ISSN (e): 2250 – 3005 || Volume, 13 || Issue, 6|| Nov.-Dec. – 2023 ||
International Journal of Computational Engineering Research (IJCER)

Overview of some of the current biodosimetry methods for evaluation of the assessment dose, after ionizing exposure in the EU laboratories. Applicable biodosimetry methods in Bulgaria.

Racheva G.[1], Kindekov I.[2], Todorova P.[3]
[1] *Military Medical Academy – Sofia – senior assistant professor, Scientific laboratory of Radiobiology and Radiation protection;*
[2] *Military Medical Academy– Sofia – senior assistant professor, clinic of Hematology, Sofia;*
[3] *Military Medical Academy – Sofia – assistant professor, Scientific laboratory of Radiobiology and Radiation protection;*
Corresponding author: Galina Racheva, PhD, galina_ra4eva@abv.bg;

Abstract
The worldwide political situation determines, the EU radiobiologists to develop and improve bidosimetry methods for analysis of the assessment dose in their own laboratories. For a short period of time every Radiobiology laboratory in the EU had to choose or improve applicable method of analysis, to develop working protocols and their own calibration curves for the applicable methods. The Scientific laboratory of Radiobiology and Radiation protection, Military Medical Academy-Sofia is responsible for biodosimetry estimation of the assessment dose to the military structures in Bulgaria. Aim of the study: The aim of the current study is to analyze and compare some of the current biodosimetry methods, used in the European laboratories. Selection of a part of the analyzed methods for current use in one of the Bulgarian radiobiology laboratories; Materials and methods: comparison of the advantages and disadvantages of the most used radiobiology methods used in the EU radiobiology laboratories such as Dicentric chromosomal assay (DCA), cytokinesis-block micronucleus assay (CBMN), premature chromosome condensation assay (PCC) and fluorescence in situ hybridization (FISH) chromosomal analysis. Results: The detailed analysis and review of the applied European methods, give the priority to the golden standard method (DCA), as the most applicable, shorten and highly effective for the needs of Scientific laboratory of Radiobiology and Radiation protection, Military Medical Academy-Sofia. The advantages of the FISH technique show a good perspective and could be performed for biodosimetry in the laboratory. Conclusion: The biodosimetry assessment of the absorbed dose is a high skilled activity that involves team of professionals, working in the laboratory, correct selection of a few appropriate methods and preliminary optimization of the process. Take into consideration all described factors for our routine work use of DCA method is the first and optimal option. The FISH-technique presents many advantages that makes it a good additional option.
Key words: *radiobiology, biodosimetry methods, DCA, CBMN, PCC, FISH;*

Date of Submission: 02-12-2023 Date of acceptance: 12-12-2023

I. Introduction

The action of the ionizing radiation, with an assessment dose over the threshold of 1 Gy to the human organism, has a severe impact to every part of it (to molecular, cellular, tissue and organismal levels). Every organism exists, reproduces and develops in an environment of radiation - from low frequency radiation to high energy γ-quants. The sources of irradiation on the human organism can be external (outside of the body) or internal (incorporated inside of it). Incorporation of the radiation material can take place by inhaling air containing radioactive aerosols or by consuming foods and liquids containing radionuclides.

To investigate the result of the radiation exposure to the irradiated human body is very important to estimate the assessment dose. After irradiation with an assessment dose above the threshold (1 Gy) or prolonged

exposure with a lot of separated low doses with total absorbed dose over the limit, appears a risk of radiation damage. In the case of the over threshold exposure (≥ 1Gy) the medical specialists expect development of deterministic effects (acute radiation syndrome, radiation cataract, skin erythema). Stochastic effects develop after certain period of time, as a result of long-term irradiation with separated low doses (malignant blood and solid tumors, anemia, reproductive damages). Estimation of the dose after over the threshold exposure (after radiation incidents) is a main part of the diagnostic and fast optimization of the therapy, because of the direct dose-effect relation. Increase of the assessment dose leads increase of the severity of the disease. In that case physicians expect development and arising of the symptoms in period of time in different range from several hours to several weeks. The estimation of the dose gives detail information about the possible effect of the irradiation, the type of the expected disease and treatment possibilities. The biodosimetry estimation of the total absorbed dose to the moment of the analysis is an important part of the assessment of the risk of stochastic effect development. The most accurate and fast way to quantify the absorbed dose is to use biodosimetry methods.

Aim

The aim of the current study is to analyze and compare some of the current biodosimetry methods, used in the European laboratories. Selection of a part of the analyzed methods for current use in one of the Bulgarian radiobiology laboratories.

II. Materials and methods

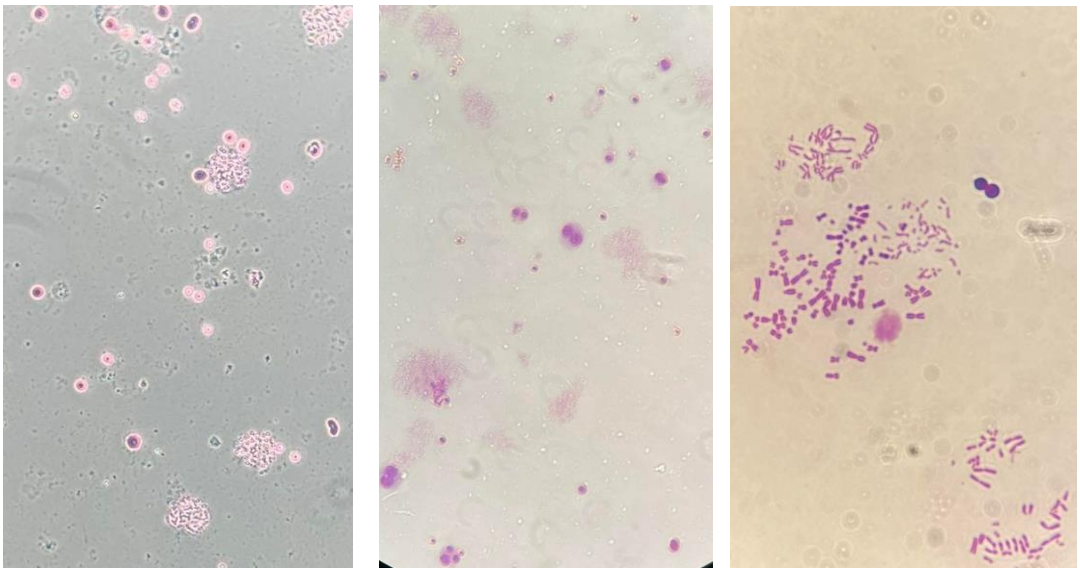
In the current study is made a comparison of the advantages and disadvantages of the most used radiobiology methods, used in the EU radiobiology laboratories, such as Dicentric chromosomal assay (DCA), cytokinesis-block micronucleus assay (CBMN), premature chromosome condensation assay (PCC) and fluorescence in- situ hybridization (FISH) chromosomal analysis. Detailed description of the methods applied for biodosimetry use in the Scientific laboratory of Radiobiology and Radiation protection, Military Medical Academy-Sofia (CBMN and FISH).

III. Results

Ionizing radiation induces a variety of DNA damages, including single-strand breaks (SSB) and double-strand breaks (DSB), change of nitrogenous base in one of the DNA's strands, cleavage of the DNA backbone. Those types of DNA damage are detected and repaired by the reparation mechanisms for the base excision repair or SSB-repair [1,4]. DSB are considered as the most dangerous and lethal DNA damage. Cells can adapt to small DNA damage by repair [12], but a single double-strand break (SSB) can be essential for cell death [17]. Double-strand breaks (DSB) are considered as typical of ionizing radiation-induced DNA damage [2]. Chromosomal aberrations are well known and studied biomarkers for genotoxic effects, resulted of irradiation. Presence of dicentric chromosomes (chromosome with two centromeres) is the main prognostic factor for the action of ionizing radiation. Much rarer is the presence of ring chromosomes (chromosome without centromere). It is a prognostic factor for very high dose irradiation. Both chromosomal structures are considered as unstable, because they are going to be lost to the next mitotic cycle.

Dicentric chromosomal assay (DCA) is the golden standard in the field of Radiobiology. DCA is a proven biodosimetry method and has been established as the golden standard of determine the irradiation DNA damage. This analysis is very specific to the action of the ionizing radiation. It is based on use of the cytogenetic karyotyping and detection of possible presence of dicentric chromosomes. The dicentric chromosome is an abnormal structure with two centromeres. It is formed after two double-strand breaks (DSB) in two different chromosomes and followed by their fusion, each with a centromere (a dicentric chromosome) and release of two acentric fragments (Fig.2 A) [10]. They are clearly visible and distinguishable in the metaphase of cell division. The action of high dose over limit radiation leads to formation of atypical chromosomal structures (such as dicentric chromosomes, ring chromosomes) and release of single, acentric fragments. The increase of overlimit dose led to increase the amount of dicentric chromosomes. It is possible to measure low dose of irradiation or to detect a partial body exposure [13]. To calculate the assessment dose is necessary every laboratory to establish its own calibration curve [12]. Military Medical Academy has been established its own calibration curve for mainly research purposes, based on the in-vitro examinations of irradiated blood cultures (peripheral blood samples of employees of the Military Medical Academy and blood donors) in different range low to high doses, during the years of research work in the laboratory. As unstable DNA damage the blood samples should be collected as fast as possible, after the exposure. The 0,5 ml blood is cultured in 4,5 ml RPMI 1640 medium, 20%FBS, L-Glu, Pen/Str, 2% PHA (the optimization of the process shows the possibility also to use 5 ml medium). The samples with the presence of 200 µl of 10 µg/ml colchicine (a mitogen agent) are incubated in 37ºC, 5% CO_2 for 48 hours. After that process, microscopic slides (as G-bands) with 4% Giemsa are prepared. They are used for observation, and counting of the dicentric chromosome amount. The manual counting requires

500-1000 metaphases to be count. That is long and slow time-consuming process, used for research work. In case of radiation incident, the process should be faster and triage should be done for a very short period of time. That is the reason to reduce the amount of scoring to 30-50 per sample [13, 16]. The equipment of the laboratory is a determinant factor. Most of the EU laboratories (including Bulgaria) use an automation of the process and accelerate it multiple times. Depending on the available equipment, the scoring process of the 50-100 cells takes 1-2.5 hours [7]. The assessment dose of the DCA is in a range of 0.1 – 5 Gy [12,15], because larger dose of irradiation produces a lot of fetal damages and a few cells in metaphases (no clear dose-effect relation). The risk assessment of stochastic effects, after periodically irradiation with low-doses radiation can be performed with this method. In those case should be performed multiple counts of samples with a larger number of cells, during prolong period. That method has been established and developed as the first major biodosimetry method selected for use at the Military Medical Academy-Sofia (fig.1A)).



A) B) C)

Fig.1: A) Giemsa staining of metaphase chromosome and performed dicentric chromosome analysis; B) Giemsa staining of anaphase cells (binuclear cells) for CBMN; C) Giemsa staining of PCC-chromosomes.

Cytokinesis-block micronucleus assay (CBMN) is used as an alternative method of DCA, mostly in the research work. The CBMN is a routine method based on the formation of small membrane-bound DNA fragments (micronuclei) in nucleated cells that have undergone only one nuclear division [19]. Micronuclei could be acentric (chromosomal fragments without a centromere) or from whole chromosomes unable to migrate with the rest of the chromosomes, during anaphase of cell division [5]. Micronuclei formation result of toxic activity and is not specific for radiation exposure [20]. For routine biodosimetry is recommended to score minimum 1000 binuclear cells with one micronuclei [18]. CBMN is used to measure over limit dose (≥1Gy) and the recommended range is 1-5 Gy [6, 15, 20]. That method is performed in Military Medical Academy-Sofia, as a research method, but is not a main choice for biodosimetry routine activity (fig.1B)).

Premature chromosome condensation assay (PCC) is used mostly as a research method. PCC combine isolated peripheral blood lymphocytes and mitotic Chinese hamster ovary (CHO) culture cells. The CHO cells are used to cause interphase chromatin condensation into visible and countable chromosomes (fig.1 C)). That premature chromosome condensation allows to visualize and count the chromosomal aberrations. That assay provides quick estimation of the assessment dose, because that method doesn't require culturing and mitogen stimulation [11]. Microscope manual scoring is difficult and time consuming. The automation of the process could speed up it and makes it good dose estimate method. Its disadvantage is the need of combination between peripheral blood cells and cell culture cells. It is very good and relatively accurate method for research work.

Used of fluorescence in-situ hybridization (FISH) analysis is based on the analysis of existed translocations (exchange of the chromosomal parts between chromatids of different chromosomes) in the cells [18] (Fig.2 B). The frequency of the radiation-induced translocations in the cells has obvious relation with increasing of the dose. Some of those translocations could persist in the next cell's generations and to become mitotically stable, as it is observed on A-bomb survivors and in patients undergo to prolong radiotherapy [3]. In a comparison with the unstable dicentric chromosomes, those mutations are better prognostic factor for retrospective analysis of the irradiation, when time has passed since the exposure or the radiation has been chronically received.

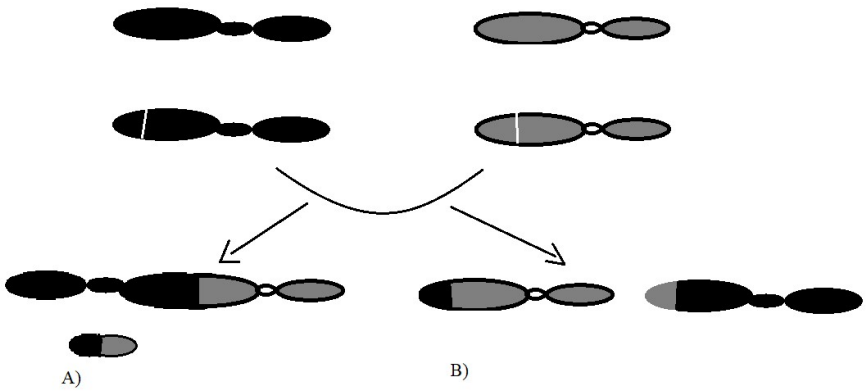


Fig.2: Chromosomal translocations – forming of unstable dicentric and free residue A) or stable translocated chromosomes B);

The principle of that method is to specifically visualized a part of the DNA with fluorescent labeling. Every pair translocation exchange, which include the visualized part of the chromosomes, are visible on the fluorescent microscope. Detected aberrations are those between the fluorescens painted and unpainted (dual colored) material, the efficiency of the method depends on the number of the colored chromosomes. The fastest and statistically efficient variant, which includes optimal financial and time-consuming efforts, is to use three pairs of chromosomes to be colored [8, 9]. The existence of dicentric chromosomes should be considered and should be included coloring of the telomere and centromere material. The combination of the described FISH techniques gives great advantage of the whole process of scoring of the chromosomal aberrations. In the Military Medical Academy – Sofia is used three chromosome pairs staining as research method (Fig.3 A), B), C)). The advantages of the combined FISH technique make that method a good perspective for additional biodosimetry method, that could be performed in the laboratory.

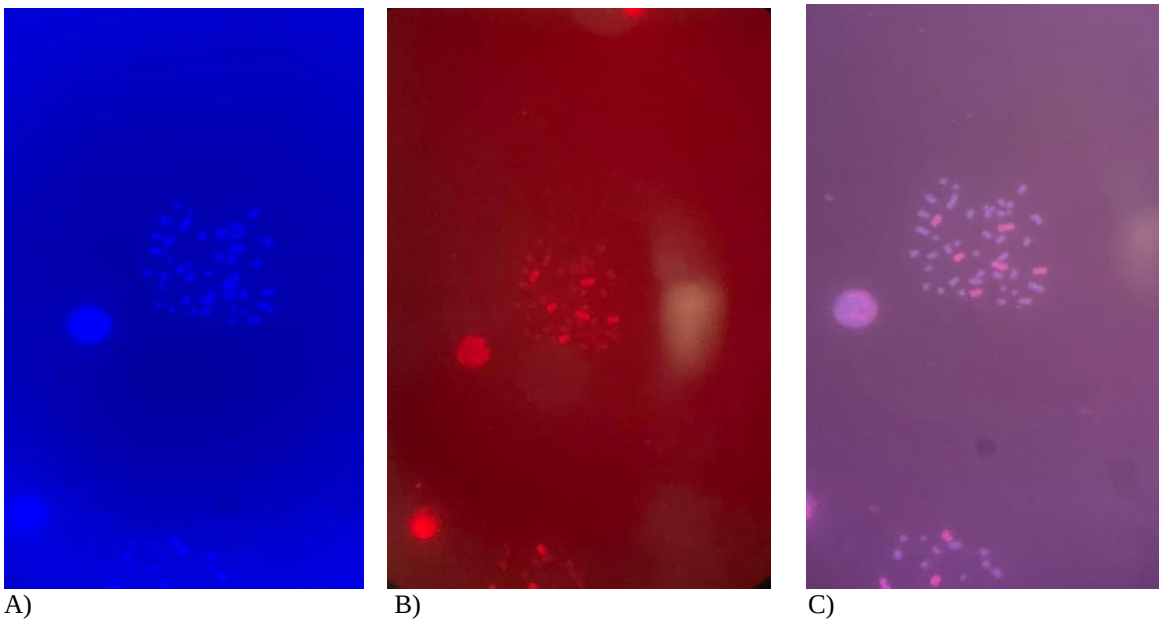


Fig. 3: Performed FISH staining of three pairs of chromosome with counterstained with DAPI: A) FISH – counterstained DAPI filter; B) FISH-red filter; C) FISH-mixed filters.

IV. Conclusion:

The biodosimetry assessment of the absorbed dose is a high skilled activity that involves team of professionals, working in the laboratory, correct selection of a few appropriate methods and preliminary optimization of the process. Take into consideration all described factors for our routine work use of DCA method is the first and optimal option. The FISH-technique presents many advantages that makes it a good additional option.

References:

[1]. Almeida KH, Sobol RW, A unified view of base excision repair: lesion-dependent protein complexes regulated by post-translational modification, DNA Repair (Amst) 2007; p.: 695–711.

[2]. Brandi L. Mahaney1, Katheryn Meek, and Susan P. Lees-Miller, Repair of ionizing radiation-induced DNA double strand breaks by non-homologous end-joining, NIH Public Access, Biochem J. 2009, p.: 639–650

[3]. Buckton, K.E.; Jacobs, P.A., Court Brown,W.M.; Doll, R. A study of the chromosome damage persisting after x-ray therapy for ankylosing spondylitis. Lancet 1962, p.: 676–682.

[4]. Dianov GL, Parsons JL, Coordination of DNA single strand break repair, DNA Repair (Amst) 2007, p:454–460.

[5]. Fenech M., The in vitro micronucleus technique, Mutation Research, 2000, p.:81-95.

[6]. Fenech M., The lymphocyte cytokinesis-block micronucleus cytome assay and its application in radiation biodosimetry. Health Phys., 2010, p:234–243

[7]. Flegal FN, Devantier Y, McNamee JP, Wilkins RC. Quickscan dicentric chromosome analysis for radiation biodosimetry. Health Phys. 2010;

[8]. Goh, V.S.T.; Fujishima, Y.; Abe, Y.; Sakai, A.; Yoshida, M.A.; Ariyoshi, K.; Kasai, K.; Wilkins, R.C.; Blakely, W.F.; Miura, T., Construction of fluorescence in situ hybridization (FISH) translocation dose-response calibration curve with multiple donor data sets using R, based on ISO 20046:2019 recommendations. Int. J. Radiat. Biol. 2019, p: 1668–1684.

[9]. Gregoire, E.; Barquinero, J.F.; Shi, L.; Tashiro, S.; Sotnik, N.V.; Azizova, T.V.; Darroudi, F., Ainsbury, E.A.,Moquet, J.E., Fomina, J., et al., Verification by the FISH translocation assay of historic doses to Mayak workers from external gamma radiation. Radiat. Environ. Biophys. 2015, p: 54, 445–451.

[10]. IAEA, Biological Dosimetry: Chromosomal aberration analysis for dose assessment. In Technical Report series N_260; IAEA: Vienna, Austria, 1986.

[11]. Julie M. Sullivan et al, Assessment of Biodosimetry Methods for a Mass-Casualty Radiological Incident: Medical Response and Management Considerations, NIH Public access, Health Phys. 2013 December , p. 105(6)

[12]. Lee D., Goldberg A., Proteasome inhibitors: valuable new tools for cell biologists, Cell press, Elsevier, Volume 8, Issue 10, 1998, p. 397–403, IAEA 2011

[13]. Lloyd DC, Edwards AA, Moquet JE, Guerrero-Carbajal YC, The role of cytogenetics in early triage of radiation casualties, Appl Radiat Isot, 2000; p: 52:1107–1112.

[14]. M'Kacher, R., Colicchio, B., Borie, C., Junker, S., Marquet, V., Heidingsfelder, L., Soehnlen, K., Najar, W., Hempel, W.M., Oudrhiri, N., et al., Telomere and Centromere Staining Followed by M-FISH Improves Diagnosis of Chromosomal Instability and Its Clinical Utility, Genes, 2020

[15]. Pinto MM, Santos NF, Amaral A., Current status of biodosimetry based on standard cytogenetic methods, Radiat Environ Biophys, 2010, p: 49:567–581

[16]. Prasanna PG, Moroni M, Pellmar TC., Triage dose assessment for partial-body exposure: Dicentric analysis. Health Phys, 2010, p: 98:244–251.

[17]. Rich T., Allen R., Wyllie A., Defying death after DNA damage, Nature, 2000, p.: 777-783.

[18]. Sorokine-Durm, I., Durand, V., Le Roy, A., Paillole, N., Roy, L., Voisin, P, Is FISH painting an appropriate biological marker for dose estimates of suspected accidental radiation overexposure? A review of cases investigated in France from 1995 to 1996, Environ, Health Perspect, 1997, p. 1427–1432.

[19]. Thierens H., Vral A., The micronucleus assay in radiation accidents: Ann. Ist. Super, Sanita, 2009, vol. 45, p. 206-264

[20]. Vral A, Fenech M, Thierens H., The micronucleus assay as a biological dosimeter of in vivo ionising radiation exposure, Mutagenesis, 2011, p: 26:11–17