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# Implementation of the Fluorescent in situ Hybridization Technique at the Northeast Regional Center for Nuclear Sciences

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## 1. Introduction

Biological dosimetry arose from the need to quantify the dose absorbed by the human body after incidents or accidents involving ionizing radiation. This type of dosimetry is used in situations where the absorbed dose to the whole body is unknown or uncertain, such as in cases of accidental or occupational exposure [1]. Among the techniques developed for this purpose are conventional cytogenetic analysis of dicentric chromosomes and the micronucleus technique by blocking cytokinesis. However, these techniques are applied to estimate recent damage to ionizing radiation, as they are representative of unstable damage and their frequencies are reduced with the elimination of lymphocytes through the rate of cell renewal [1].

For this reason, cytogenetic markers such as translocations are used to recognize and study the most persistent damage to cells. Reciprocal translocations have been used for exposures that occurred years to decades in the past [1,2]. Some case studies in retrospective dosimetry confirm the use of FISH - Fluorescent *in situ* Hybridization - as the most reliable test for retrospective biodosimetry [2,3,4].

As there is still no retrospective dosimetry service in Brazil, this work aimed to implement the FISH technique in the Biological Dosimetry Laboratory of the Northeast Regional Nuclear Science Center - CRCN-NE/CNEN, as the first step towards establishing a dose-response calibration curve for this biodosimetric technique.

## 2. Methodology

Firstly, 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.

An important point suggested both by the IAEA and in the webinar is that the chromosomes to be chosen for marking with the probes should be the largest (i.e. 1 to 12), as they represent around 20% of the genome, leading to approximately 33% efficiency in detecting translocations [1]. In view of this, specific probes were acquired 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 of $^{60}$ Co (Gammacel 220 Irradiator), located in the Department of Nuclear Energy (DEN/UFPE), 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 fluorescent *in situ* hybridization - FISH - process begin, according to the protocol to be implemented.

## 3. Results and Discussion

When analyzing the protocols, some differences were observed in relation to the procedures, reagents and steps required to carry out the hybridization. According to the laboratory protocol provided by the hospital and the IAEA manual, the addition of pepsin and post-fix solution would be necessary for hybridization and, in the case of the hospital protocol, the use of IGEPAL CA-630 for washing. According to the recommendations of the probe manufacturer (Metasystem), it would not be necessary to use pepsin to denature the proteins, making the process simpler. In addition, as recommended by the IAEA manual [1], the material can be washed with 2X SSC buffer solution and Tween-20, replacing IGEPAL.

Another point suggested by the hospital's methodology is the stage of marking the region with the highest concentration of metaphases on "white slides", by visualizing it under a light-field microscope, for later application of the probe. With this, the volume of the probe to be applied is reduced to 3 μΛ, allowing for greater savings in the use of this input since the one recommended by the IAEA is ~7 μΛ and by the manufacturer is 10 mL. For the type of analysis carried out in this laboratory, the practice is viable, since they don't need to analyze a large number of metaphases. However, with the reduction in the amount of probe to 3μL, a loss in marking quality was observed in metaphases further away from the application area, with heterogeneous staining. As a result, there was a reduction in the number of viable metaphases on the slides, which would make it difficult to reach the number of metaphases needed for dosimetry, making this reduction unfeasible. Therefore, this stage of marking the white slides was discarded. Therefore, the manufacturer's instructions will be followed, applying 10μL at 2 equidistant points on the slide to cover the entire region in order to obtain the maximum number of well-marked metaphases.

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

The previously fixed material was dripped onto the slide followed by a drop of the fixative methanol:acetic acid (3:1). The slides were dried using the flame technique to evaporate the excess fixative.

After 24 hours of dripping, the slides containing the material were bathed for 2 minutes in four solutions 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 Mixe probe was applied and a 22x22cm coverslip was placed over the area where the probe had been added, fixing it with the aid of Polytubes glue. The whole set

was then placed on the heating plate at a temperature of 75±1°C, where it remained for 2 minutes. After this process, the slides were stored in a humidified box to control humidity and temperature, and placed 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 a temperature of 72±1°C in a water bath for 2 minutes, followed by a 30 second wash with a solution of 2X SSC 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 for counterstaining in the same region as the probe and a 24x32mm coverslip was placed and sealed with enamel. After 10 minutes, the slides were viewed under a fluorescence microscope (Leica DM 6000 B), as shown in figure 1.

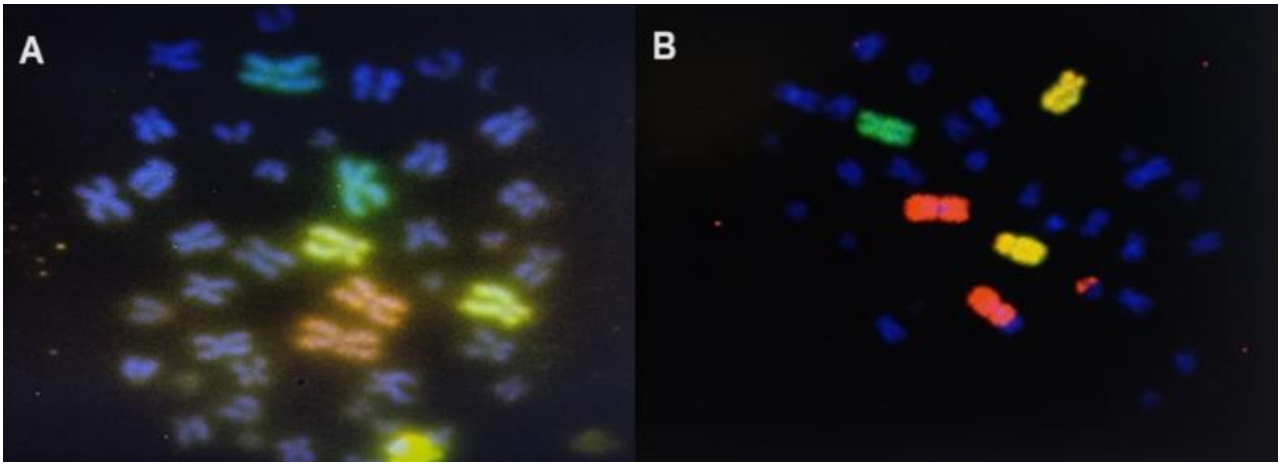


Figure 1: (A) Metaphase fluorescence showing chromosomes 1 (orange), 2 (green) and 4 (yellow); (B) Metaphase showing a reciprocal translocation [t (Ab):t(Ba)] between chromosome 1 (orange) and a chromosome counterstained with DAPI.

## 4. Conclusions

The FISH technique protocol was modified and satisfactorily implemented at the Biological Dosimetry Laboratory of the Northeast Regional Nuclear Science Center (CRCN-NE/CNEN). As a result, the dose-response calibration curve for the FISH technique will be constructed in order to enable the estimation of retrospective absorbed doses of individuals exposed to ionizing radiation in Brazil.

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