# BIOLOGICAL DOSIMETRY METHODS FOR EXCESS EXPOSURE MANAGEMENT AND DOSE ASSESSMENT

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## 1. Introduction:

It is a well-established fact that at low doses of ionizing radiation such as less than 100-200 mSv, no serious medical concern exists. Many myths surrounding radiation effects have spread over the time due to misunderstandings and incomplete scientific information. Peaceful applications of radiation including those in medical, industrial and power sectors have become prohibitively expensive due to such wrongfully spread information. For scientific community, it is of paramount importance to eliminate such wrong information, present the reality, work towards achieving better and more realistic radiation protection procedures. To avoid any untoward radiation exposure, it is a mandatory to monitor the workers in all sectors of radiation applications where there are possibilities of accidental / occupational exposure. In most cases, exposures are trivial in nature. However, these cases are scientifically investigated to avoid and minimize the causes. Every radiation worker is assigned with a personnel monitoring device to monitor the possible exposures. These are read 4-12 times in a year and dose information is secured in digital form.

In India, if a radiation worker is suspected of exposed to more than 10 mSv in a monitoring period, it calls for investigation as a precautionary measure. A radiation worker has the dose limit of 100 mSv in five calendar years with average of 20 mSv in a year while restricting maximum of 30 mSv in a calendar year. However, to avoid further exposures, investigations are called upon to strengthen the safe operations and appropriate steps are taken to prevent unnecessary exposure which are avoidable. With the increasing applications of radiation, number of radiation workers using radiation generating devices such as X-rays, accelerators, therapy units and radioactive sources is also on constant raise. Inadvertent exposure to radiation in the working professionals is almost impossible when safety procedures are meticulously followed. Besides, inbuilt safety features prevent workers from getting serious exposures. However, small scale incidents can yield doses in the range of few mSv to couple of hundreds of mSv. Such incidents are seriously viewed from regulatory point of view as

they deviate from safe operations. Majority of such incidents are being reported from industrial and X-ray workers. Rate of such incident are trivial in facilities such as Department of Atomic Energy installations, radiotherapy facilities and other industries with well-trained radiation workers.

Many complex scenarios of excess exposures, including false positives due to mis-handling of personnel dosimeters demand alternate and reliable methods to ascertain the genuineness of exposure and also estimate the actual dose. Besides, protracted exposure, non-uniformity in radiation fields leading to partial body exposure, internal exposures due to radioactive contamination, mixed radiation fields and LET of radiation and type of radiation add to the complexities while estimating the dose. Biological dosimetry methods relaying on biological signals in response to radiation exposure are helpful to address most of these challenges. These methods help to assess such scenarios.

Biodosimetry lab of BARC has established these assays with high throughput and automated facilities to cater the regulatory needs of occupational exposures, emergency preparedness for radiological incidents and render tools for clinical applications. Radiation-induced DNA damage assessment through repair proteins like γH2AX and 53BP1 is being explored not only for biodosimetry but also as a clinical tool. These assays offer rapid radiation dose estimation. Beyond biodosimetry, their applications encompass addressing various clinical challenges like determining an individual's radio-sensitivity, estimating bone marrow dose, assessing whole-body radiation exposure during diagnostic and therapeutic procedures, and detecting genetic abnormalities. Moreover, employing multiple assays for multi-parametric analysis has enhanced the robustness and contributed to more accurate dose estimations in diverse scenarios, emphasizing their potential in both emergency response and clinical settings. The versatility of these assays highlights their importance in radiation research and radiological medicine.

Each scenario of excess exposure offers new technical challenges for dose estimation. An ideal biological dosimetry assays should be sensitive over a wide dose range of 20 mGy to several Gy, ability to detect radiation specific changes, exhibit reproducible dose response, must occur early but without fading over long durations, should respond to different types of radiation such as low and high LET, should be able to distinguish partial body irradiations, should be less-invasive, repeatability, rapid and amenable to automations. To meet the challenges of dose estimation, often it becomes necessary to adopt multiple assays through multi-parametric approach. This helps to eliminate limitations of some assays and help to ascertain biological dose independently by set of assays.

Biodosimetry lab in BARC has established various cytogenetic techniques to address these requirements. Advantages and features of these techniques are listed in Table 1.

**Table 1: Comparison of cytogenetic techniques**

| Assay | Dose range | Advantages/applications | Limitations |
|---|---|---|---|
| **Chromosome aberration analysis** | 0.1 – 6 Gy | Radiation specific, very low back ground, amenable to automation. | Labor intensive, requires skilled manpower. |
| **Micronucleus assay** | 0.25 – 6 Gy | Less labour intensive, better amenability to automation. | Not useful for non-uniform exposure and localized exposure, higher MDL relatively high background and inter-individual variation of response. Culture durations are longer. |
| **Fluorescent *in situ* hybridization** | 0.25 – 5 Gy | This technique has the capability to identify reciprocal chromosomal translocations, which are recognized as stable signals over an extended period (up to several decades), making it highly advantageous for retrospective biodosimetry, assessing internal exposure, and evaluating high linear energy transfer (LET) radiation effects. | Relatively lacking specificity, characterized by a notable background signal, a labour-intensive and time-consuming process, and associated with high costs. |
| **Mitotic cell fusion induced G0-PCC** | 0.25-25Gy | Same day dose estimation even with application of FISH, most suitable for high doses, most suitable for partial body exposures. | Lots of expertise is required, low dose uncertainty until 1Gy due to high background. |
| **Drug induced Premature chromosome condensation assay(G2/M-PCC)** | 3 – 25 Gy | Useful high-dose exposures, simple assay | Not sensitive at low doses limited applicability for non-uniform exposures |
| **γH2AX assay** | 0.05 -> 0.6 Gy | Rapid outcomes, suitable for automation, capable of detecting low doses <100 mGy if blood samples are available immediately (cell culturing not required), very sensitive, and a valuable tool for clinical applications. | Signal diminishes with DNA double-strand break repair, necessitates execution within a specific time frame following radiation exposure, and may not be suitable for prolonged exposure scenarios. |

Details of these assays, including methodology, samples pictures of cells used for analysis and specific advantages are discussed in the next sections.

## 2. Cytogenetic techniques in biodosimetry:

### *2.1 Dicentric chromosomal aberration (DCA) analysis:*

Dicentrics are products of DNA mis-repair by cells. While joining the broken ends of DNA through Non-Homologous End Joining (NHEJ) repair process, sometimes, though rarely, will lead to crosslinking of chromosomal bodies leading structures known as dicentrics. These chromosomal bodies will contain two centromeres due to crosslinking. They are also associated with an acentric fragment. Examples of dicentrics are shown in Fig 1b while 1a shows the comparative pic of normal cell without any aberrated chromosomes. In normal unexposed individuals, background dicentric frequencies are found to be in the range of 0.0005 to 0.001. The frequency increases with dose linearly at low doses and linear-quadratically with doses beyond about 1 Gy for low LET radiations. For high LET radiation such as neutrons, response will be linear throughout the dose range with higher slope. The frequency of dicentrics "y" varies with the dose "D" as follows:

$$y = c + \alpha D + \beta D^2$$

This dose refers to the equivalent whole-body dose received acutely in an accidental exposure. The lower limit for detection is 100 mGy for low LET radiation and 10 mGy for fast fission neutrons. It is necessary to establish calibration curves for many different radiations by the laboratory performing this assay. The calibration curves for different radiations are described in Fig 3. Range of dose that can be assessed by this assay is 0.1 - 6 Gy.

DCA has proven very reliable during the Chernobyl and Goiania accidents. Dicentric yield remains unaltered for several weeks but reduces over the time due to replacement of cells in circulatory blood. The signal goes down with two subsets of half time, one with fast component, 180 days at high doses and the second component, more slowly with an approximate half-life of 3 years[1–3]. Some individual differences in the response for the induction of CA may arise due to variations in genetic composition and DNA repair capacity but it is observed that the individual variations are negligible over the age group. Rare genetic and immune related disorders can enhance the induction of these aberrations.

An important requirement for performing this assay is a well-experienced scorer. These analyses are labor intensive, time consuming and not amenable to complete automation due to inherent variation of morphology of chromosomes, condensation levels, over lapping of chromosomes, staining variations, artefacts etc., which can be easily identified manually but pose challenges to automation. Nonetheless, many recent advances such as metaphase finders, centromeric FISH painting by anti-kinetochore antibodies have helped to achieve better throughput and reduced the fatigue on human resource.

It is necessary to score large number of cells (500-2000) when expected doses are low (<200 mSv). However, during radiological incidents involving large dose and for triage purpose, scoring of 20-50 cells will suffice the need. Preliminary dose estimates can be provided with this kind of approach for medical management. Whenever low doses (a few mGy) has to be detected, the Poisson error associated with the estimates can be very large due to detection of small number of dicentrics. At high doses (>3-5 Gy), severe leukopenia which may appear

within 1-2 days may lead to sparse availability of viable lymphocytes leading to difficulty in harvesting metaphase cells.

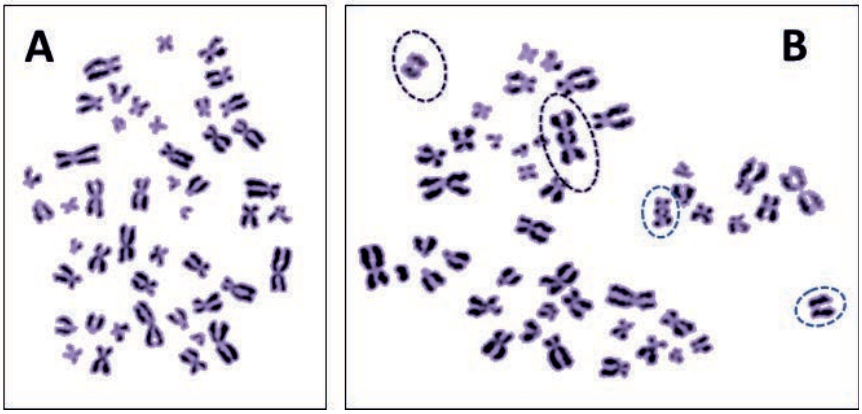


**Fig 1 (A). Metaphase chromosomes with NO aberrations. (B). Metaphase chromosomes with two dicentrics accompanied with two fragments.**

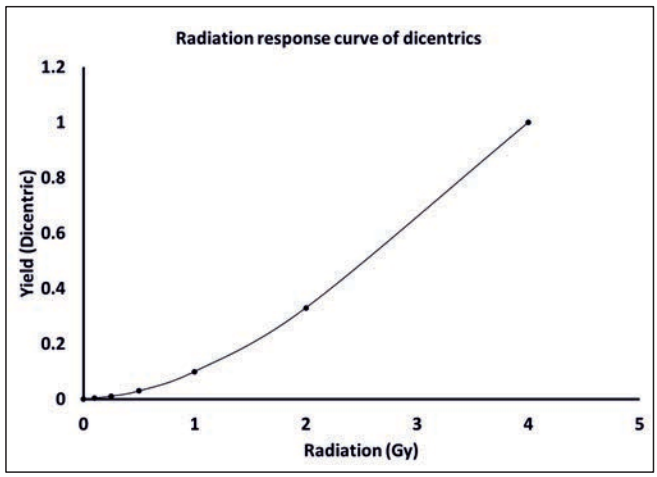


**Fig 2a. Response curve for gamma exposure, b. Confidence level of dose estimation using CA for low doses**

## DCA for non-uniform exposures:

Dicentrics are distributed in cells. When large number of cells observed, majority of the cells do not show any aberrations at low doses. As dose increases, more and more cells show single dicentric while very few with multiple dicentrics. This distribution follows Poisson distribution when the exposure is uniform to the whole body. The distribution gets deviated from the Poisson distribution when the exposure is non-uniform or partial. In most of the radiological incidents involving large dose, the exposures are non-uniform or even localized due to partial shielding or contact exposures. This will result in large doses to the exposed lymphoid pool while remaining portion may receive negligible dose. Dispersion analysis to derive the degree of over-dispersion can be done by u coefficient.

$$u = \frac{\left[\sigma^2/y - 1\right]\left[N - 1\right]}{\sqrt{2(N-1)(1 - 1/Ny)}}$$

Where, N is number of cells scored; y the frequency of dicentrics and $\sigma^2$ its variance. This coefficient will be greater than 1.96 for over dispersion suggesting either partial body or localized exposure.

### *2.2 Micronucleus Assay (MN assay):*

During division of cells, two main nuclei will get formed and finally lead to cytokinesis for equal distribution of genetic materials to the two daughter cells. Chromosomal breaks without any centromeres will fail to migrate into these main nuclei and remain outside in the cytoplasm and eventually lead to formation of small nuclei known as micronuclei. Sometimes, certain aneugenic factors may also lead to micronuclei by whole chromosomes. Cells getting exposed to radiation and other clastogenic agents can exhibit higher micronuclei yields. To visualize them,[4] cytokinesis blocking method by cytochalasin B is used.

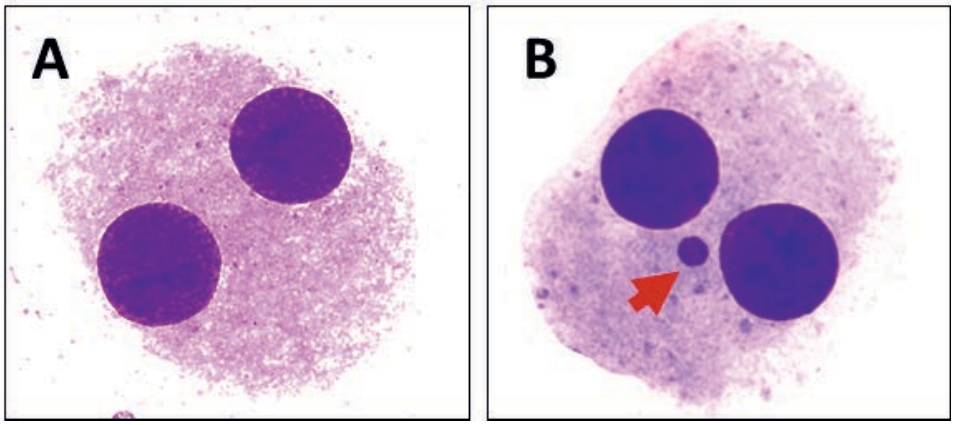


**Fig 3 (A). A binucleated cell with NO micronuclei**
**(B). A binucleated cell with ONE micronucleus**

Since micronuclei can be formed by other clastogenic agents too, many life style factors can affect the base line frequency and thereby leading to higher detection limit (250 mGy). In triage situation, this may be helpful as scoring is relatively easier and also more amenable to automation compared to DCA[5].

### *2.3 Premature Chromosome Condensation (PCC):*

At high doses cell cycle arrest at various checkpoints and mitotic cell death does not allow enough metaphases to be available for biodosimetry. In such situation chromosomal aberrations can be analyzed outside mitotic phase after premature chromosome condensation (PCC). PCC can be induced by two ways one is drug induced and another is mitotic cell fusion induced. Both are described below:

### *2.3.1 Drug Induced PCC ($G_2$/M-PCC):*

Drug induced PCC utilizes ability of few chemicals to remove effect of checkpoint inhibitor enzymes. Calyculin-A, Okadaic acid, caffeine are popular chemicals used for drug induced PCC at $G_2$-arrest[6,7]. The advantage with drug induced PCC is that it is a simple method and scoring of aberration (ring chromosomes) after PCC is also un-complicated. Ring chromosomes are known to follow linear response after radiation in the range of 1-25Gy. Though rings are as specific as dicentrics, yield of ring chromosome is lower hence, they are more conveniently used above 3Gy. Another limitation to drug induced PCC is time, it requires 48h assay duration excluding harvesting, slide preparation and analysis time. Further, it's utility in non-uniform exposure is limited.

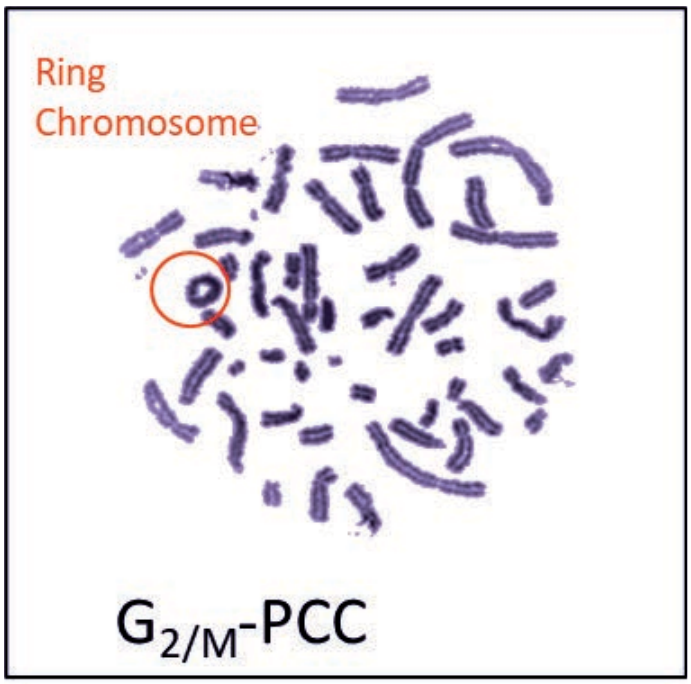


**Fig 4. Drug induced PCC spread with ring type of aberration**

### *2.3.2 Mitotic cell fusion induced PCC ($G_0$-PCC):*

Mitotic cell fusion induced PCC induces chromosome condensation within few hours after blood collection and whole analysis can be completed within 6-8h [8]. Mitotic cells are collected from Chinese hamster ovary cell culture and then preserved until use under cryopreservation. The method can be used to assess ring chromosomes, gross level fragmentation in each cell with Geimsa staining or dicentrics as well as chromosome specific breaks and translocations with the application of fluorescent in-situ hybridization[9].

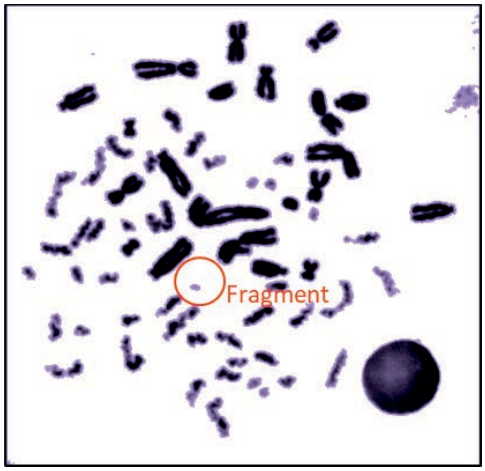


**Fig 5. Mitotic cell fusion induced $G_0$-PCC spread after Geimsa staining. Dark color metaphase chromosomes are from mitotic CHO cells whereas single chromatid chromosomes are prematurely condensed chromosomes from lymphocytes. Number of PCC bodies can be counted to conclude number of fragments (bodies excess to 46)**

### *2.3.3 $G_0$-PCC- Centromere-FISH*

Dicentrics being the specific marker of radiation, are most reliable endpoint. Due to morphological indistinctness, the dicentrics cannot be identified by uniform staining of chromosomes in $G_0$-PCC. For that purpose, centromere specific fluorescent pan centromere probes are used to stain the centromeres. It allows counting of dicentrics in $G_0$-PCC.

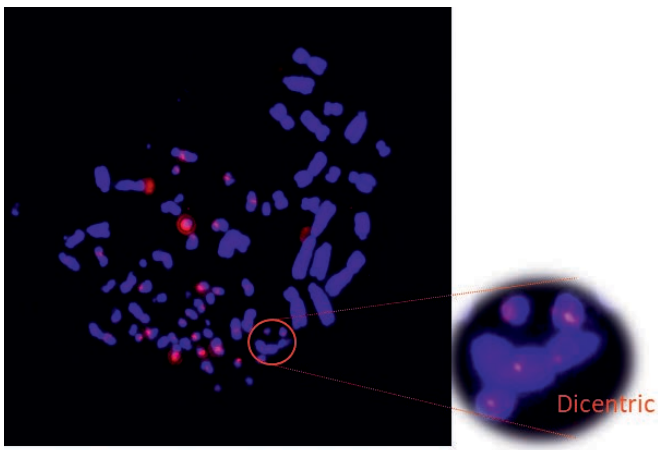


**Fig 6. $G_0$-PCC Spread after pan-centromere FISH and DAPI counter staining. Since the probes are specific to humans, they do not give signal at centromere of hamster chromosomes. Dicentric chromosomes can be easily identified by presence of more than one centromere (circled and enlarged)**

### *2.3.4 $G_0$-PCC-FISH (Whole chromosome Painting)*

$G_0$-PCC-FISH can also identify translocations and chromosome specific breaks by application whole chromosome painting probes similar to metaphase FISH. The probes can

be used for all the 24 chromosomes or only for few chromosomes of choice depending on the need.

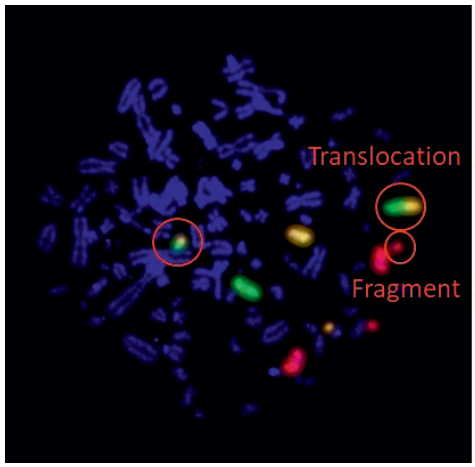


**Fig 7. $G_0$-PCC Spread after whole chromosome painting FISH of chromosomes 1, 2 & 4 in red, green and yellow colors respectively. A reciprocal translocation between chromosomes 2 & 4 is clearly visible. In addition, fragments of chromosome 1 & 4 are also visible**

### *2.3.5 $G_0$-PCC-Multi-color banding FISH:*

Multicolor banding is another advanced FISH based technique used to assess involvement of specific region of chromosome in a rearrangement. In this technique, a chromosome is divided in multiple regions and regions of a chromosome is painted in different colors. It is best suited for studying inversions. It is also useful in studying high LET radiation exposures. High LET radiations produce dense ionization, hence intra-chromosomal rearrangements are more frequent than that in Low LET exposures.

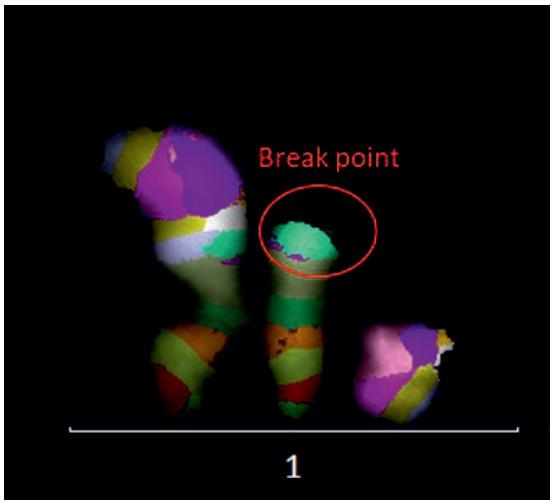


**Fig 8: Multicolour banding of chromosome 1 pair after $G_0$-PCC. One of the homologues can be seen broken and the band at the breakpoint can be identified. This can help in identifying highly sensitive regions of chromosomes**

## 2.4 Fluorescence In Situ Hybridization (FISH) Assay:

Fluorescence in situ hybridization (FISH) is emerging as a powerful tool for radiation dosimetry due to its remarkable ability to precisely detect and quantify radiation-induced translocations and other structural changes within chromosomes. Translocations are chromosomal rearrangements that occur when two different chromosomes exchange genetic material. These translocations are considered stable aberrations, which means they persist over time, making them valuable indicators of past radiation exposure. Here, we delve into why FISH is gaining prominence as a biological dosimeter and the advantages it offers over traditional methods.

First and foremost, FISH provides unparalleled accuracy in identifying and measuring radiation-induced translocations. The technique involves labelling specific DNA sequences with fluorescent probes, allowing researchers to visualize these chromosomal changes directly. This direct visualization not only simplifies the identification of translocations but also enhances sensitivity. Even subtle translocations that might go unnoticed using conventional methods become readily detectable with FISH. This increased sensitivity is vital for assessing low-dose radiation exposure accurately. Furthermore, FISH offers a considerable advantage in terms of speed and efficiency. Traditional methods for scoring translocations, such as the banding technique, are dishonourable for being labour-intensive and time-consuming. In contrast, FISH streamlines the process by enabling researchers to paint individual chromosomes with fluorescent probes, reducing the time and effort required for analysis. This efficiency is particularly important when dealing with a large number of samples, as is often the case in radiation dosimetry studies.

### *2.4.1 Detecting Chromosomal Changes with Interphase FISH (I-FISH) Without Cell Culturing:*

Interphase Fluorescence In Situ Hybridization (I-FISH) is a powerful molecular technique used for the detection of chromosomal changes in cells without the need for cell culturing. Unlike traditional cytogenetic methods that often require cell division and culture, I-FISH can directly examine the changes in genetic material in interphase cells, which are cells not actively undergoing cell division. The process involves using fluorescently labelled DNA probes that are complementary to specific target DNA sequences on the chromosomes of interest. These probes bind to their respective DNA sequences, and when viewed under a fluorescence microscope, they produce distinct signals, highlighting the presence of specific genetic alterations, such as chromosomal translocations, deletions, or duplications.

I-FISH as shown in Fig 6 is particularly valuable in clinical and other research settings like biodosimetry and syndrome detection. It allows for the rapid and accurate assessment of chromosomal changes in patient samples, aiding in the diagnosis and prognosis of genetic diseases and various types of cancers. Additionally, I-FISH is essential in studies involving rare or difficult-to-culture cells, where traditional cell culturing methods may not be feasible. Overall, Interphase FISH is a versatile and sensitive tool that has revolutionized the field of cytogenetics by enabling the detection of chromosomal abnormalities directly in interphase cells, offering faster and more convenient analysis without the need for cell division or culture.

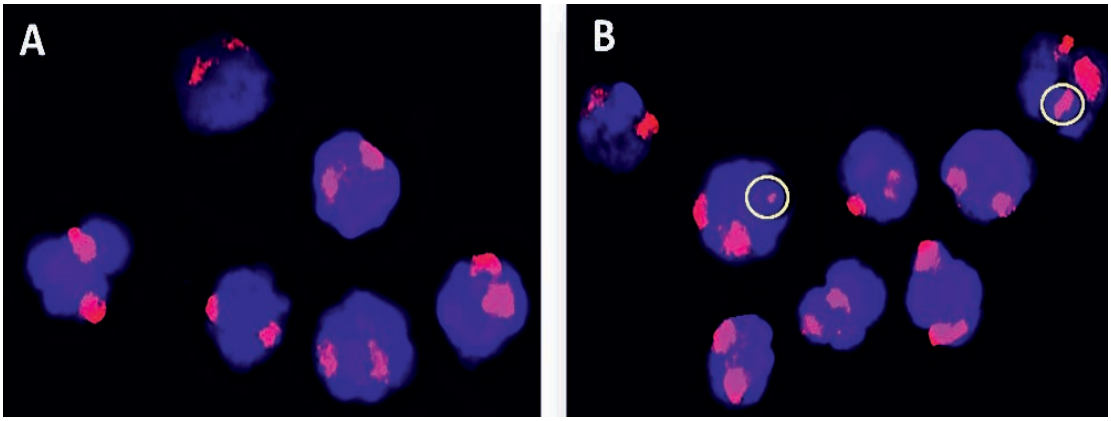


**Fig 9. Illustration of interphase-lymphocytes processed with single-color FISH. In panel (A), a control interphase-lymphocyte without aberrations is shown. In panel (B), an irradiated (4Gy) interphase-lymphocyte exhibits chromosomal breaks (highlighted in yellow circles).**

### *2.4.2 Enhancing chromosomal change detection and quantification with whole chromosome painting and centromere labeling:*

FISH has transformed biodosimetry by enabling precise and highly sensitive detection of chromosomal rearrangements[10–13]. Two key applications of FISH involve painting entire chromosomes and targeting centromeres as shown in Fig. 7.

Whole chromosome paint probes represent a significant improvement in the ability to identify and quantify translocations, crucial indicators of radiation exposure. These probes, coupled with advanced imaging techniques, can detect translocations at remarkably low levels, even down to the kilobase range. By fluorescently labeling entire chromosomes, researchers can visually capture translocations involving exchanges between painted and unpainted chromosomes, offering precise quantification. This enhances our capacity to evaluate radiation exposure, yielding biodosimetry data with exceptional kilobase-level resolution. This level of detail is invaluable for accurately gauging the extent of radiation-induced damage.

On the other hand, centromere-targeted FISH (Centromere-FISH Fig 8) plays a vital role in identifying dicentric chromosomes, another crucial radiation exposure marker. Centromere-FISH achieves this by specifically homing in on centromeric regions using fluorescent probes, substantially improving dicentric identification accuracy. Dicentric chromosomes are a telltale sign of ionizing radiation exposure, and their precise detection furnishes valuable insights into the type and extent of genetic damage inflicted by radiation. In summary, the application of FISH techniques for painting whole chromosomes and centromere targeting has not only revolutionized biodosimetry but also expanded our capabilities in assessing radiation exposure and genetic damage. These methods provide unmatched sensitivity, precision, and resolution, making them indispensable tools in radiation research and protection.

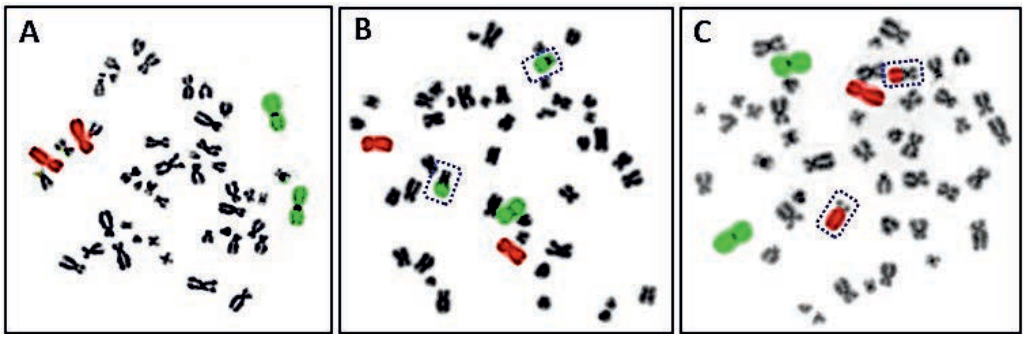


**Figure 10. Illustration of metaphase spreads processed with two-color FISH, with chromosome pair 1st and 2nd painted in green and red fluorophores, respectively. In panel (A), control metaphases are depicted. In panel (B), metaphases exhibit one inter-chromosomal translocation involving green and black chromosomes, while panel (C) shows metaphase with one inter-chromosomal translocation between red and black chromosomes.**

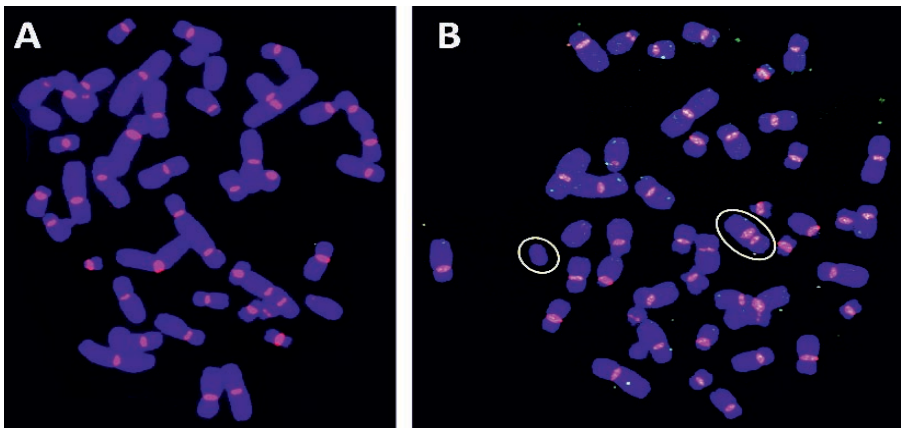


**Figure 11. Metaphase spreads processed for pan-centromere FISH, where the centromeres of all chromosome pairs are labeled with red fluorophores. In panel (A), control metaphases with no chromosomal aberrations are illustrated. In panel (B), metaphases exhibit one dicentric chromosome accompanied with an acentric fragment.**

### 2.4.2 Advanced chromosomal analysis techniques in biodosimetry: Multiplex FISH and spectral karyotyping:

Multiplex Fluorescence In Situ Hybridization (mFISH) is a cutting-edge technique used in biodosimetry and other clinical investigations (Fig. 9). It involves employing a set of fluorescent probes, each designed to bind to one of the 24 different types of chromosomes in the human genome. These probes are labeled with distinct colors, allowing researchers to simultaneously visualize all the chromosomes in a cell. When mFISH is combined with spectral karyotyping (SKY), each homologous pair of chromosomes is assigned a unique color code. This sophisticated labeling system enables researchers to detect and quantify a wide range of chromosomal changes or rearrangements in a single experiment/metaphase. These changes can include translocations (where genetic material exchanges between chromosomes), deletions (loss of genetic material), duplications (extra copies of genetic

material), and inversions (reversals in the genetic sequence). The power of mFISH lies in its ability to provide a high-resolution view of these complex genomic alterations.

One of the significant advantages of mFISH is that it offers a comprehensive and detailed analysis of chromosomal changes. By visually distinguishing each chromosome and tracking their interactions, researchers gain valuable insights into genetic abnormalities. This technique has undergone extensive validation and is widely used in both cytogenetics research and clinical diagnostics and investigations. In summary, mFISH is a powerful tool in biodosimetry due to its ability to simultaneously examine all 24 human chromosomes, each with its unique color label. When combined with SKY, it becomes an invaluable method for detecting and quantifying various chromosomal changes. This sophisticated approach offers a high-resolution view of complex genomic alterations, making it a valuable asset in both research and clinical settings for understanding genetic abnormalities resulting from radiation exposure.

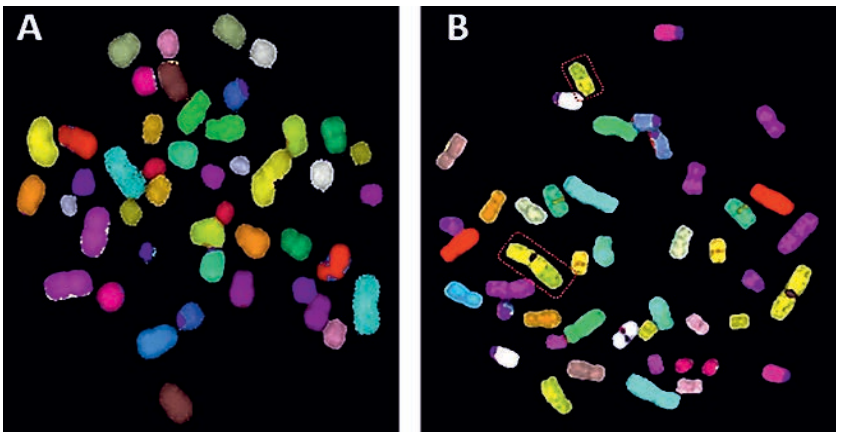


**Figure 12. Depiction of metaphase spreads analyzed using multiplex FISH, where all 24 types of chromosomes are distinctly labeled with different colors. Panel (A) displays control metaphases without any observable chromosomal aberrations, while Panel (B) illustrates metaphases featuring a single inter-chromosomal translocation involving chromosomes colored yellow and light green.**

### 2.4.3 mBAND-FISH: Advancing biodosimetry and clinical research:

Multiplex BAND Fluorescence In Situ Hybridization (mBAND-FISH) is a pioneering technique that combines the power of multiplex fluorescence in situ hybridization (FISH) with chromosome banding. Its primary aim is to detect and quantify intrachromosomal rearrangements, which are alterations occurring within the structure of a single chromosome. These rearrangements are predominantly triggered by high linear energy transfer (LET) radiation, making mBAND-FISH especially relevant in the context of high LET radiation exposure. The advantage of mBAND-FISH lies in its ability to use distinct colors to label different regions within the same chromosomes (Fig. 10). This labeling system enables precise visualization and identification of intrachromosomal rearrangements, which can be challenging to detect using conventional methods. These rearrangements are significant because they reflect the complex molecular changes happening within chromosomes in response to radiation exposure.

This innovative technique has found successful application in the study of radiation-induced genomic alterations. It serves as a valuable tool in biodosimetry research, helping scientists better understand the intricate molecular mechanisms that underlie radiation-induced genomic instability. By shedding light on these genomic alterations, mBAND-FISH contributes to a deeper understanding of the biological effects of radiation exposure. In summary, Multiplex BAND Fluorescence In Situ Hybridization (mBAND-FISH) is a cutting-edge method that combines multiplex FISH with chromosome banding to detect intrachromosomal rearrangements induced by high-LET radiation. Its ability to provide precise visualization and identification of these rearrangements makes it an invaluable asset in biodosimetry research and other clinical investigations where understanding the molecular consequences of radiation exposure is crucial.

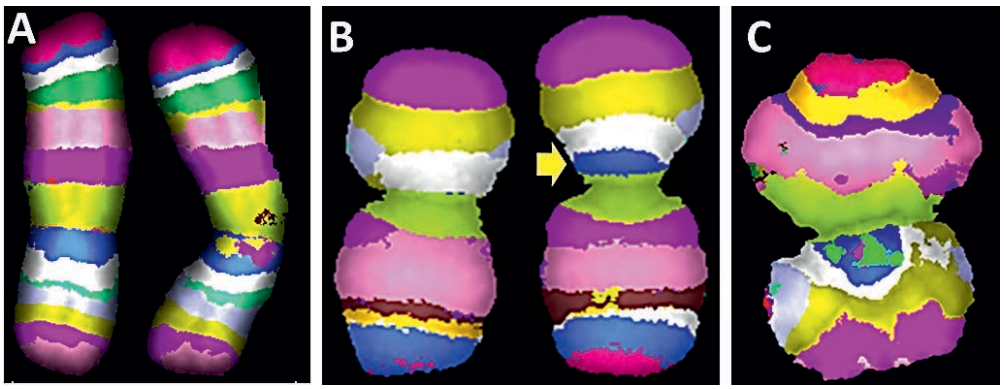


**Figure 13. Illustration of chromosome pairs processed with multiplex-BAND-FISH, where distinct colors are used to highlight different regions within the same chromosome. In panel (A), a control chromosome pair exhibits an unaltered banding pattern. In panel (B), a chromosome pair displays one interstitial deletion, identifiable by the blue band. In panel (C), a chromosome exhibits complex rearrangements, resulting in a complete loss of the banding pattern. These cells were exposed to Am-α radiation.**

## 2.5 Immuno-fluorescence methods:

Radiation exposure is known to cause DNA double-strand breaks (DSBs). These DSBs trigger a prompt response from DNA repair proteins such as γH2AX, 53BP1, ATM, RAD 51, and others. These DSB repair proteins hold promise as potential markers for quantifying DNA DSBs, offering the potential for correlation with the absorbed radiation dose. These repair proteins can be detected and quantified by immune fluorescence methods.

### *2.5.1 Phospho-γH2AX: A Rapid Biodosimetry Tool for DNA Double-Strand Break Repair:*

Phospho-γH2AX, a type of modified histone H2AX, has become a vital tool in biodosimetry due to its ability to detect DNA double-strand breaks (DSBs) and its role in repairing them [14]. When DSBs happen, H2AX gets phosphorylated at the damaged DNA site, which can be detected as γH2AX foci. These foci act as precise markers for assessing DNA damage caused by radiation (Fig. 11). To detect phospho-γH2AX foci, researchers use methods like immunofluorescence staining with fluorescent tagged antibodies, followed by examination under a fluorescence microscope or flow cytometry. By counting these foci and analyzing

their distribution in cells, we can estimate radiation doses and evaluate the complexity of radiation-induced DNA damage.

Phospho-γH2AX is preferred as a biodosimetry tool for its speed and sensitivity in biodosimetry. It can detect DNA damage shortly (within 4-5 hours) after exposure, making it valuable for immediate assessment. Moreover, its sensitivity to low-dose radiation allows its use in scenarios involving diagnostic exposures or accidental incidents with relatively low radiation doses. In addition, efforts are being made to develop protocols that can halt biological processes and preserve sample integrity during the transportation of blood samples to analyzing laboratories. This approach aims to minimize changes or degradation that might occur during sample transport.

In essence, phospho-γH2AX is a crucial biodosimetry tool, providing a fast and dependable method to assess radiation-induced DNA damage. Continuous improvements and refinements promise to enhance our ability to accurately estimate radiation doses and evaluate potential health risks linked to radiation exposure.

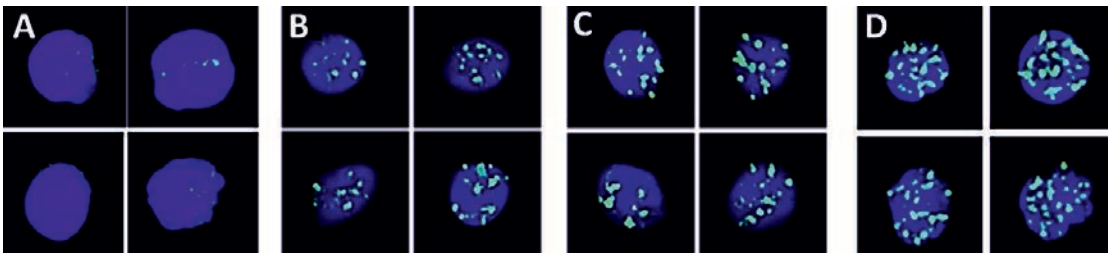


**Figure 14. Lymphocytes with a increasing count of phospho-γH2AX foci in the nucleus as the radiation dose increases (A-0 Gy, B-0.5 Gy, C-1.0 Gy, and D-2.0 Gy), one hour after exposure.**

### *2.5.2 53BP1 as a Rapid Biodosimetry Tool for DNA Damage Assessment:*

In the domain of rapid biodosimetry, phosphorylated-53BP1 (p-53BP1) emerges as a striking proteomic marker, complementing the utility of γ-H2AX in detecting DNA double-strand breaks (DSBs)[15]. These two markers, phosphorylated histone H2AX (γ-H2AX) and p53 binding protein-1 (53BP1), are widely recognized molecular indicators of ionizing radiation-induced foci. They play essential roles in revealing the presence of DSB repair and assessing the extent of DNA damage. When DSBs occur, a cascade of events starts within the cell's nucleus. Initially, histone H2AX molecules located within chromatin domains containing DSBs undergo phosphorylation, resulting in the formation of γ-H2AX foci. Subsequently, 53BP1, a downstream protein, is recruited to the precise locations of induced DSBs, effectively serving as an additional marker for DSBs.

The most advanced method currently available for the sensitive and specific analysis of DSBs involves quantifying these small but critical foci, which consist of both γ-H2AX and 53BP1. The colocalization of these foci further enhances the precision of radiation-induced damage assessment at the molecular level. By harnessing the combined potential of γ-H2AX and 53BP1, researchers can rapidly and accurately evaluate the impact of ionizing radiation on DNA integrity. This dual-marker approach not only provides valuable insights into the extent of radiation-induced damage but also holds promise for advancing the field of biodosimetry, ultimately contributing to better radiation risk assessment and emergency response planning.

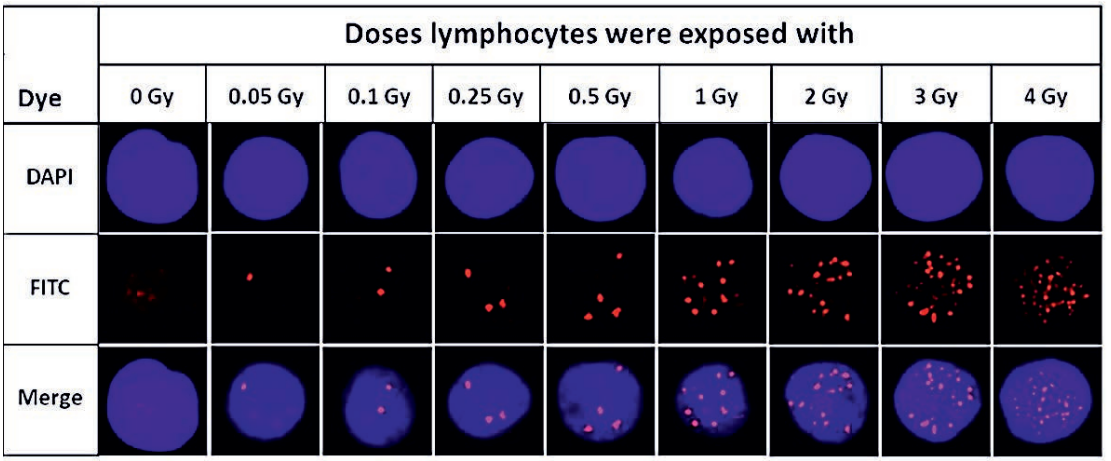


**Figure 15. Lymphocytes exhibiting a rising count of phospho-53BP1 foci within the nucleus as the radiation doses increase (0.05 to 2.0 Gy after 1 hour and 3 and 4 Gy after 4 hours of incubation post-irradiation).**

## 2.6 Pseudo-Pelger Huët (PHA) Neutrophils: A novel permanent biomarker for radiation exposure:

Pseudo-Pelger Huët Anomaly (PHA) has emerged as a novel marker for detecting radiation exposure within circulating blood neutrophils[16]. In contrast to regular neutrophils, which typically feature 3 to 4 lobed nuclei, PHA neutrophils display distinctive nuclei that are round, oval, bean-shaped, or symmetrically bi-lobed and connected by a thin mitotic bridge (Fig 13). This anomaly arises from a mutation in the lamin-B receptor (LBR) gene, resulting in reduced LBR expression.

PHA is noteworthy for its specificity to *in vivo* radiation exposure, originating within the bone marrow, and its inability to manifest in cells outside the body (*ex vivo*). In a significant study involving archived blood samples from the 1958 Y-12 criticality accident in Oak Ridge, Tennessee, researchers observed the appearance of Pseudo-Pelger Huët morphology in circulating blood within just 12 hours after radiation exposure. This suggests its potential as an indicator of acute radiation exposure. Impressively, in one case from the same accident, the PHA marker persisted for up to 16 years, serving as a stable and permanent mutation marker induced by radiation exposure and offering retrospective dosimetry capabilities. To establish a comprehensive dose-response curve for PHA, researchers have analyzed circulating blood neutrophils from individuals exposed to radiation. However, constructing this curve poses challenges, primarily because it necessitates the involvement of radiation-exposed human volunteers for *in vivo* exposure. Nonetheless, ongoing global research endeavours are focused on further investigating and validating PHA as a dependable biodosimeter for assessing radiation exposure. This promising biomarker holds the potential to revolutionize radiation dosimetry by providing a permanent and reliable indicator of past radiation exposure events.

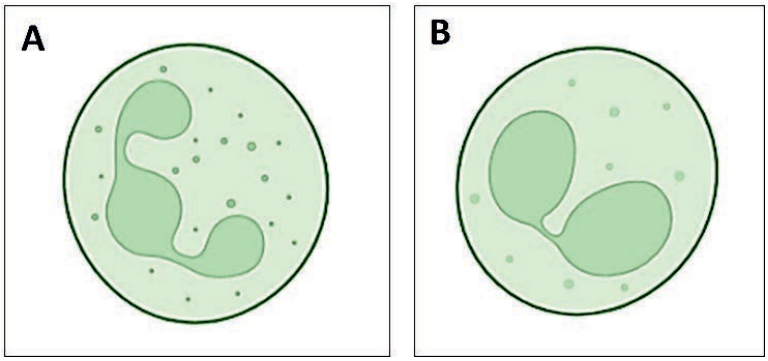


**Figure 16. (A) Depiction of a typical healthy neutrophil featuring a multilobed nucleus and (B) A Pseudo-Pelger Huët (PHA) neutrophil characterized by a bilobed (goggle-shaped) nucleus.**

## 2.7 Multiparametric approach for Biodosimetry of complex scenarios:

No single Biodosimetry assay can suffice to address many complex scenarios of radiation exposure. It becomes necessary to perform multiple assays to meet the challenges. Few of the assays are still in development stage but offer themselves as excellent tools in certain scenarios.

## 2.8 Summary:

The described assays help to conclude and ascertain genuineness of most excess exposure scenarios. These assays are helpful:

- To confirm or reject the findings of physical dosimeters and to distinguish between genuine and non-genuine exposures.
- To detect a suspected exposure when there is no information from physical dosimeters, such as in the case of a person who is not routinely monitored or a radiation worker not wearing a personal dosimeter.
- To provide reassurance to those who are false positive on physical dosimeters which got exposed during storage; or maliciously irradiated.
- To detect the average dose to the body in the case of non-uniform exposures. In such situations the dosimeters may indicate a very high or low dose depending upon the irradiation geometry.
- To distinguish between protracted and acute exposures in accidents. DCA provides a purely biological response taking into consideration the duration of exposure and accounting for the possible repair of radiation damage.
- To detect the dose to the exposed part of the body and the fraction of the body exposed, in accidents involving inhomogeneous exposure (Partial-body, or localized). Dispersion analysis of the chromosome aberration data indicates the deviation from a Poisson distribution, which in turn is a measure of the non-uniformity of exposure.
- To assist the triage of victims in accidents involving large number of exposed individuals. In the medical management of radiation accidents DCA can serve as a reliable prognostic indicator.

- To address complex scenarios of excess exposure, multi parametric approach with combination of assays can be employed.

Besides biological dose assessment, these assays are also useful to estimate radio-sensitivity prior to treatment of cancer, plan in advance for hypersensitivity, measure bone marrow doses in past therapeutic exposures needing repeated administration of nuclear medicine and measure body burden. Such clinical applications are currently gaining importance and are expected to help individualization of treatment protocols.

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