ADVANTAGES OF MICRONUCLEI ANALYSIS THROUGH IMAGES AUTOCAPTURING AND SCREEN SCORING

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ABSTRACT

The cytokinesis-block micronucleus (CBMN) test is a quantitative assay for genetic toxicity assessment. One of the advantages of the MN assay is its amenability for automation. Different type of cells has been used to evaluate genetic damage through MN assay, such as, human lymphocytes and rodent cell lines (i.e. CHO, V79, CHL and L5178Y). The MN quantification is a time consuming process and several efforts has been conducted for its automation. Some of them include an operator checking step, like PathFinder CellScan System, or are fully automated such as MNScore from MetaSytems. Usually, fully automated systems detect two or three times less MN than visual scoring. In some cases, the impact of false positive detection is reduced with a visual detection step. In the present work we have tested a combination of image autocapturing of CHOK1 cells previously treated with bleomycin (0, 2.5, 5.0 and 10.0 μg/ml) or UVC (0, 4, 8 and 16 J/m^2) with a screen scoring. Capturing images using the AutoCapture option from Metafer 4 from MetaSystems (GmbH, Germany) plus screen scoring render similar results in terms of MN cells frequency than microscopic live scoring. The resultant bias from the Bland–Altman analysis was -1.1% with confidence intervals between -2.2% and -0.1%, indicating an acceptable agreement between both MN scoring method. However, the mean time devoted to live microscope scoring per sample was 159 minutes compared to 39 minutes for microscope images autocapturing and screen scoring. Therefore, it become advantageous to combine autocapturing of microscope images plus screen scoring when many samples have to be analyzed for radiological biodosimetry purposes.

1. INTRODUCTION

The in vitro micronucleus (MN) test is a quantitative assay for the genetic toxicity assessment, either for population or environmental biomonitoring in human or mammalian cells (i.e. CHO, V79, CHL or L5178Y) [1,2]. Therefore, there is a strong interest in developing automated systems to score micronuclei as a biomarker of chromosome breakage or loss. Besides, due to the existence of advanced image analysis systems, MN assay become suitable for automation [3,4]. The MN quantification is a time consuming process and several efforts has been conducted for the automation of the MN assay. Some of them include an operator checking step (PathFinder CellScan System) and other are fully automated (MNScore from MetaSytems [5]. Many efforts has been carried out to standardize slide preparations as well as to establish scoring criteria for automated scoring of micronuclei by automated image cytometry using different commercially available platforms [6,7]. In general, automatic systems detect two or three times less MN than visual scoring. Some systems reduce the impact of false positive detection with a visual detection step [5]. In the present contribution we have tested a combination of both approaches establishing a link between image autocapture and image screen scoring for the CBMN *in vitro* assay employing Cytochalsin-B for blocking cytokinesis [8] in CHO-K1 cells exposed to different clastogenic agents.

2. MATERIALS AND METHODS

2.1. Cells

Chinese hamster ovary cells (CHO-K1) were routinely maintained in Ham's nutrient F12 medium (Life Technologies) with L-glutamate supplemented with 10% fetal calf serum (FCS) (Life Technologies) and 100 U penicillin and 100 μg/ml streptomycin at 37°C in a 5% CO_2 humidified atmosphere.

2.2. Chemicals

Bleomycin (BLM) and Cytochalasin B (Cyt B) were purchased from Sigma. BLM was dissolved in ultrapure water at a 20 mg/ml concentration and Cyt B in dimethylsulfoxide (DMSO) (Merck) at a 10 mg/ml concentration. The final concentration of DMSO represented 0.5% of the final volume.

2.3. Cell treatment and UVC irradiation

Exponentially growing cells were plated in 100 mm diameter Petri dishes and cultured for 24 h prior to BLM treatment. Three independent cultures were established for each BLM concentration (2.5, 5, 10 μg/mL) or UVC dose (4, 8, 16 J/m^2). The cell cultures were exposed to BLM for 3 h. UVC irradiation was performed at a 0.4 J/sec rate.

2.4. *In vitro* CBMN assay

At the end of treatment, cells were washed twice with phosphate-buffered saline (PBS) prior to a 20 h incubation in fresh medium containing 10% FCS and 3 μg/ml cyt B. At the end of cyt B treatment, cells were washed twice with PBS. Then, cells were stained with 10 μg/ml of propidium iodide for 5 min [9]. Glass coverslips were mounted and slides were observed

under an Axioplan 2 Mot epifluorescence microscope from Carl Zeiss Company (GmbH, Germany).

2.5. Slide scoring

All slides were coded prior to scoring. Scoring was carried out using a Zeiss AxioPlan 2 Mot microscope system equipped with a complete epifluorescence system at 1000X magnification under oil immersion. Criteria for cell and MN scoring were done as described by Fenech (2000) [10]. Briefly, only binucleated cells with an intact nuclear membrane and located within the same cytoplasmic boundary. Both nuclei should be approximately equal in size, staining pattern and intensity. MN should be morphologically identical to, but smaller than, nuclei (their diameter usually varies between one-sixth and one-third of the mean diameter of the main nuclei). MN may be readily distinguished and should not be linked or connected to nuclei.

2.6. Metafer 4 autocapture and image screen scoring

Digitalized images at 63x magnification were obtained automatically with a Zeiss Axioplan 2 Mot microscope system coupled to CCD camera controlled by the image analysis software called Metafer 4 from MetaSystems (GmbH, Germany) in MSearch and fluorescent modes. A total of 1240 images per experimental point were captured using the AutoCapture option from Metafer 4. The images were exported as black and white images in TIF format with high resolution. The same requisites for live microscope scoring were followed with the rejection of incomplete cells in the border of the image to avoid their impact in the speckle counting [10].

2.7. Statistical Analysis

A Bland-Altman analysis was performed to the agreement between live microscope scoring and the screen scoring of images captured. The dose-response was tested by ANOVA followed by Dunnett test. p-Values < 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

The capture of microscope images by the autocapture option of Metafer 4 software from MetaSystems (GmbH, Germany) plus screen scoring render similar results than microscopic live scoring. The resultant bias from the Bland-Altman analysis was -1.1% with confidence intervals between -2.2% and -0.1%.

Two different clastogenic agents (BLM and UVC irradiation) were used to induce MN on CHO-K1 cells to test the impact of both scoring methods. The concentration-response (BLM) or dose-response (UVC) was similar for both scoring methods (Figure 1).

The time consumed by the operator for live microscope scoring or microscope capturing by means of autocapture option from Metafer 4 plus image scoring were compared. The mean time devoted to manual microscope scoring per sample was 159 minutes and the time required for image capturing by the autocapture option from Metafer 4 plus image scoring was just 39 minutes.

The main concern about exported images screen scoring is that only one plane composed the image compared with the multiple focus possibility of live microscope scoring performed by the operator. A possible solution could be to capture multiple microscope planes using the autocapture option from Metafer 4 with the aim to create an integrated 2D image, although this process could increase the time for automated analysis of MN [11]. In the present work we have tested the impact of image screen scoring when the images were obtained automatically in one plane without observing any difference respect to live microscope scoring.

Therefore, we have observed a significant reduction of microscope operational time increasing the rapidness of the response and reducing the maintenance cost per assay four times when automated image analysis is coupled with visual scoring of captured images at high resolution.

CONCLUSIONS

We concluded that the combination of image capture and image screen scoring in the CBMN *in vitro* assay using CHO-K1 cells give similar results than live microscope scoring and saves significant operational time**.**

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Figure 1.- The number of Binucleated cells (BN) with Micronuclei (MN) was plotted against the dose or concentration of two model clastogens. The scoring of BN cells with MN was performed by two approaches, live microscope scoring(squares) and screen scoring of autocaptured images(circles) A. UV radiation dose response B. Bleomycin (BLM) concentration response.