CHAPTER 12

CYTOGENETIC BIODOSIMETRY

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INTRODUCTION

Biological dosimetry is the measurement of radiation-induced changes in the human body to assess acute- and long-term health risks. Biological dose estimation provides an independent means of obtaining dose information otherwise exclusively based on computer modeling, dose reconstruction, and physical dosimetry. Various biodosimetry tools are available and certain characteristics make some more valuable than others (Exhibit 12-1).

EXHIBIT 12-1

CHARACTERISTICS OF AN IDEAL BIODOSIMETER

- Shows dose-effect relationship
- Demonstrates radiation specificity
- Persists after exposure
- Shows low interindividual variation
- Provides results within a clinically relevant time
- Estimates fraction of the body irradiated and dose to that fraction in partial-body exposures
- Can assess in fractionated and chronic exposures
- Has known radiation quality effects
- Uses sampling that is noninvasive or semi-invasive
- Is amenable to automation

Cytogenetic methods now occupy a unique and valuable niche in biological dosimetry.[1] When available, cytogenetic analysis can complement physical dosimetry by confirming or ruling out a radiological exposure. When physical dosimetry is unavailable, cytogenetic analysis is often the only available dose estimation method. Cytogenetic biodosimetry using human peripheral blood lymphocytes (HPBLs) following an accidental overexposure was first used in the 1962 Recuplex criticality accident in Hanford, Washington.[2] Since then it has been used in response to several radiation accidents, such as that at Chernobyl (Ukraine), Goiânia (Brazil), and Tokaimura (Japan), for dose assessment, as well to resolve suspected occupational overexposures.

Estimated doses using cytogenetic methods correlate well with the severity of acute radiation syndrome (ARS).[3] In the Chernobyl accident, dosimetry was approximated by rapid preliminary examination of 50 lymphocyte metaphases per person for several individuals,[4] although accurate dose assessment involves analysis of 500 to 1,000 metaphase spreads taken from the peripheral blood lymphocyte (PBL) cultures obtained from a radiation-exposed individual. The radiation accidents above emphasized the importance of cytogenetic methods in early dose assessment after a radiological event in influencing treatment decisions; as a result, many countries have set up laboratories for biological dosimetry. Cytogenetic methods, which are standardized and routinely used, are also employed to assess the late effects of irradiation. A technical manual and standards for laboratory accreditations are also available.[5–7]

Small volumes (less than 10 mL) of peripheral blood are obtained by phlebotomy from exposed subjects as soon as practical (generally 1 day after exposure) and sent to a cytogenetic biodosimetry laboratory for dose assessment. The laboratory processes samples according to its established protocols. Cytogenetic damage is then assessed by experts and dose assessment is made by comparison with an appropriate calibration curve, taking into consideration radiation type, dose rate, whole- or partial-body exposures, delay between samplings, and specific cytogenetic assessment.[5]

BIODOSIMETRY PRINCIPLES

Lymphocyte Cell Cycle

PBLs are routinely used for cytogenetic biodosimetry. They have diploid deoxyribonucleic acid (DNA) content (2n) and are predominantly in a "quiescent" state; therefore, they do not normally undergo cell division. There are two types of circulating PBLs: T and B lymphocytes. T lymphocytes (specifically the $CD4^+$ and $CD8^+$ subtypes) can be stimulated by mitogen (eg, phytohemagglutinin) to grow in culture. First-division cycle metaphases are harvested from lymphocyte cultures for assessing radiation dose. Upon stimulation, T lymphocytes undergo a cell-division cycle, which is divided into two brief periods: interphase (gap 1 [G_1], synthesis [S], and gap 2 [G_2] phases) and mitosis (M phase). In interphase, the cell grows and replicates its DNA; in mitosis, it divides into two distinct daughter cells. In general, regulatory molecules, cyclins, and cyclin-dependent kinases (CDKs) regulate eukaryotic cell cycles.[8] Cyclin D is the first cyclin produced in response to extracellular growth signals. When activated by a bound cyclin, CDKs perform phosphorylation, which in turn activates or inactivates target proteins to a synchronized entry into the next phase of the cell cycle.

Cell-cycle regulation is mediated via cell-cycle

checkpoints[9] such that entry and exit of cells from each phase depends on the proper progression and completion of the previous phase. Cell-cycle checkpoints prevent cycle progression at specific points, allowing completion verification of the necessary phase and DNA damage repair. The cell does not proceed to the next phase until all checkpoint requirements are met. The presence of several checkpoints ensures that damaged or incomplete DNA is not passed on to daughter cells. Two main checkpoints are the G_1/S checkpoint and the G_2/M checkpoint. Genes such as protein 53 play important roles in triggering the control mechanisms at both the G_1/S and G_2/M checkpoints.

G_1 Phase

Within interphase, the stage from the end of the previous M phase until the beginning of DNA synthesis is called the G_1 phase. This phase is marked by the synthesis of enzymes that are required for DNA replication in the S phase. Upon receiving a promitotic extracellular signal, G_1 cyclin-CDK complexes activate to prepare the cell for the S phase, promoting the expression of transcription factors that in turn promote the expression of S cyclins and enzymes required for DNA replication. The G_1 cyclin-CDK complexes also promote the degradation of molecules that function as S-phase inhibitors.

S Phase

The S phase follows the G_1 phase, commencing with DNA synthesis. Upon completion of the S phase, all chromosomes are replicated, quadruplicating the DNA content. Each chromosome now consists of two (sister) chromatids. Ribonucleic acid transcription and protein synthesis rates are very low, barring histone production, which is crucial for chromatin packaging. Active S cyclin-CDK complexes phosphorylate proteins in the prereplication complexes, which are assembled during the G_1 phase. The phosphorylation serves two purposes: (1) to activate the already-assembled prereplication complex, and (2) to prevent new complexes from forming. This ensures that every portion of the cell's genome is replicated only once.

G_2 Phase

The G_2 phase follows the S phase, which lasts until the cell enters mitosis. Again, significant protein synthesis occurs during this phase, mainly involving the production of microtubules that are required for transporting sister chromatids to opposite poles to divide the nucleus. Inhibition of protein synthesis during the G_2 phase prevents the cell from undergoing mitosis.

M Phase

The M phase follows the G_2 phase and sequentially consists of prophase, metaphase, anaphase, and telophase. In mitosis, karyokinesis (division of chromosomes between the two daughter cells), and cytokinesis (division of cytoplasm) occur. Mitotic cyclin-CDK complexes promote the initiation of mitosis by stimulating the synthesis of downstream proteins involved in chromosome condensation and mitotic spindle assembly, preparing for chromosome segregation. Anaphase-promoting complex, a critical protein complex, is activated during this phase, promoting degradation of structural proteins associated with the chromosomal kinetochore. Anaphase-promoting complex also targets the mitotic cyclins for degradation, ensuring the progression of telophase culminates in cytokinesis.

HPBLs are highly differentiated and are in a synchronized quiescent state, called "G_0." Following stimulation in culture, this synchrony is maintained; at least until the first-division cycle is complete. Although the lymphocyte cell-cycle time depends on culture conditions, DNA synthesis starts around 26 hours after culture initiation, and first-division mitoses start to appear around 36 hours after stimulation. DNA synthesis peaks at 34 and 40 hours, resulting in two peaks of respective mitotic activity around 44 and 49 hours of culture initiation.[5] Irradiation of lymphocytes as well as the presence of chromosomal aberrations induce a delay in cell-cycle progression as well as asynchrony, to some extent.

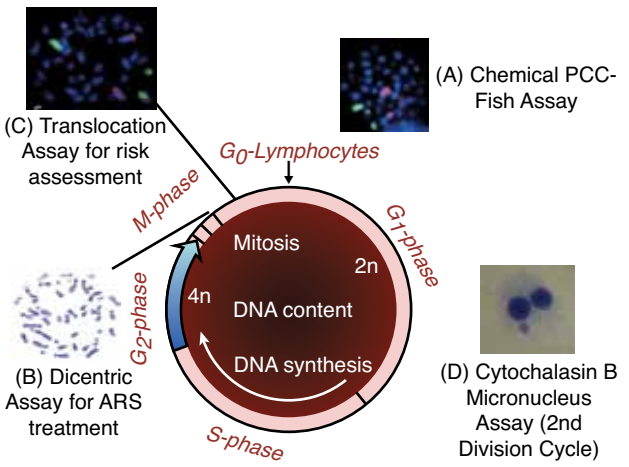


Figure 12-1. Various cytogenetic assays performed using human peripheral blood lymphocytes in different cell cycle phases. ***(a)*** Chemical premature chromosome condensation assay. ***(b)*** Dicentric assay. ***(c)*** Translocation assay. ***(d)*** Cytochalasin B micronucleus assay.
ARS: acute radiation syndrome
DNA: deoxyribonucleic acid

Various cytogenetic assays (Figure 12-1) can be performed using HPBLs. Premature chromosome condensation (PCC) assay is performed in the G_0/G_1 phase, where chromatin material is condensed prematurely by means of mitotic cell fusion,[10] phosphatase inhibitors,[11] or mitotisis-promoting factors in conjunction with phosphatase inhibitors[12] to study radiation-induced chromosome damage. Dicentric and chromosome translocation assays are performed after DNA replication, using metaphase spreads specifically for analyzing structural chromosomal aberrations (see Figure 12-1, b and c). In the second-division cycle, cytome assay[13] (including micronucleus and nucleoplasmic bridges) is performed (see Figure 12-1, d).

Human Karyotype

Based on the relative size of chromosomes and the position of the centromere (the point of spindle attachment during mitosis or the primary region of constriction) along the longitudinal axis, chromosomes are arranged in the form of a karyotype. The human karyotype consists of 46 chromosomes (44 autosomes and 2 sex chromosomes) and are classified into 7 groups: A, B, C, D, E, F, and G (Figure 12-2).

Group A consists of three pairs of large, metacentric (centromere position is in the middle of the longitudinal axis) chromosomes, 1, 2, and 3. Chromosome 1 is the largest pair of metacentric chromosomes in the human karyotype. Group B consists of chromosomes 4 and 5, which are large, submetacentric chromosomes (the centromere is located off center, dividing the chromosome's arms into "short" and "long" arms). Chromosomes are arranged in the karyotype with the short arm on the top and long arm on the bottom. Group C consists of chromosomes 6 through 12. These are all mid-sized, submetacentric chromosomes relative to group B chromosomes. The D group consists of chromosomes 13 through 15. All three pairs are large acrocentrics, where the centromere is positioned toward the terminal end of the chromosomes. Often these chromosomes display a "satellite," small chromatin material in the form of a dot. A smaller metacentric chromosome (16) and two smaller submetacentric chromosomes (17 and 18) constitute group E. Group F consists of chromosome pairs 19 and 20; the smallest metacentrics. Chromosome pairs 21 and 22, the smallest acrocentics, form group G. The G group chromosomes frequently display satellites. Sex chromosomes in a male karyotype consist of an X chromosome, which is medium sized (similar to C group chromosomes) and a Y chromosome, which is a small, acrocentric chromosome similar to G group chromosomes. Sex chromosomes in a female karyotype consist of two X chromosomes.

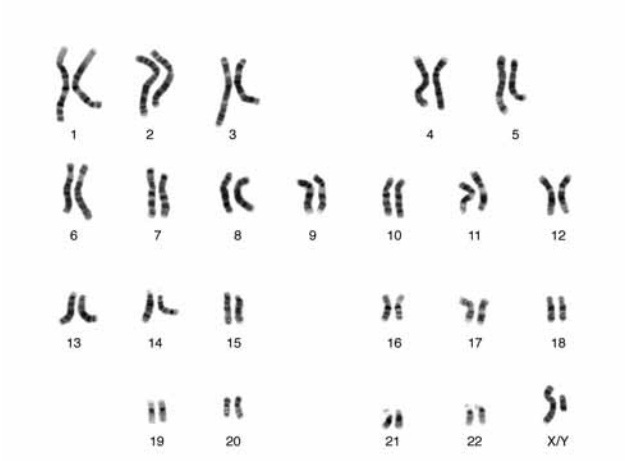


Figure 12-2. The human karyotype.
Courtesy of the National Library of Medicine.

Simultaneous visualization of all pairs of chromosomes in different colors is now possible with the use of molecular cytogenetic techniques, using combinatorial labeling to generate many different colors unique to specific chromosomes with limited, spectrally distinct fluorophores. Spectral differences among chromosomes are captured using a fluorescent microscope to analyze structural aberrations.

Chromosome Structure

The DNA backbone is made up of sugar, phosphates, and holding bases, as well as adenine, thymine, guanine, and cytosine, which carry genetic information. The basic premise of cell biology is that the chromosomes are dynamically modified in interphase and condense during mitosis. Cytogenetic examination of radiation-induced damage is mostly analyzed using condensed chromosomes, such as metaphase chromosomes. Historically, three different conceptual classes of models for metaphase chromosome architecture have evolved. They are the chromatin network, hierarchical folding, and radial loop ("scaffold") models, which are quite different in terms of structural motifs, giving rise to chromosome condensation. In the chromatin network model, chromosomes are stabilized by protein cross-links between adjacent chromatin fibers every 15 kilobases, on average.[14] In the hierarchical models of chromosome folding, 10- and 30-nm chromatin fibers fold progressively in larger fibers (chromonema) that coil and form the metaphase chromosomes.[15]

The scaffold model assumes loops of chromatin are attached to an axial chromosome structure, or "scaffold," formed by nonhistone proteins, topoisomerase IIα, and structural maintenance of chro-

mosomes (SMCs).[16] This model is consistent with the relationships of observed structural dimensions and the "central dogma of molecular biology" related to transcription, replication, and matrix attachment domains.[17] The DNA double helix is folded in alternating coiling-and-loop formation, induced by the packaging of histones and nonhistone proteins spooled in a tight helix. Histones H2A, H2B, H3, and H4 form "core" histones, and H1 and H5 form "linker" histones. Two of each core histones form a nucleosome core by wrapping the DNA double helix. The DNA is locked into place by binding the nucleosome, and entry and exit sites of the DNA double helix by the linker histone, H1.

Condensins and topoisomerase II appear to play an important role in the dynamics of chromosome condensation. The axial distribution of topoisomerase IIα and the condensing subunit, SMC 2, in unextracted metaphase chromosomes, with SMC 2 localizing to 150 to 200 nm diameter central core, is now confirmed by examination of interphase chromosomes.[16] Early prophase condensation occurs through the folding of large-scale chromatin fibers into condensed masses. These resolve into linear, middle prophase chromatids measuring 200 to 300 nm in diameter that double in diameter by late prophase. Hierarchical levels of chromatin folding are stabilized late in mitosis by this axial "glue" of topoisomerase IIα and SMC 2.[17]

Gene analyses are often based on short stretches of only a few kilobases of DNA; however, an orderly transcription and replication can also involve highly folded chromosomal domains containing hundreds of kilobases of DNA. Three-dimensional chromosomal domains within the nucleus may also contribute to phenotypic expression of genes and induced aberrations.

Radiation-Induced Chromosome Aberrations

For cytogenetic biological dosimetry using HPBLs, it is important to quantify aberrations in first-division cycle metaphase spreads, where structural changes are observed in their entirety without the confounding effects of elimination and dilution of aberrations associated with cell division. Classification and relationships of induced chromosomal structural changes are discussed in great detail by Savage.[18] Generally, there are two broad categories of structural chromosomal aberrations induced by irradiation: chromosome type and chromatid type. In the former, the induced changes are always visualized in both the sister chromatids of a chromosome, whereas in the latter only one of the sister chromatids is affected. Chromosome-type aberrations arise from damage of the chromatid thread in its pre-DNA synthesis stage (unreplicated), and this damage is duplicated along with the chromosome during cell-cycle progression through the S phase. Since HPBLs are largely in a presynthetic phase of the cell cycle, irradiation produces only chromosome-type aberrations (ie, damage affecting both the chromatids); therefore, the description below focuses only on chromosome-type structural aberrations. Chromatid-types of structural aberrations are produced only when the cells are irradiated during or after chromosome duplication. Chromosome-type structural aberrations are rarely found after chemical or drug exposure, when cells are examined in their first-division cycle.

Exchanges

Interchanges. Asymmetrical interchanges (dicentrics) result in a chromosome with two centromeres along with a single acentric fragment. In order to produce a dicentric aberration, DNA lesions are necessary in two unreplicated chromosomes (circulating lymphocytes are in their pre-DNA synthetic stage) in close proximity with respect to time and space so that the damaged chromosomes can undergo an exchange. The exchange is radiation specific and can occur either as a result of a misrepair of DNA strand breaks induced directly by radiation, or as a result of misrepair during excision repair of base damage. The distance between centromeres can vary from being indistinguishable to spanning almost the total length of the arms involved. Dicentrics are the most easily recognizable and unambiguous aberrations to score in the spectrum of radiation-induced chromosomal aberrations. The cells containing dicentrics are rapidly lost from the cell population because of mechanical difficulties during cell division. The associated acentric fragment is usually excluded from the daughter nuclei, often forming a micronucleus resulting in a genetically deficient daughter nucleus.

Symmetrical interchange (reciprocal translocation), the symmetrical counterpart of the dicentric, is a reciprocal transfer of terminal portions of two separate chromosomes. These are often undetected with conventional staining methods. Reciprocal translocations can be transmitted to subsequent cell generations; therefore, they are often referred to as "stable" translocations.

Intrachanges. Interarm intrachanges, when asymmetric and complete, form centric rings and are often scored along with dicentrics for dose estimation. They are analogous to asymmetrical interchanges, where two lesions in different arms of the same chromosomes form a loop around the centromere. An acentric fragment is also formed. At anaphase, because of failure to freely separate, these may form interlocking rings, leading to the formation of bridges.

Symmetrical interarm intrachanges (pericentric inversion), when complete, lead to a pericentric inversion involving inversion of the arms by 180°. Unless this results in a very obvious change in centromere index or the arm ratio, such aberration is undetectable.

In intraarm intrachange (interstitial deletions), the interaction of two lesions within an unduplicated chromosome arm can result in deletion of a region interstitial to the centromere and the telomere. Ends of the deleted portion may rejoin, forming an acentric ring, whereas the chromosome arm may be shortened. At anaphase, interstitial deletions, which lack a centromere or point of spindle attachment like other acentric fragments or terminal deletions (see Breaks, below), lag and form a micronucleus. Occasionally, the interstitial segment between two segments may be reversed or inverted. Since there is no change in the arm ratio, such aberrations are also undetectable.

Breaks (terminal deletions, chromosome breaks). Breaks arise because of a complete severance of a terminal region of a chromosome arm. The size of the deletion may vary. Small deletions are difficult to identify; therefore, often in cytogenetic biodosimetry, these are categorized together with interstitial deletions and called "fragments" or "deletions." However, when scoring, acentric fragments arising from dicentrics or centric rings are invariably excluded from the category of fragments or deletions.

BIODOSIMETRY BY DICENTRIC ASSAY

Because of their radiation specificity, dicentrics, a common structural aberration, in an individual's PBLs indicate radiation exposure. They show a very good dose-effect relationship for different radiation types. For low-LET (linear energy transfer) radiation, the dose-effect relationship is linear–quadratic; for high-LET radiation, the relationship is linear (Figure 12-3). Lymphocyte exposure in vitro or in vivo produces similar levels of dicentrics per gray.[5] Therefore, observed dicentric yield in an exposed person's PBLs can be converted to absorbed dose by comparison with an appropriate calibration curve. Because of low background levels (about 1 dicentric chromosome in 1,000 cells), high sensitivity (a threshold dose of 0.05 Gy), very low interindividual variation, and ability to assess partial-body exposure, dicentric assay (DCA) is considered the "gold standard" biodosimetry method. Estimated doses using DCA correlate well with the severity of ARS.

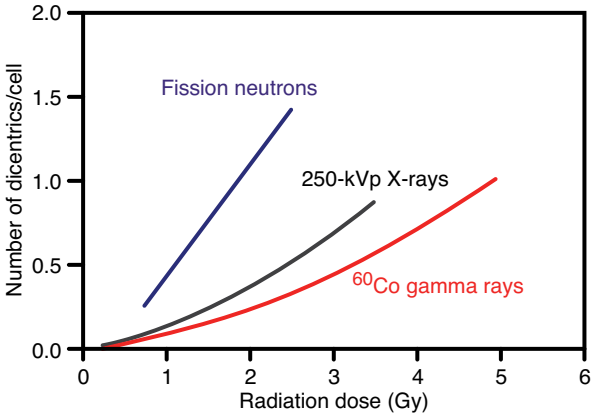


Figure 12-3. Dose-response relationship for dicentrics in human lymphocytes for three radiation qualities.

Blood Sampling, Culturing, and Analysis

Small volumes (less than 10 mL) of peripheral blood are collected from exposed subjects in vacutainers containing a suitable anticoagulant as soon as practical, generally 1 day after exposure, and sent to a cytogenetic biodosimetry laboratory for blood culturing, metaphase spread harvesting, and DCA for dose estimation. The laboratory processes samples in accordance with internationally accepted protocols and guidelines. Briefly, either whole blood, lymphocyte-enriched buffy coat, or isolated PBLs are stimulated by a mitogen (eg, phytohemagglutinin) to grow in culture and cells in first-division cycle metaphases are collected on glass slides. Specimen collection procedures for cytogenetic biodosimetry are described elsewhere in the literature.[19] Metaphase spreads are then stained and dicentric chromosomes are counted by microscopy to estimate dose by comparison with an appropriate calibration.

Influencing Factors for Dose Assessment

Radiation Type and Dose Rate

Most accidental radiation exposures involve gamma or X-rays. Since there is a difference in the yield of dicentrics with energy between gamma and X-rays, it is imperative to equate the dicentric yield with an appropriate calibration curve for dose assessment. Occasionally radiation accidents may also involve degraded neutrons. Since the energy spectrum for degraded neutrons is similar to that for fission spectrum neutrons, the linear dose-effect calibration curve for fission neutrons is normally used for dose estimation. For low-LET radiations, dose rate is an important determinant of dicentric yield, particu-

larly in the quadratic component of a dose-response curve. The dicentric yield reduces with dose rate. For biodosimetry triage during a nuclear detonation, most radiation-only injuries will be caused by fallout. While there may be some neutron component, the dose estimation is done assuming only gamma-ray exposure.

Sampling Delay

The persistence of dicentrics in HPBLs is closely related to the life span of the blood lymphocytes. Renewal of circulating lymphocytes from bone marrow stem cells will result in dilution of dicentric frequency with time following exposure, and hence, dose underestimation. Lymphocyte half-life can vary among individuals; however, no correction for the assessed dose is necessary when sampling is done before 4 to 6 weeks after exposure. Dose assessment is still possible by DCA up to 3 years after exposure, with the appropriate correction based on lymphocyte half-life in circulating pool.

Heterogeneity of Irradiation

Accidental irradiations often result in inhomogeneous dose distribution and irradiated and unirradiated lymphocytes are mixed. In such cases, the overall dicentric frequency following a high-dose exposure of a small part of the body can be equal to the overall frequency after exposure of a large portion of the body to a lower dose. With uniform whole-body exposures to low-LET radiations, the dicentrics follow a Poisson distribution; with significant partial-body exposures, the distribution is non-Poisson. Two statistical methods are generally used to assess partial-body exposures: Dolphin's contaminated Poisson method and Sasaki's QDR (Quantity of Dicentrics and Rings) method.[5] The frequency of metaphase spreads without dicentric aberrations can be used to identify patients with partial-body exposure and cohorts suitable for cytokine therapy after radiation accidents.[20] Dose estimations following internal radionuclide contamination are difficult and estimated doses are less certain. However, since incidence of dicentrics in circulating lymphocytes is radiation-specific, the presence of dicentrics may be used in identifying internal contamination of radionuclides.

Statistical Considerations

Dose estimation requires constructing a calibration curve by the maximum likelihood method and deriving dose and confidence intervals by comparing observed dicentric yield with a chosen calibration curve. Deriving a dose from the measured yield of dicentrics is relatively easy, but the degree of accuracy and precision on the assessed dose depends on the confidence limits of the calibration curve used, number of metaphase spreads analyzed, or number of dicentrics observed in a given number of metaphase spreads. Generally, a 95% confidence limit is chosen to express uncertainty on the assessed dose, and at lower doses, dose estimate is based on the analysis of at least 500 metaphases.

However, for risk-based stratification in radiation mass casualty and emergency situations, scoring 20 to 50 metaphase spreads is adequate to provide information on dose and the nature of dose distribution (ie, whether the irradiation is partial- or whole-body based on the distribution of dicentrics among the analyzed cell population).[20] Statistical methods for constructing calibration curves and assessing dose are not available in routine statistical software. Several laboratories have generic programs, which are not especially user-friendly, quality controlled, or widely available. Two cytogenetic dose assessment software tools, Chromosomal Aberration Calculation Software (CABAS; this free program can be downloaded at http://www.ujk.edu.pl/ibiol/cabas/index.htm)[21] and Dose Estimate (Health Protection Agency Centre for Radiation, Chemical and Environmental Hazards, Didcot, England)[22] are now available.

Other factors that influence dose assessment (to a lesser degree) in DCA include age, whether or not the individual smokes, and genetic predisposition.

Assay Harmonization, Quality Control, and Assurance

The DCA's variability and accuracy among different cytogenetic laboratories was determined in an interlaboratory comparison study. Minimum variability was found in calibration curves among established laboratories, and biologically predicted dose was accurate against physical doses (Figure 12-4).[1] However, it is important to determine dose based on each laboratory's own calibration. For a given number of dicentrics, comparison with another laboratory's calibration curve may lead to erroneous dose assessment if the calibration curves are inherently different.

Laboratory protocols and quality-control standards are available. The International Atomic Energy Agency revised technical details of laboratory protocols, standardizing methodologies,[19,23] and the International Organization for Standardization developed compliance standards for laboratory accreditation.[6] Performance criteria for cytogenetic triage in a radiation mass casualty situation are also available.[7]

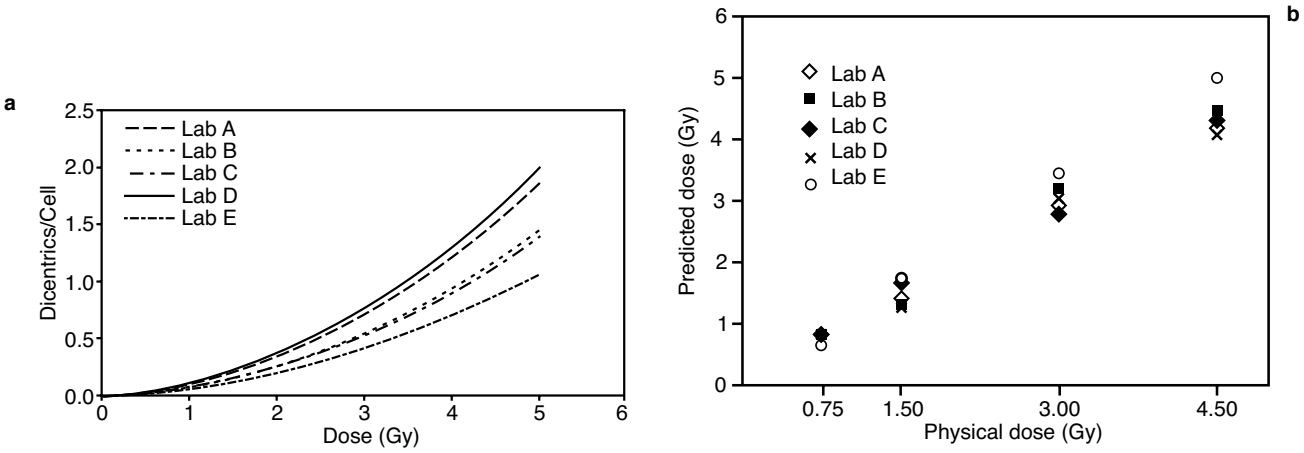


Figure 12-4. Comparison of radiation dose-effect calibration curves for **(*a*)** cobalt-60 gamma radiation for dicentric yield among established cytogenetic laboratories and **(*b*)** distribution of predicted biological doses to dose-blinded samples for actual physical doses in all laboratories. Reproduced with permission from: Wilkins RC, Romm H, Kao TC, et al. Interlaboratory comparison of the dicentric assay for radiation biodosimetry in mass casualty events. *Radiat Res*. 2008;169:551–560.

Laboratory Automation and Information Management

Laboratory automation and information management is essential for practical applications of cytogenetic assays in radiation mass casualties. Cytogenetic sample preparation, DCAs for dose assessments, and cytogenic data management are time-consuming and laborious in a large-scale disaster. For dose-based stratification of exposed subjects to estimate risk for developing ARS in mass casualties, whole blood is processed through a cytogenetic laboratory's various automated equipment stations. A laboratory information management system (LIMS) will allow sample tracking and prioritization as well as data and resource management. LIMS should be flexible, scalable, and upgradable for automated cytogenetic sample processing of metaphase spreads from whole blood. Automation by customization and integration of commercial, off-the-shelf technologies can support quality control and assurance, as well as increase throughput and the occupational safety of laboratory personnel in a biologically hazardous, high-throughput laboratory environment.[24] In the Armed Forces Radiobiology Research Institute's automated cytogenetic laboratory, a customized, automated, liquid-handling robot enclosed in an engineered Biosafety Level 2 environment and integrated with an automated cell viability analyzer and automated centrifuge performs high-throughput blood-sample processing, eliminating an important rate-limiting bottleneck in sample processing for cytogenetic dose assessment and maintaining sample chain-of-custody via barcoding and sample tracking in LIMS. Because there is no difference in radiation-induced dicentric yield between whole blood and isolated lymphocyte cultures,[25] a whole-blood culture method may be preferred in mass casualty situations to enhance throughput. Metaphase harvesters are used to eliminate the labor-intensive and repetitive tasks involved in metaphase harvesting from blood cultures (ie, centrifugation, aspiration and disposal of supernatant, treatment with hypotonic and fixative solutions) under controlled environmental conditions in a one-step protocol, thus enhancing quality and reproducibility. Similarly, a metaphase spreader provides optimal environmental conditions of temperature and humidity for spreading cell suspension on glass slides. An autostainer provides a rapid and consistent method of staining slides with Giemsa and requires minimal human involvement. Intelligent, flexible, and tandem sample scheduling can allow up to 1,000 samples per week in such an automated cytogenetic laboratory. A sample priority assignment feature in LIMS can allow specific sample batches to be queued and processed ahead of others with no user involvement.

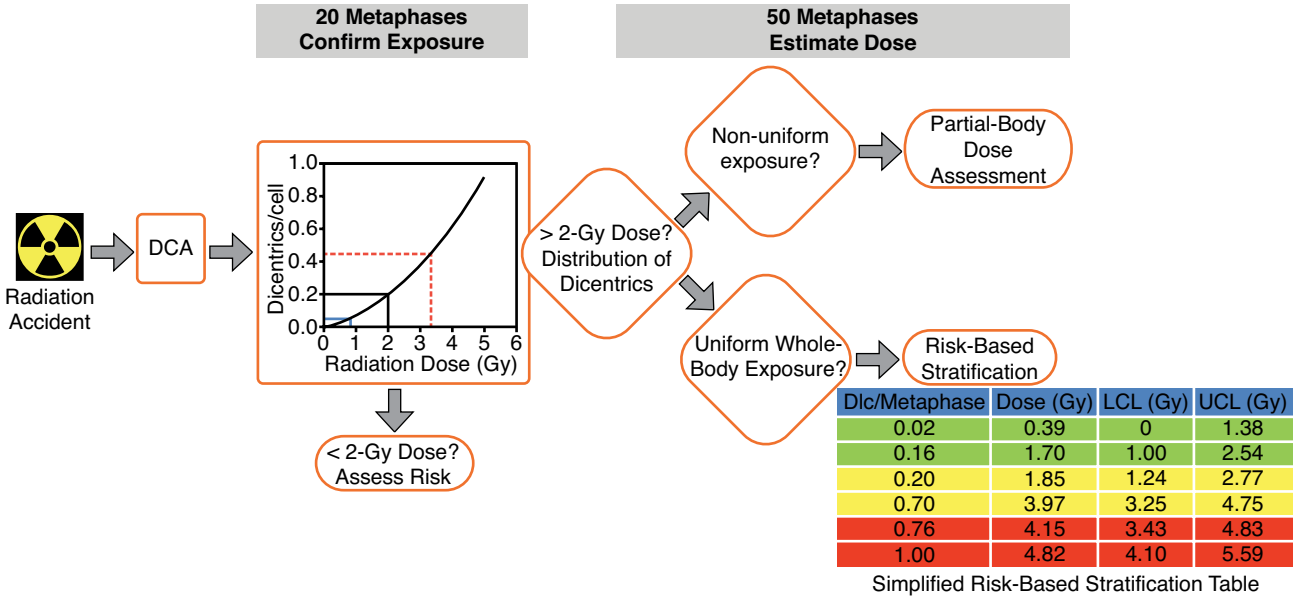
Automated metaphase spread analysis is currently limited to differentially locating metaphase spreads on microscope slides and computer-assisted manual scoring, at best.[26] Nevertheless, automated metaphase finders further enhance a laboratory's sample analysis throughput.[27] A metaphase finder generally consists of a high-end computer, a digital camera, a high-quality microscope, an automated stage with autofocus, and a robotic slide delivery system. The computer is loaded with automated metaphase-finding software and interactive automated scoring and annotation software

for chromosome aberration analysis. Such metaphase finders can scan up to 250 slides per run, locating metaphase spreads on slides. As it scans, the data (pictures and locations of metaphase spreads on slides) are stored on the hard disk or a centralized server for subsequent relocation and analysis of metaphases either at the metaphase finder station itself or at multiple remote satellite scoring stations.[28] While automated sample processing for cytogenetic analysis increases throughput, downstream DCA for dose estimation may rely on one or all of the following:

- A physical transfer of slides to various satellite laboratories or laboratories in a network for manual analysis.
- Digital encryption and transfer via a virtual private network for downstream analysis and assessment (for virtual high-resolution images of metaphase spreads acquired by metaphase finders). The technological advances in terms of required bandwidth and capability to stream data and images are already available. Virtual digitization can be coupled with real-time data and image monitoring, further enhancing the speed and accuracy at which samples from irradiated personnel can be treated in a mass casualty event.
- Development of an automatic, ultrafast, high-capacity digital-pathology scanning platform to build and validate a reliable and walk-away analysis system based on artificial intelligence for rapid downstream DCA.

Triage Dose Prediction

A triage dose prediction model (Figure 12-5) uses the dose-response calibration curve data from the interlaboratory comparison study[1] for rapid, risk-based stratification of a radiation-exposed population. It applies DCA after whole-body and partial-body exposures following a radiation mass casualty event.[29] A single HPBL count (after 12 hours) or serial counts are used to estimate dose, as is the individual's medical history. DCA would be used selectively after a radiation mass casualty event. DCA is proposed following receipt of blood samples from a radiation event to confirm irradiation by an initial screening involving analysis of only 20 metaphase spreads. Accordingly, radiation doses greater than 2 Gy are confirmed by the presence of four dicentrics in 20 metaphases. For cases with confirmed doses of 2 Gy or more, analysis is then increased to 50 metaphases to evaluate homogeneity of the dicentric distribution. Partial-body exposures are indicated by variation from the expected dose-dependent distribution of the number of dicentrics per cell. In cases of uniform whole-body exposures, samples



Dic/Metaphase	Dose (Gy)	LCL (Gy)	UCL (Gy)
0.02	0.39	0	1.38
0.16	1.70	1.00	2.54
0.20	1.85	1.24	2.77
0.70	3.97	3.25	4.75
0.76	4.15	3.43	4.83
1.00	4.82	4.10	5.59

Simplified Risk-Based Stratification Table

Figure 12-5. Rapid risk-based stratification by cytogenetic dose assessment in radiation mass casualties. Reproduced with permission from: Prasanna PG, Moroni M, Pellmar TC. Triage dose assessment for partial-body exposure: dicentric analysis. *Health Phys*. 2009;98:244–251.
DCA: dicentric assay; Dic: dicentric; LCL: lower confidence limit; UCL: upper confidence limit

are categorized based on a risk-based stratification table into the following categories: not life threatening, potentially life threatening, and significantly life threatening (see Figure 12-5). For acute moderate exposure, DCA is more suitable.

Acute High-Dose Exposures

HPBLs and complete blood counts (white count, differential, and platelets) will be used in triage decision-making. In cases of acute, high-dose, life-threatening exposures, the PCC assay is useful for estimating dose because radiation-induced cell death and cell-cycle progression delay will not interfere. Traditionally, PCC is induced in HPBLs by fusing Chinese hamster ovary mitotic cells, obtained from cell cultures, using polyethylene glycol as a fusogen to allow radiation-induced chromosome damage in an extended dose range to be measured.[30] Specific inhibitors of protein phosphatases (eg, calyculin A or okadaic acid) are also used to induce PCC in various cell-cycle stages in proliferating cells, such as mitogen-stimulated HPBLs.[11,31] Further, chromosome damage in chemically induced PCC can also be studied using whole-chromosome–specific hybridization probes.[32] Differentiated and nonproliferating cells, such as resting HPBLs, do not normally respond to phosphatase inhibitor treatment and do not induce PCC. However, incubating resting HPBLs in a cell culture medium containing a phosphatase inhibitor, such as calyculin A, along with a mitosis-promoting factor, p34cd2/cyclin B kinase, and adenosine triphosphate, induces PCC without mitogen stimulation. This method results in a high yield of PCC suitable for fluorescence in situ hybridization (FISH) and biological dosimetry.[12,33] Chromosome-specific aberrations are detected using a single whole chromosome probe[12] or a set of whole chromosome probes.[33] Upon FISH, undamaged (normal) cells display two fluorescent spots representing specific chromosomes, and cells with an aberrant specific chromosome show more than two spots per chromosome. Irradiation increases the frequency of damaged cells. The PCC-FISH method was useful in estimating dose in cases of localized high-dose irradiation to skin.[34] Nevertheless, because of limited sample processing and analysis throughput, as well as requirements for expert application of the PCC-FISH method, use in radiation mass casualties is limited.

Acute Low-Dose Exposures

Managing radiation mass casualties will require early dose estimation, both for treatment and assessment of long-term health risks. The cytochalasin-B blocked micronucleus assay[35] may be suitable for early dose estimation. In this assay, radiation-induced chromosome damage is measured as micronuclei in cytokinesis-blocked HPBL. It has undergone extensive development and evolved as a "cytome" assay.[13] The cytome assay is a "catch all" method for measuring induced genetic insult and includes a radiation-specific biomarker: nucleoplasmic bridges. A strong correlation was observed between nucleoplasmic bridges and dicentric chromosmes and centric rings.[36] Therefore, nucleoplasmic bridges can be considered "surrogate" radiation exposure markers for early dose assessment, particularly at doses below 2 Gy in radiological mass casualties. In addition, micronuclei in interphase cells represent chromosome damage transmitted through cell division to daughter cells after radiation exposure; concurrent measurement of micronuclei can also serve as an early biomarker for late effects.

Retrospective Biological Dosimetry

Analysis of persistent chromosomal aberrations, such as stable translocations, is relevant for retrospective biological dosimetry. With advances in molecular cytogenetic techniques, translocations are easily recognizable by chromosome painting. Specific DNA sequences attached to flourochromes are used as probes to either detect a part of a chromosome or paint entire chromosomes, enabling observation of chromosome rearrangements. Translocation analysis is normally used for retrospective estimation of dose rather than for acute exposures in populations without prior personal dosimetry. However, important confounders for using translocation analysis for prospective acute biodosimetry include (*a*) interindividual differences in radiosensitivity, (*b*) inability to distinguish between chronic and acute exposures, (*c*) whole- and partial-body exposures, (*d*) reproducibility of data among laboratories, (*e*) want of standardized scoring criteria, and (*f*) possible radiation-quality–dependent variation in persistency.[5]

SUMMARY: TREATMENT IMPLICATIONS

Medical management of radiation exposure depends on the dose received, organs exposed, and individual susceptibility. The scenario of exposure is critical, ranging from a potential overdose from a diagnostic procedure or radiation therapy, to an industrial accident, to a mass casualty event as large as a nuclear detonation. The extent of diagnostic and therapeutic resources and personnel available will be

determined by the event. For medical or industrial incidents, there will likely be sufficient resources so that healthcare workers can conduct a medical history, physical examination, and laboratory studies on each person involved. For a mass casualty event, priorities will be established based on the resources available.

Details on medical evaluation are available from the Radiation Event Medical Management Web site (www.remm.nlm.gov).[37] In general, medical management is based on both dose and organ dysfunction score.[38] Radiation syndromes (eg, ARS) are organ based, with the hematological system predominating at the lower doses, gastrointestinal and cutaneous syndrome next, and central nervous system syndrome at doses of about 10 Gy. It is now recognized that all organs are affected by irradiation to some extent, so radiation sickness really is a multiorgan injury.[39] Radiation doses greater than 2 Gy to a substantial part of the body would raise the issue of prompt initiation of mitigating agents; doses in excess of 4 Gy would require immediate medical attention. For mitigation to be effective, it must be administered in a timely manner, probably within the first 24 hours. For people with lower exposure not at risk for ARS, the concern is for radiation-induced cancers, the discussion of which is beyond the scope of this chapter.

If time of an exposure is known, the initial dose assessment will be made on symptoms and possible physical dosimetry. The initial laboratory assessment will include a complete blood count, and the decline in lymphocytes and possibly the ratio of neutrophils to lymphocytes will be used to estimate dose. Should these data indicate the need for immediate treatment, a blood sample would be stored, if possible, for eventual cytogenetic assay. For those who have received a dose that does not require medical intervention, at least in a mass casualty setting, no further evaluation would be done at this time, although later a blood sample may be taken to estimate long-term risk. There may be a group of victims with doses between 2 and 4 Gy for whom the need for ARS treatment is uncertain. Since the hematological syndrome has an onset of 2 to 4 weeks, it is logical that further blood analysis, including blood count and cytogenetic biodosimetry, be done as rapidly as possible, and those at risk for developing bone marrow dysfunction be sent to the appropriate experts for management (for example, to the Radiation Injury Treatment Network).[40]

Potentially exposed victims concerned about radiation-induced cancer could undergo cytogenetic biodosimetry. For a mass casualty event, the cutoff may be estimated exposure of 0.75 Gy, but that decision would be made based on the size of the event and the laboratory capacity. Individuals deemed at increased risk of radiation-induced cancer could undergo counseling to improve their general health (eg, eliminate smoking) and to understand their increase in lifetime cancer risk in terms they could understand. The latter may help reduce anxiety and stress-related illnesses.

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