ESTABLISHING WORKING STANDARDS OF CHROMOSOME ABERRATIONS ANALYSIS FOR BIOLOGICAL DOSIMETRY

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ABSTRACT: Biological dosimetry is an dose assessment method using specify biomarkers of radiation. IAEA (International Atomic Energy Agency) and ISO (International Organization for Standardization) defined that dicentric chromosome is specify for radiation, it is a "gold standard" for biodosimetry. Along with the documents published by IAEA, WHO, ISO and OECD, our results of study on the chromosome aberrations induced by radiation were organized systematically in nine standards that dealing with chromosome aberration test and micronucleus test in human peripheral blood lymphocytes *in vitro*. This standard addresses: the reference dose-effect for dose estimation, the minimum detection levels, cell culture, slide preparation, scoring procedure for chromosome aberrations use for biodosimetry, the criteria for converting aberration frequency into absorbed dose, reporting of results. Following these standards, the automatic analysis devices were calibrated for improving biological dosimetry method. This standard will be used to acquire and maintain accreditation of the Biological Dosimetry laboratory in Nuclear Research Institute.

***Keyword**: Biological dosimetry, Chromosome aberration, Micronuclei, Radiation risk assessment, Standard.*

1. INTRODUCTION

The individual radiation dose estimation is an important step in the radiation risk assessment. In some cases of radiation incident or radiation accident, physical dosimetry method can not be used for calculating the individual radiation dose, the other complement method such as biological dosimetry is a well replacement method. Using the cytogenetic techniques for biological dosimetry were recommended by IAEA with some documents: Technical reports 160 (1986); 405 (2001); IAEA / WHO EPR (2011). The International Organization for Standardization published *ISO 19238:2004 - Radiation protection-performance criteria for service laboratories performing biological dosimetry by cytogenetics*. The task for compiling some biological dosimetry standards including: standard for chromosome aberration test and standard for micronucleus test in human peripheral blood lymphocytes *in vitro*. These standards are intended to standardize the protocols and analysis methods, providing the basis for the implementation and development of biological dosimetry techniques in Nuclear Research Institute and Vietnam.

2. METHOD

The standards for biodosimetry were compiled base on the documents published by IAEA, WHO, ISO standards and OECD including:

- The IAEA documents: IAEA – EPR – Biodosimetry, "*Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies*", WHO, IAEA, Vienna, 2011; IAEA Technical Reports, Series No. 405, "*Biological dosimetry: Cytogenetic analysis for radiation dose assessment*", A manual, IAEA, Vienna, 2001; IAEA Technical reports, Series No.

260, "*Biological dosimetry: Chromosomal aberration analysis for dose assessment*", IAEA, Vienna, 1986.

- The ISO documents: ISO 19238:2004: *Radiation protection – Performance criteria for service laboratories performing biological dosimetry by cytogenetics*; ISO 21243:2008: *Radiation protection – Performance criteria for laboratories performing cytogenetic triage for assessment of mass casualties in radiological or nuclear emergencies – General principles and application to dicentric assay.*

- The OECD documents: OECD 473 *In vitro* mammalian chromosome aberration test; OECD 487 *In vitro* mammalian cell micronucleus test.

The result of studies on biological dosimetry carried out in Nuclear Research Institute from 1990 up to date were used for these standards including: the dose – effect respond curve of some radiation sources (gamma Co-60, thermal neutron and mixed gamma neutron fields), epidemiology of chromosome aberrations, chromosome aberrations induced by some chemical agents…

3. RESULTS

3.1. Some standards for biological dosimetry including

- Chromosome aberration test in human peripheral blood lymphocytes *in vitro* – Part 1: Standards for culture of human peripheral blood lymphocytes.

- Chromosome aberration test in human peripheral blood lymphocytes in vitro – Part 2: Standards for microscope slides of conventional Giemsa – stained chromosomes in human peripheral blood lymphocytes.

- Chromosome aberration test in human peripheral blood lymphocytes in vitro – Part 3: Standards for karyotyping of human peripheral blood lymphocytes using conventional Giemsa staining.

- Chromosome aberration test in human peripheral blood lymphocytes in vitro – Part 4: Standards for analysing of chromosome aberrations in human peripheral blood lymphocytes exposed to ionizing radiation.

- Chromosome aberration test in human peripheral blood lymphocytes in vitro –Part 5: Standards for biodosimetry using chromosome aberration analysis in peripheral blood lymphocytes.

- Chromosome aberration test in human peripheral blood lymphocytes in vitro –Part 6: Standards for analysing of chromosome aberrations in human peripheral blood lymphocytes exposed to chemical agents.

- Micronucleus test in human peripheral blood lymphocytes in vitro – Part 1: Standards for culture of human peripheral blood lymphocytes.

- Micronucleus test in human peripheral blood lymphocytes in vitro – Part 2: Standards for microscope slides of conventional Giemsa- stained micronuclei in human peripheral blood lymphocytes.

- Micronucleus test in human peripheral blood lymphocytes in vitro – Part 3: Standards for analysing of micronuclei in human peripheral blood lymphocytes.

3.2. Testing the protocol of chromosome aberration test and micronucleus test in human peripheral blood lymphocytes in vitro

Chromosome aberration test and micronucleus test *in vitro* in human peripheral blood lymphocytes irradiated by 3.0 Gy of gamma ^{60}Co and control following the standards.

- Chromosome aberration test in human peripheral blood lymphocytes *in vitro*:

The requirements for cell culture technique and microscope slide of human peripheral blood lymphocytes *in vitro* including: Mitotic index – MI (%), the proportion of first cell cycle metaphase to second cell cycle metaphase – P (%), the shape of metaphase and chromosome. Following the protocols in standards, all of these criteria got well for biological dosimetry.

We used the slides from this test for calibration of Carl Zeiss AXIO Imager Z2 microscope controlled by Metafer 4.0 and DCscore (Metasystem). After calibration, this system was compatible with slides for scoring the chromosome aberration automatically.

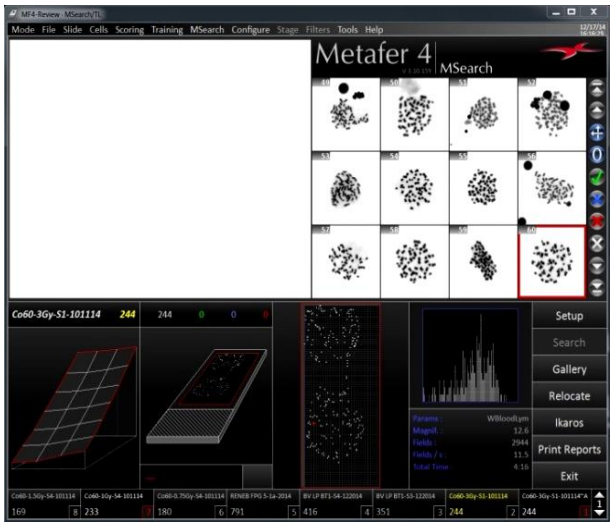

Figure 1a: Metafer 4.0 scanning metaphase automatically.

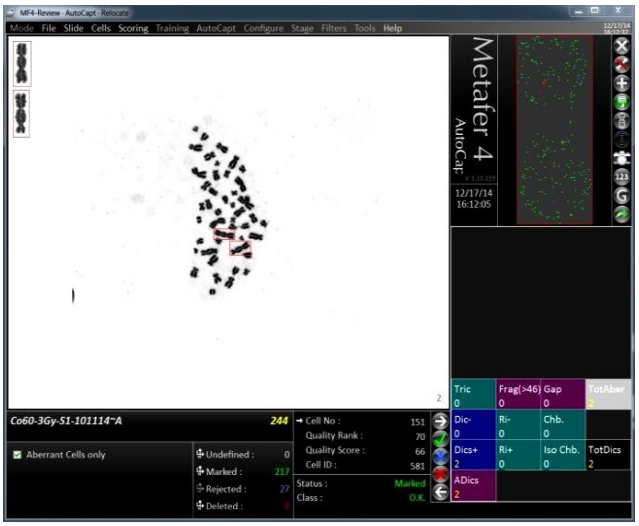

Figure 1b: DCscore scoring dicentric chromosome automatically.

- Micronucleus test in human peripheral blood lymphocytes *in vitro*:

The requirements for cell culture technique and microscope slide of human peripheral blood lymphocytes *in vitro* including: Nuclear division index – NDI (%), the proportion of mononuclei, binuclei, trinuclei and tetranulei, the shape of binuclei cell. Following the protocols in standards, all of these criteria got well for biological dosimetry.

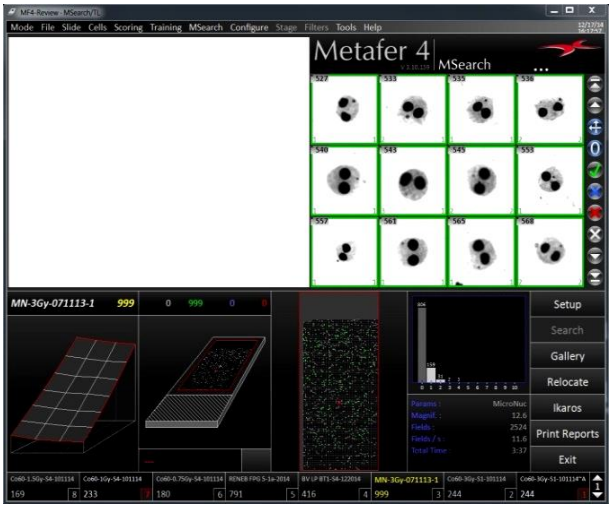

Figure 2a: Metafer 4.0 scanning binuclei automatically.

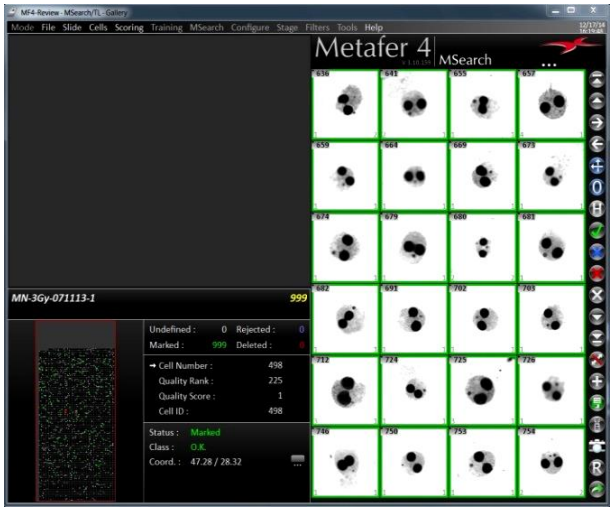

Figure 2b: MNscore scoring micronuclei in binuclei cell automatically.

We used the slides from this test for calibration of Carl Zeiss AXIO Imager Z2 microscope controlled by Metafer 4.0 and MNscore (Metasystem). After calibration, this system was compatible with slides for scoring micronuclei automatically.

4. EVALUATION

The cytogenetic biological dosimetry is a dose assessment method which fills a gap in dosimetric techniques, particularly in cases that persons or radiation workers not wearing dosimeters have been exposed to radiation, or in cases of overexposure in an individual working time. Many biomarkers were recognized for biological dosimetry, however the dicentric chromosome is the best biomarker, so called " the gold standard" for some characteristics: shows close dose – effect relationship, was very specify for radiation, shows the radiation quality effects, persists after exposure, determines the whole body or partial body exposure, can assess in fraction and chronic exposures, is amenable to automation. The studies carried out in Nuclear Research Institute brought about protocols and data used for biological dosimetry such as: cell culture, slide preparation, analysis method, automatic analysis system, dose – effect curves… These standards were built base on our data, these documents are expected to contribute to the radiation protection area.

5. CONCLUSIONS

The results of this task were completing nine standards for chromosome aberration test and micronucleus test in human peripheral blood lymphocytes *in vitro*, standardizing the protocols and calibrating the automatic analysis devices for biological dosimetry. These standards will be used for radiation risk assessment, the necessary thing in radiation safety.

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