THE DEVELOPMENT OF AN IN-HOUSE FLOW CYTOMETRY-BASED MICRONUCLEUS ASSAY

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The *in vitro* micronucleus test (MNvit) is vastly used to estimate damages on the genetic material, and micronuclei (MN) frequencies reflect DNA damage levels. MN can be quantified visually using microscopy or automatically by flow cytometry. The automatic measurement allows for high-throughput screening of chemical genotoxicity; however, the assay kit is sold by only one company, and it is commercially available in a few countries. To overcome this limitation, we develop an in-house flow cytometry-based MN assay based on published methods. V-79 cells, cultured in 24-well plates, were exposed to known direct genotoxicants (mitomycin C (MMC), methyl methanesulfonate (MMS), vinblastine (VB)) for 24 h, for MN induction. To establish the protocol, the following variables were considered: cell density, cell death markers (7-AAD, PI, EMA), nucleus staining and MN (4',6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI), Sytox green), concentration of nucleus markers, MN and cell death, lysis solution and threshold parameter in the acquisition of Data. Data acquisition was performed on the BD FACSVerse™ cytometer, and the FlowJo™ software was used for data analysis. Cell density strongly influences the quality of MN acquisition, and an initial cell density of $7x10^4$ cells/well was the best condition. EMA at 8.5 µg/ml was the most appropriate for labeling necrotic/apoptotic cells due to its covalently binding into DNA to distingue live and dead cells. The in-house method was able to distingue MN subpopulation from integry nuclei and discriminated MN frequencies between control and treatment groups. The implementation of this protocol adds value since the kit sold is extremely expensive and is not sold in countries like Brazil, this being an alternative of open protocol to be used by several researchers to analyze MNvit.