAS®) were developed. Using irradiated whole blood samples, it was shown that the ISX-CBMN method could automatically image and enumerate BNCs and MN. Dose response curves between 0-4 Gy were generated and followed a linear quadratic dependence with dose, similar to what is observed when performing the assay using traditional protocols. Two limitations of the CBMN assay are the requirement of large blood culture volumes (500 μL - 2 mL) and a 72 h culture time. To address these limitations, a reduction in both initial blood volume (200 μL) and culture time (48 h) were investigated to determine whether this method could generate dose estimates within 0.5 Gy of the delivered dose.

Amnis provided an early stage prototype of features under development for the analysis of MN, which were used to create a new IDEAS analysis template for improved detection of MN in BNCs. Dose response curves and blinded samples were reanalyzed using this improved analysis template. The results demonstrated a significant increase in the detection of MN in BNCs and subsequently enhanced the ability of this assay to provide accurate dose estimates. A comparison of the results before and after improvements in the analysis software will be presented.

Conclusion: The results indicated that MN in BNCs can be clearly imaged and enumerated automatically using the ISX, allowing for rapid dose estimations. In addition, data collection and analysis is completely automated, requiring no user intervention. The adaptation of the CBMN assay to an imaging flow cytometry method greatly increases its applicability in high throughput triage radiation biodosimetry.

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