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Implementation of the Fluorescent in situ Hybridization Technique at the Centro Regional de Ciências Nucleares do Nordeste (CRCN-NE/CNEN)

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Introduction

Biological dosimetry is crucial for measuring the radiation absorbed by the human body after exposure to ionizing radiation [1]. Studies indicate that FISH - Fluorescent In Situ Hybridization - is a reliable method for retrospective biodosimetry, allowing the assessment of persistent damage [2,3,4]. This study aims to implement FISH in the Biological Dosimetry Laboratory at CRCN-NE/CNEN, starting with the construction of a dose-response calibration curve for this technique, in order to fill the existing gap in retrospective dosimetry in Brazil.

Methodology

A survey was carried out of different protocols for the FISH technique, including the protocol suggested by the International Atomic Energy Agency - IAEA [1], the one presented by the Cytogenetic Dosimetry Laboratory of the Chilean Nuclear Energy Commission through a webinar and the protocol of the Cytogenetics Laboratory of a local hospital, which uses this technique for different applications. Both emphasized selecting the largest chromosomes (1 to 12) for probe labeling, optimizing translocation detection efficiency to approximately 33%. Consequently, specific probes were obtained for chromosome pairs 1, 2, and 4.

The materials and common points of the 3 protocols studied were selected and correlated with the indications suggested by the probe manufacturer (Metasystem) in order to establish a protocol that was simpler, faster, capable of quality marking and at a lower cost. To carry out the final protocol, we collected peripheral blood from a healthy non-smoking individual, separating one sample to be irradiated at an absorbed dose of 3Gy in a source of60 Co (Gammacel 220 Irradiator), and another kept as a control in the laboratory. Cytological preparation for chromosome analysis was carried out using lymphocyte culture according to the protocol already established in the laboratory and based on the IAEA protocol [1]. Only after the cells had been cultured and fixed did the FISH - process begin, according to the protocol to be implemented.

Results and Discussion

When analyzing the protocols, differences were observed in the procedures, reagents and steps for hybridization. The hospital protocol and the IAEA manual [1], suggested adding pepsin and post-fix solution, along with IGEPAL CA-630 for washing, while the manufacturer's recommendations simplified the process by dispensing with pepsin and indicated that the material can be washed with 2X SSC buffer solution and Tween-20, replacing IGEPAL. The hospital's methodology suggested marking the region with the highest concentration of metaphases on "white slides" to reduce the volume of the probe to 3µL, with a view to reducing costs. However, this led to a loss in marking quality and reduced metaphase viability, making this approach unfeasible. Consequently, the manufacturer's instructionswere followed, applying 10µL at two equidistant points on the slide to cover the entire region in order to obtain the maximum number of well-labeled metaphases.

Therefore, with the respective alterations, the protocol was adjusted with the aim of obtaining results that enable cytogenetic analysis aimed at biological dosimetry and was implemented as described below.

After 24 hours of dripping, the slides containing the material were immersed in a series of four solutions for 2 minutes each, in the following order: 1) 2XSSC buffer; 2) 70% alcohol; 3) 85% alcohol; 4) 100% alcohol. After drying the slides at room temperature, 10µL of the XCP-Mix #10-#2G-#460 probe was applied, covered by a 22x22cm coverslip fixed with Polytubes glue. The whole set was then heated to 75±1°C for 2 minutes on a hotplate. The slides were then stored in a humidified box to control humidity and temperature, and kept in an oven at 37±1°C overnight for the hybridization process. After this period, the coverslips and glue were removed and the slides were immersed in 0.4XSSC buffer at 72±1°C in a water bath for 2 minutes, followed by a 30-second wash with a solution of 2XSSC buffer + 0.05% Tween pH 7.0 and then with plenty of distilled water. After drying the slides at room temperature, 10µL of 49,6-diamidino-2-phenylindol (DAPI) was applied to counterstain the same region where the probe was applied, and a 24x32mm coverslip was placed and sealed with nail polish. After 10 minutes, the slides were viewed under a fluorescence microscope (Leica DM 6000 B), as shown in figure 1.

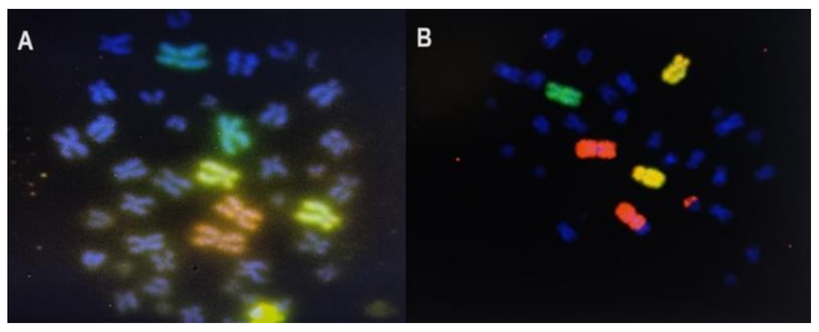

Figure 1: (A) Metaphase fluorescence showing chromosomes 1 (orange), 2 (green) and 4 (yellow); (B) Metaphase showing a reciprocal translocation [t (Ab):t(Ba)] between chromosome 1 (orange) and a chromosome counterstained with DAPI.

Conclusions

The FISH technique protocol was modified and satisfactorily implemented at the Biological Dosimetry Laboratory of the Centro Regional de Ciências Nucleares do Nordeste (CRCN-NE/CNEN). As a result, the dose-response calibration curve for the FISH technique is being constructed in order to enable the estimation of retrospective absorbed doses of individuals exposed to ionizing radiation in Brazil.

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CNEN Comissão Nacional de Energia Nuclear · CAPES · Finep Inovação e Pesquisa

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