Rapid and High-Throughput Detection of Peripheral Blood Chromosome Aberrations in Radiation Workers

Jinling Bi1,2, Hong Dai1, Junchao Feng1, Huahui Bian1, Weibo Chen1, Youyou Wang1, Yulong Liu1,3,4, and Yong Huang2

Abstract
There is a pressing need to establish automated solutions for the rapid, high-throughput, and automatic detection of chromosome aberrations (CAs) in the occupational health surveillance of large-scale radiation workers. Here, we described and verified the accuracy of a new measurement system based on the automatic scanning and analysis of dicentric chromosomes (DICs). The effects of cell number on DIC detection by automatic scanning and analysis were studied, and the distribution of DIC values per cell was calculated. In total, 1088 cases were detected by automatic DIC scanning and analysis in 26 663 radiation workers, and 73 cases were further confirmed by a technician, including 5 cases in which radiation exposure lead to harmful medical consequences. Our approach reduces the workload by 96% and increases the speed of assessment approximately 7-fold. Overall, this study validates the utility of a novel rapid and high-throughput CA detection procedure as a means of occupational health surveillance of large-scale radiation workers.

Keywords
high-throughput, automatic dicentric chromosome analysis, radiation workers, occupational health surveillance

Introduction
The detection of chromosome aberration (CA) is not only used as a biomarker of radiation exposure but also in establishing the relationship between radiation exposure and cellular responses in vivo, in dose, and dose-rate responses, as well as potential health problems in humans.1 The traditional measurement of CA requires hundreds of cells to be visually analyzed under the microscope for each tested sample, and each technician can only analyze 1 or 2 samples per workday. Automation offers an effective means to solve this problem. In the 1990s, with the development of the electron microscope and computer image processing technology, a CA automatic scanning analysis system was developed to automatically search for peripheral blood cells in metaphase and acquire high-resolution images, to identify cases of dicentric chromosomes (DICs). In 2009, automation DIC analysis has been achieved by Vaurijoux and his colleagues through establishing the dose–effect curve for automatic DIC analysis, and the analysis time was greatly reduced.2 At present, according to the literature, automation DIC analysis is usually for estimation of the biological dose, but its application in occupational health examination of the large-scale radiation workers is rarely reported. The main purpose of the present study was to design and achieve a rapid, accurate, and high-throughput CA detection method for the occupational health surveillance of radiation workers using an analytical approach. We present a detection procedure, which is considerably less time-consuming than previous methodologies.

1 Department of Nuclear Accident Medical Emergency, The Second Affiliated Hospital of Soochow University, Suzhou, China
2 Department of Oncology, The Second Peoples Hospital of Hefei, Hefei, People’s Republic of China
3 State Key Laboratory of Radiation Medicine and Protection, School of Radiation Medicine and Protection, Soochow University, Suzhou, China
4 Collaborative Innovation Center of Radiation Medicine of Jiangsu Higher Education Institutions, Suzhou, China

Received 01 September 2018; received revised 27 February 2019; accepted 05 March 2019

Corresponding Authors:
Yulong Liu, Department of Nuclear Accident Medical Emergency, The Second Affiliated Hospital of Soochow University, Suzhou 215004, China.
Yong Huang, Department of Oncology, The Second People’s Hospital of Hefei, 246 Heping Road, Hefei 230001, China.
Emails: yulongliu2002@suda.edu.cn; hy670716@163.com

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (http://www.creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).
Materials and Methods

Blood Sample Collection

In 2012, peripheral blood samples were collected from 20 healthy volunteers for analysis of the automatic detection of DIC rate and establishment of a high-throughput detection method. From 2013 to 2017, peripheral blood samples were collected from 26,663 radiation workers in batches for analysis by the high-throughput detection method. The 26,663 radiation workers consisted of 718 radiation workers from industrial testing and medical institutions whose average annual effective dose was $0.097 \pm 0.020$ mSv, 25,945 workers from nuclear power plants whose average annual effective dose was $0.243 \pm 0.100$ mSv. In the 25,945 workers who were from nuclear power plants, there were 10,664 from operation department, 6,343 from maintenance department, 3,198 from equipment management department, 1,546 from radiation protection department, 1,607 from production planning department, 1,884 from technical support department, and 703 from security department.

Irradiation Conditions

The blood samples which from healthy volunteers were irradiated in the IAEA/WHO Network of Secondary Standard Dosimetry Laboratories Shanghai, China. Three dose points (0.5, 2, 4 Gy) were set, and the absorbed dose rate was 0.39 Gy/min.

Cell Culture and Chromosome Specimen Preparation

The blood samples of healthy volunteers were placed in a water bath of $37^\circ C \pm 0.5^\circ C$ for 2 hours after irradiation, while those of radiation workers were not irradiated and were cultured for less than 48 hours. Next, lymphocytes were cultured in Roswell Park Memorial Institute (RPMI) 1640 culture medium containing fetal bovine serum, phytoagglutinin (PHA), 1% penicillin and 100 μg/mL streptomycin, and 0.04 g/mL colchicine at $37^\circ C$ in 5% CO$_2$ in a humidified incubator (Thermo Scientific, Scotts Valley, California) for 50 hours. The whole blood culture method was used, and the proportion of blood to culture medium was 1:10. Heparin lithium (0.5 mL) was added to 5 mL lymphocyte culture medium as an anticoagulant. Cell suspensions were prepared using a CP-II-64 automatic cell harvester (Lechen Biotechnology, Shanghai, China). Cells were subjected to hypotonic treatment by 5 mL KCl solution twice for 30 min/time, then fixed 4 times with Carnoy solution for 5 minutes each. Slides were produced using a CP-AS-40 automatic slide-making machine (Lechen Biotechnology) and subjected to Giemsa staining using a CP-G-24 automatic dyeing machine (Lechen Biotechnology). The parameters of the instrument were set according to the results of preliminary experiments.

Effective Cell Rate Analysis

The effective cell rate was calculated according to the following formula:

$$\text{Effective cell rate (\%)} = \left( \frac{\text{Artificial cell number}}{\text{Photographed cell number}} \right) \times 100.$$  

Automatic Detection of DIC Rate Analysis

The Metafer 4 (V.3.11.6) chromosome scanning and analyzing system (MetaSystems, Altusheim, Germany) was used to search for cells in metaphase and acquire high-resolution images. The sensitivity parameter for automatic metaphase cell searching was set to 6, and 3 regions were set for the search window, with the 15% area proximal to tab, 35% area of the central slide, and 50% area distal to tab (Figure 1). The collected high-resolution images were subjected to DIC analysis using DCScore software (MetaSystems). The detected DIC was further confirmed by a laboratory technician. The numbers of

![Figure 1. Distribution of DIC values on 0th to 199th cells. DIC indicates dicentric chromosomes.](image-url)
DIC and marked cells were recorded after the elimination of false positives. Dicentric chromosome was confirmed using experience and/or Ikaros software (MetaSystems) based on the following principles: (1) no count for those not suggested by the software, (2) not considering whether the 46 chromosomes were complete in metaphase cells, (3) only the DICs in the main cell were counted when more than one cell was present in a high-resolution image, and (4) scattered DICs released from the broken cells were not counted. The numbers of DICs artificially confirmed and the numbers of marked cells by software were recorded. Then, the high-resolution images were artificially analyzed the same, and the artificial analysis of the DIC number was recorded. The DIC automatic rate was calculated according to the following formula:

\[
\text{Automatic detection of DIC rate} (\%) = \left( \frac{\text{Automatic DIC number}}{\text{Artificial DIC number}} \right) \times 100
\]

**Dicentric Chromosome Distribution Interval Analysis**

We analyzed 142 samples containing one automatic DIC per 200 cells in our laboratory and marked the locations of DIC on the 0th to 99th and 100th to 199th cells in each sample. There were 72 cases in which DIC appeared on the 0th to 99th cell, and 70 cases in which DIC appeared on the 100th to 199th cell (Figure 1). Therefore, some DICs were missed when only 100 cells were analyzed by the automatic scanning and analysis system. We therefore suggest that at least 200 cells should be analyzed in each case, and 250 or more high-resolution images should be acquired.

**Results**

**Automatic Detection of DIC Rate**

According to the DIC numbers acquired by the automatic scanning and analysis system, and confirmed by a technician, as well as the artificial DIC numbers from the same high-resolution images, the automatic detection of DIC rate was in the range of 50% to 75%. The results are shown in Table 1.

**Efficiency Comparison Between the New and the Traditional Method**

The number of samples containing DIC ≥1 accounted for about 4% of the total cases, in our laboratory, as determined by the automatic scanning and analysis system. Three in 80 cases...
required confirmation by a technician, and the process took about 1.5 days. It only took 6.23 days in total for a technician to finish the report of the 80 cases, while the traditional method requires 43 days. The workload has therefore been reduced by 96\%, and the speed has been increased about 7-fold. A comparison of the time required for the new and traditional methods is shown in Table 3.

**Discussion**

The traditional measurement of CA requires hundreds of cells to be visually analyzed under the microscope for each tested sample, and each technician can only analyze 1 or 2 samples per workday. Automation offers an effective means to solve this problem. The commercialized DIC automatic analysis software (DCScore software, MetaSystems, Altlussheim, Germany) has been on the market for 20 years since 1998. It can only analyze one of the DIC distortion automatically, and false positive or false negative might be present, so it was considered valueless. Automatic biological dose estimation has been realized by Vaurijoux and his colleagues through establishing the dose–effect curve by the software, and the analysis time was greatly reduced. At present, according to the literature, automation DIC analysis is usually for estimation of the biological dose, but its application in occupational health examination of the large-scale radiation workers is rarely reported.

In this study, DIC is used as a marker to screen out those who have DIC in their blood samples by the software, then confirmed by artificially analysis, the results showed that the workload was reduced by 96\%, and the speed has been increased about 7-fold. The detection accuracy of DIC by a technician will also decrease if the technician is having fatigue, especially when the number of samples is very large; however, the method outlined herein obviates this difficulty, it is less likely than traditional analysis to miss detection.

The DIC analysis technology is the method which DIC is recognized by the computer image analysis technology basing on the morphology of DIC from the collected chromosome digital images, and which has been used to evaluate radiation damage for more than 40 years. It is well known that the number of DIC can be changed by different dose, but there is no research to claim that the morphology of DIC can be affected by the dose. In theory, no matter how the dose, the morphology of the DIC must be same as long as the DIC appear. In fact, it is possible to choose any dose for irradiation, as long as it can produce sufficient DICs for analysis. In our study, we think that the morphology of the DIC is same as that high dose, although the average annual effective dose of radiation workers is very low. In order to analyze automatic detection rate of DIC of the software, we selected 0.5, 2, and 4 Gy for irradiation in vitro to obtain sufficient DICs. It is different from fitting dose–response curve with more than 10 irradiation dose points. In fact, it is feasible to choose low dose (<0.1 Gy). But the number of DIC produced under low dose will be very few compared with high dose in the same time. If we want to obtain enough number of DICs to analyze automatic detection rate of DIC of

---

**Table 3. Efficiency Comparison Between the New and the Traditional Method.**

| Method     | Sample | Culture Time (d) | Slide Time (d) | Image Time (d) | Automatic DICs (d) | Artificial DICs (d) | Report (d) |
|------------|--------|------------------|---------------|---------------|-------------------|---------------------|------------|
| New        | 80     | 2.000            | 1.000         | 1.670         | 0.069             | 1.500               | 6.239      |
| Traditional| 80     | 2.000            | 1.000         | 0             | 0                 | 0                   | 43         |

Abbreviation: DIC, dicentric chromosomes.

*It takes 1 day to finish the report of 2 cases by the traditional method.
the software quickly, it would be time-consuming to acquire a
large number of metaphase digital images of chromosomes for
detaching DIC.

Radiation-induced chromosomal structural aberrations
include acentric fragments, double minutes, dicentric frag-
ments, acentric fragments, dicentric and polycentric chromo-
somes, inversions, and reciprocal translocations. Analyses of
these 7 kinds of CA are required for the long-term personal
monitoring of radiation workers. However, currently, software
can only analyze DIC events automatically and cannot detect
the other forms of aberrations. Dicentric chromosome and
acentric fragments account for a much larger portion of the
radiation-induced CA, while the others smaller. It plays an
important role in the diagnosis of chronic radiation injury,
while centric rings and reciprocal translocations together
account for 1% or more. If the exposure dose exceeds a certain
threshold level, DICs are expected to appear. As long as DIC
exists, it can be detected by automated software. Therefore, it is
feasible to use DIC as the primary marker for whether chronic
or nonchronic radiation injury has occurred. The data for an
abnormal case report are ultimately acquired by a chromosome
scanning and analysis system and confirmed by a technician, so
it coincides with the standard of the GBZ/T248-2014.

Although the chromosome auto-scanning analysis system
for DIC displays a high degree of reproducibility, and it is a
powerful tool for dose-estimation as reported by Wang et al.,
the data of the automatic DIC cannot be directly used in the
assessment of CA for the occupational health surveillance of
radiation workers. However, a previous report has shown that
there is a good correlation between the dose–response curve
established by automatic analysis and the artificial dose–
response curve, and the automatic detection of DIC rate is
about 50% to 70%. Results from our study indicated that the
automatic detection of DIC rate was similar to previous reports
and was about 50% to 75%. In theory, if the automatic detec-
tion of DIC rate is 50%, one DIC may be missed by the new
method. According to the standard of GBZ/T248-2014, one
DIC is typically found in 200 analyzed cells. Therefore, when
DIC cannot be found in 200 analyzed cells, the test and assess-
ment of CA for radiation workers is considered normal.

When only acentric fragments but no DIC occur, as in
some cases of chronic radiation injury, the injury may not be
correctly diagnosed. However, according to GBZ/T248-
2014, CA is not a specific index of external chronic radiation
disease, and the diagnosis must be based on clinical symp-
toms; for example, using the white blood cell count. Therefore,
the new method may miss some cases of CA but will not
affect the diagnosis. In this study, 5 cases exposed to medical
radiation and being suffered harm were successfully diag-
nosed among the 2663 radiation workers. However, not all
2663 cases were analyzed individually by technicians, so
further research is needed in the future.

Rapid and high-throughput analysis of CA is probably an
ideal screening tool for peripheral blood CAs in radiation work-
ners. In this study, the results showed a higher sensitivity of
diagnosis of external chronic radiation disease with our
approach. Furthermore, the workload was reduced by 96%, and
the speed has been increased about 7-fold. This technical
scheme can meet the requirements of fast and accurate package
solution with practical value for the rapid and high-throughput
detection of CA for the occupational health surveillance of
radiation workers, especially when the number of sample is
very large.

Authors’ Note
J.B. and H.D. contributed equally to this work.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to
the research, authorship, and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for
the research, authorship, and/or publication of this article: This work
was supported by Jiangsu Health and Family Planning Commission
Preventive Medicine Research Projects in 2014-2015 (no. Y2015024).

References
1. International Commission on Radiological. The 2007 Recommen-
dations of the International Radiation Protection Committee. Beij-
ing, China: Atomic Energy Press; 2008.
2. Vaurijoux A, Gruel G, Pouzoulet F, et al. Strategy for population
triage based on dicentric analysis. Radiat Res. 2009;171(5):
541-548.
3. Chen DQ, Zhang CY. A simple and convenient method for gaining
pure populations of lymphocytes at the first mitotic division in
vitro. Mutat Res. 1992;282(3):227-229.
4. National Health and Family Planning Commission of PRC. GBZ/
T248-2014 Test and Assessment of Chromosomal Aberrations on
Occupational Health Examinations for Radiation Workers. Beij-
ing, China: Standards Press of China; 2014.
5. Wang Y, Xu C, Du LQ, et al. Evaluation of the comet assay for
assessing the dose-response relationship of DNA damage induced
by ionizing radiation. Int J Mol Sci. 2013;14(11):22449-22461.
6. Wang H, Liu Q, Wan D, et al. BioDoser: improved dose-estimation
software for biological radiation dosimetry. Comput Methods Pro-
grams Biomed. 2012;108(1):402-406.
7. Cytogenetic Dosimetry. Applications in Preparedness for and
Response to Radiation Emergencies. Vienna, Austria: IAEA; 2011.
8. National Health and Family Planning Commission of PRC.
GBZ205-2017 Diagnostic Criteria for Chronic Radiation Sickness
From External Exposure. Beijing, China: Standards Press of
China; 2017.