Retrospective Biological Dosimetry by FISH and Alternative Techniques

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Abstract: Cytogenetical dosimetry based on the stable chromosome aberrations frequency was one of the major challenges until ten years ago, when G or R banding were the only inexpensive but extremely time consuming tools available. Since the development of the pioneer molecular cytogenetic techniques, Fluorescence in situ Hybridization (FISH), much work has been done. Even though FISH is an easy to score technique, it is very expensive. Curiously, now the circle appears to close by using FISH to get multicolor banded chromosomes, which reminds, to some extent, to the classical banding techniques allowing us to recognize more subtle chromosomal abnormalities (inversions and deletions). Those, very well known by cytogeneticists through G or R banding, are still undetectable by means of Whole Chromosome Painting (WCP) a traditional FISH technique. In this work we present some alternatives to perform retrospective dosimetry according to different scenarios, explored extensively by our group. Possibly, these alternatives will be useful in countries with different biological dosimetry laboratory infrastructures, different budgets and different personnel capabilities in banding techniques.

Introduction

Chromosome aberration frequency measured in peripheral lymphocytes of persons exposed to ionizing radiation has been used since 1960s for dose assessment. Suspected overexposure is usually evaluated by the frequency of dicentrics and centric rings using an appropriate in vitro calibration curve, according to the radiation quality. However, these chromosome aberrations are unstable with time after exposure and dose reconstruction may encounter uncertainties when the time between the exposure and the analysis is considerable or even unknown.

If those called stable chromosome aberrations (translocations and inversions) certainly persist with time after exposure then, they may be used in retrospective dosimetry for evaluating acute past overexposures and, moreover, to show the accumulation of cytogenetical information which correlates with dose received under fractionated, chronic or occupational exposure conditions. Nowadays, the stability of translocations and inversions is practically accepted [1] [2].

All type of stable chromosome aberrations may be detected using G-banding, an inexpensive simple and rapid staining technique although it is a time consuming method for aberration scoring and requires a highly trained personnel.

For ten years, the combined application of conventional cytogenetics and molecular biology have been allowing the identification, through Fluorescence in situ Hybridization (FISH) technique, of some structural chromosome aberrations.

The most promising alternative is the so called "chromosome painting", a method based on painting only some chromosome pairs with specific whole chromosome DNA probes (WCP) and then extrapolating the observed translocation frequencies to the full genome [3].

However, this method is unable to recognize inversions and deletions.

In general, FISH is expensive and it is not easily available yet for the majority of the laboratories, but allows a faster aberration scoring than banding techniques and appears as a promissory tool in biodosimetry, particularly when it is necessary to assess low doses and, consequently, to score a large number of metaphases, i.e. radiation workers exposed within dose limits.
Anyway, assessing doses retrospectively is a must for a biological dosimetry laboratory and it should be faced in some way.

In this work we propose different alternative techniques in order to perform retrospective dosimetry in three possible laboratory scenarios each one with some particular handicap.

(1) A conventional citogenetics laboratory with neither FISH equipment nor supplies but with very well trained personnel in conventional banding techniques.
(2) A complete FISH laboratory infrastructure (including fluorescence microscope) but not enough budgets to acquire commercial DNA probes and with personnel untrained in banding techniques.
(3) A complete laboratory (see below, in FISH equipments) but without fluoresce microscope or trained personnel in banding techniques.

Materials and Methods

Cultures

We use a modified micromethod [4]. Briefly, whole heparinized blood cultures for in vivo or in vitro studies were set up, using phytohemagglutinin P (PHA P, 60 g/ml) as a mitogen. Stimulated lymphocytes were grown in 10 ml RPMI 1640 medium enriched with 15% of fetal calf serum, supplemented with 2mM L-glutamine and 20 µM 5-bromo-deoxyuridine. The incubation time was 48 h at 37 °C. Colchicine was added in a concentration of 0.4 g/ml 2 h before harvesting. After hypotonic treatment with KCl (0.075 M) for 10 min at 37°C, the lymphocytes were fixed by three changes in methanol - acetic acid (3:1), air dried and stored at 60 °C overnight.

Aging and storage

Slide aging is necessary for G banding; in our experience, at least two weeks.
FISH does not require previous aging but it is possible to use slides stored at -20ºC several months later.
G banding is storable at 0ºC and FISH slides at -20ºC for several months.

FISH Equipment

Starting with a full conventional dosimetry laboratory, the minimum requirement is:

-Fluorescent microscope + filters

Probe development equipment

-Micro dissection device + Inverted microscope
-Thermo circler
-Electrophoresis cube
-Puller
Performing retrospective biological dosimetry

Scenario 1

Let us consider three approaches depending on cytogeneticists expertise.

(a) To build an in vitro dose response relationship scoring translocations, inversions or both on the whole G or R banded karyotype with at least 350 bands according to the International System for human Cytogenetic Nomenclature (ISCN) [5]. For special studies the same slides, conveniently stored, can be used later for scoring small deletions, insertions or complex arrangements. For these analyses it is convenient increase the band number to 500 bands (FIG. 1) shortening time and increasing colchicine (or colcemid) concentration. Obviously, Premature Chromosome Condensation (PCC) technique allows 800 bands, but it is out of the scope of this work and our experience.

![FIG. 1. G banded metaphase, 500 bands (ISCN). Arrows show a reciprocal translocation t7 q34::9q12](image)

(b) As a second approach we have proposed a less time consuming method mimicking FISH by scoring translocations using only three pairs of chromosomes and extrapolating to the whole karyotype. In this scenario it is very easily to score inversions too. If scoring includes inversions, it is necessary a corrected extrapolation formula [6].

\[
F_G = \frac{F_p}{2.05 \times f_p \times (1-f_p) \times (f_p)^2}
\]

where

- \( F_G \) is the full genome translocation and inversion frequency
- \( F_p \) is the translocation and inversion frequency detected by G banding
- \( f_p \) is the fraction of genome (number of pairs of chromosomes) used in the analysis

The above mentioned approaches are possible only for very well trained cytogenetists.
Software support for “not so well trained” cytogeneticists.

We have developed software which helps to observe more bands and to detect inversions and small deletions, e.g. as biomarkers of past exposure to densely ionizing radiation [7]. Based on the fact that the human eye can distinguish 50 of 256 possible gray tones and unable to separate 5 very close tones we have developed software to “see” more bands than G banding images offers to the naked eye, pseudocoloring those tones. Briefly, the binary transformed microscope image has 256 gray tones. In order to assign pseudo-colors to gray tones a particular algorithm was built relating the cubic matrix $(256)^3$ with the maximum number of tones. Due to the fact that it is impossible to establish a bi-univocal relationship between gray tones and colors, the software automatically associates a color range from a discrimination level. It is noted that “light” pseudo-colored bands correspond to “dark” G bands.

![Images of chromosomes with G banding, ideogram, and pseudo-colored bands](image)

**FIG 2.** Human chromosome 2. (a) G banded chromosome (19 bands), (b) Ideogram (27 bands), (c) pseudo-colored chromosome (24 bands), (d) and (e) G banded and pseudo-colored chromosome with inversion. Arrows show break points: inv 2p1 :: 2q23.

**Scenario 2**

In this special case we propose to perform ISH (In situ Hybridization) using DNA probes which result, after an enzymatic process, in brown staining detectable by conventional light microscope. So fluorescent microscope is not required.

![ISH image](image)

**FIG. 3.** ISH. Arrow shows human chromosome 11

This method is not so impressive but results substantially cheaper than FISH, particularly if probes are synthesized by micro dissection (see later, Scenario 3).
FISH applications reach different fields: basic genetic research in man and other species, medical diagnosis and prognosis related to constitutive and somatic cell genetics and, of course, retrospective dosimetry.

Performing FISH in general or particularly “Chromosome Painting” with commercial DNA direct probes is easier than the traditional indirect method but, apart from their cost, commercial DNA probes are storable usually up to six months. Up to now, in our country as in Latin America, FISH is performed using commercial DNA probes.

In a joint effort between CONICET, CNEA and ARN, Argentina has concluded the project to set up the Synthesis of chromosome probes by Micro dissection stained with Fluorescent dyes (SMF). It is suitable for any species lowering costs to about one sixth of the equivalent commercial probes.

It is remarkable that SMF not only lowers costs but also expands the possibilities of working with other species besides man. At the same time improves the traditional Chromosome Painting staining by using different fluorescent dyes and increases sensitivities developing probes for only parts (arms or less) of chromosomes.

The general SMF protocol, the cost analysis comparing with commercial probes and the minimum requirement for technology transfer and implementation of this technique in Latin American countries have been presented [7].

We show in the present work some additional images of human and other species SMF probes. (FIG. 4,5).

**Human SMF**

*FIG.4. (a) WCP with SMF chromosome 3 (green). (b) reciprocal translocation. (c) deletion. (d) ring.*
FIG. 5. (a) both human X chromosomes (yellow) show insertions and translocations induced in vitro after 7 Gy of gamma rays. [8]. (b) Complex arrangement between chromosome 2 (red) and chromosome 3 (green).

Other species SMF

FIG. 6. Turtle Trachemys scripta. (a) WCP Reciprocal translocation chromosome 1. [9]. (b) WCP 1 green, 3 red.

Conclusions

As can be seen in the present work, retrospective dosimetry is a matter of choice. It depends on where every laboratory has its strong point: the facilities and budgets or training in cytogenetics. Not always the expensive alternatives give better results.
References


