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Source: Radiation Research, 196(5) : 455-467

Published By: Radiation Research Society

URL: https://doi.org/10.1667/RADE-21-00072.1

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RADIATION RESEARCH **196**, 455–467 (2021)
0033-7587/21 $15.00
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DOI: 10.1667/RADE-21-00072.1

Challenges and Strategies in the Development of Radiation Biodosimetry Tests for Patient Management

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Satyamitra, M., Reyes Turcu, F. E., Pantoja-Galicia, N. and Wathen, L. Challenges and Strategies in the Development of Radiation Biodosimetry Tests for Patient Management. *Radiat. Res*. 196, 455–467 (2021).

The public health and medical response to a radiological or nuclear incident requires the capability to sort, assess, treat, triage and ultimately discharge, as well as to refer or transport people to their next step in medical care. The Public Health Emergency Medical Countermeasures Enterprise (PHEMCE), directed by the U.S. Department of Health and Human Services (HHS), facilitates a comprehensive, multi-agency effort to develop and deploy radiation biodosimetry tests. Within HHS, discovery and development of biodosimetry tests includes the National Institute of Allergy and Infectious Diseases (NIAID) National Institutes of Health (NIH), the Office of the Assistant Secretary of Preparedness and Response (ASPR), Biomedical Advanced Research and Development Authority (BARDA), and the Food and Drug Administration (FDA) as primary partners in this endeavor. The study of radiation biodosimetry has advanced significantly, with expansion into the fields of cytogenetics, genomics, proteomics, metabolomics, lipidomics and transcriptomics. In addition, expansion of traditional cytogenetic assessment methods using automated platforms, and development of laboratory surge capacity networks have helped to advance biodefense preparedness. This article describes various programs and coordinating efforts between NIAID, BARDA and FDA in the development of radiation biodosimetry approaches to respond to radiological and nuclear threats. **© 2021 by Radiation Research Society**

INTRODUCTION

In the wake of the terrorist attacks on September 11, 2001, the United States government re-focused attention on the potential threat from a radiological or nuclear incident on U.S. soil. A large nuclear disaster will necessitate evaluation and clinical management of potentially hundreds of thousands to millions of individuals (*1–3*). The U.S. government agencies support development of biodosimetry, the measurement of the biological response to an absorbed dose of ionizing radiation, as it will offer an added clinical benefit to patient care for postirradiation symptoms (*4*). Initial triage of individuals will likely consist of multiparametric approaches that include evaluation of approximate exposure location, pre-existing medical conditions (*5, 6*), and basic clinical assessment of symptoms associated with radiation exposure, such as vomiting, diarrhea, headache, loss of consciousness, blood counts and fever (*7*).

Diagnosis of radiation exposure and delayed injury can be achieved using various biodosimetry tests, which measure physiological, chemical or biological markers (i.e., biomarkers) of exposure of human tissues to ionizing radiation, for the purpose of reconstructing doses received by individuals or anticipating major outcome(s) resulting from irradiation. Biodosimeters or radiation biodosimetry devices include tests intended to measure absorbed radiation dose or predict outcome through testing of clinical specimens (e.g., blood, saliva, and urine).

In response to growing concerns about the ability of the U.S. government to mount a medical response to such a disaster, several agencies were tasked with the mission to support research for developing biodosimetry approaches and medical countermeasures (MCMs) to diagnose and treat radiation injuries after a mass casualty, public health emergency. The National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), the Biomedical Advanced Research and Development Authority (BARDA), and the U.S Food and Drug Administration (FDA) are agencies within the Department of Health and Human Services (HHS) that have been working closely together to facilitate radiation biodosimetry advances. Since 2004, the Radiation and Nuclear Countermeasures Program (RNCP) within NIAID has supported work across the entire spectrum of radiation research, including basic research to

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identify and target biological pathways involved in the radiation damage response, generating animal models, and advanced development of approaches needed to obtain licensure by the FDA. In 2009, BARDA was initiated, and tasked with supporting late-stage activities needed for product licensure. BARDA is also responsible for procurement of devices and biodosimetry tests/assays to be placed in the U.S. Strategic National Stockpile (SNS). In parallel, the FDA has provided guidance to drug developers seeking approval of products for a radiation indication, for which efficacy studies in humans cannot be feasibly or ethically performed, and to assist developers by providing assistance regarding recommendations for development and validation of biodosimetry devices.

Development of medical countermeasures (MCMs) to address radiation lethality, injuries, and biodosimetry tests to assess exposure to radiation are two sides of the same coin. It is critical to national preparedness to have sufficient MCMs approved, and simultaneously, to have biodosimetry tests authorized or approved by the FDA to best respond to a radiological mass casualty incident. With regard to drugs and biologics, the FDA pathway for radiation countermeasure development using the ''Animal Rule'' (*8*), has resulted in approval of four MCMs to treat hematopoietic complications resulting from radiation exposure: filgrastim (Neupogen®, FDA approved March 2015; Amgen, Thousand Oaks, CA),[2] pegfilgrastim (Neulasta®, FDA approved November 2015; Amgen),[3] sargramostim (Leukine®, FDA approved March 2018; Partner Therapeutics, Lexington, MA)[4] and romiplostim (Nplate®, FDA approved January 2021, Amgen).[5] To date, no device or test for radiation biodosimetry has been authorized, cleared or approved by the FDA for use in the event of a large-scale nuclear/radiological incident. However, in 2016, the FDA published a guidance document entitled ''Radiation biodosimetry medical countermeasure devices. Guidance for industry and Food and Drug Administration staff'' (*3*), to provide recommendations to support the validation of biodosimeters intended for clinical use and to assist developers of radiation biodosimetry devices.

Dr. Anthony Fauci, Director of the NIAID, recently stated, ''The COVID-19 outbreak is a stark reminder of the ongoing challenge of emerging and reemerging infectious pathogens and the need for constant surveillance, prompt diagnosis, and robust research to understand the basic biology of new organisms and our susceptibilities to them, as well as to develop effective countermeasures.''(*9*). This statement holds true for the HHS aim to identify and deploy a radiation biodosimetry device to respond to any unanticipated nuclear/radiological challenge. The first step in managing a crisis involving a large part of the population is identifying those persons at risk. Therefore, diagnostics that can rapidly identify persons who have sustained significant radiation injury and require urgent treatment will be essential in effectively managing the crisis. The purpose of this article is to highlight key programmatic elements among U.S. government agency partners that can inform the broader research community and test developers about the recommendations and challenges for radiation biodosimetry research at different strata of the development pathway. The discussion here is also limited to radiation biodosimetry devices that would be used in the event of large-scale, radiation public health emergency, such as the detonation of an improvised nuclear device, or as a consequence of a nuclear power-plant incident, either man-made or due to a natural disaster. This article does not address the use of these tests to assess radiation absorbed dose as a result of radiotherapy.

Triage, Definitive Dose, and Predictive Biodosimetry

Biodosimetry assays/tests cover the continuum of radiological response, spanning from initial triage to medical management and further clinical evaluations. To maintain congruency of terminologies used in this article, and based on potential intended use(s) of a given test, ''triage'', ''definitive dose'' and ''predictive biodosimetry'' assays are described here:

1. Point-of-care (POC) tests for triage are qualitative assays that can be deployed for field triage or at the patient's bedside primarily for the purpose of distinguishing between exposed and non-exposed populations.
2. High-throughput (HT) devices to measure definitive dose refer to those biodosimetry devices intended to quantify the radiation dose in an exposed individual.
3. Predictive biodosimetry tests are intended to inform the consequences of exposure to radiation, for example, indicating clinically significant injury to major organs and its potential sequalae, such as predicting neutropenia after acute total-body irradiation (TBI) or pneumonitis or pulmonary fibrosis after significant irradiation of the thorax.

All three indications are characterized by specific, measurable biological patterns such as a biomarker or set of biomarkers defined as a ''signature''.

RADIATION BIODOSIMETRY PROGRAM AT THE RNCP, NIAID

NIH Goals for Radiation Biodosimetry

In 2005, a blue-ribbon panel was convened by the NIH to create a Strategic Plan and Research Agenda for Medical Countermeasures against Radiological and Nuclear Threats.[6] That panel directed the program to pursue research

[2] https://bit.ly/2ZJO9KH.
[3] https://bit.ly/2U8OwdE.
[4] https://bit.ly/2XYai6h.
[5] https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/125268s167lbl.pdf.
[6] https://bit.ly/3nwDdw4.

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TABLE 1
Current Biodosimetry Approaches Supported by the RNCP/NIAID

Approach	Model	Potential use
Discovery stage		
Senescence markers	Mouse	I
Aptamer	Mouse	I
Radionuclides Algorithm for retrospective biodosimetry	Historical data	N/A
Gene expression	*Ex vivo* human blood	II
Early development stage		
RNS, miRNA, lncRNA signature	Mice, NHP	I, II
Lifetime study-urine metabolomics	Mice	III
Metabolomics for lung, cardiac DEARE	Mice, rats	III
Biomarkers of CRI	Engrafted mice	II, III
Transcriptomics for DEARE	Mice	III
Mid-development stage		
CBC and proteomics	Human blood	I, II
Genomics in POC device	NHO, clinical	I, II
Proteomics in leukocytes	Human blood	I, II
RABiT-II	Human blood	II
Transcriptomics for DEARE	NHP, clinical	III
Proteomics	NHP, clinical	I, II

Notes. I = triage, II = definitive dose, III = predictive test.

in the area of radiation biodosimetry using off-the-shelf products automated for a radiation biodosimetry purpose, and support development of novel biomarker assays and biodosimetry devices/techniques. In addition, the following goals were identified:

Immediate Goals

- Support rigorous quality assurance/quality control studies of current leading biodosimetry technologies to validate their use.
- Increase the speed and efficiency of current assays to determine radiation doses received due to internal or external contamination with radioactive material.

Long-term Goals

- Develop new bioassays that can provide rapid and accurate radiation dose assessments, enabling optimal triage, medical management, or predictive outcome.
- Develop biodosimetry tools and assays to evaluate radiation-related injuries and recovery processes of different physiological systems.
- Develop and validate methods to estimate radiation dose and future risk following exposure to radioactive materials by various routes, including inhalation, ingestion, skin contact, or contamination of wounds.

Several of the goals of the RNCP, NIAID are to support basic research to identify biomarkers of radiation injury, and fund mid-to-advanced-stage development of assays and biodosimetry devices for triage and medical management. The NIAID also has oversight for a translational research component, to facilitate evolution of fundamental research knowledge into the development of mature and successful biodosimetry approaches within the RNCP mission.

Described above, radiation biodosimetry is defined as the estimation of received dose from past radiation exposure, through observation of biologic variables or measurements (*10*). Over the years, the term has expanded to include devices that can be used to conduct qualitative or categorical assessments (*3*). Evidence of radiation exposure can be found on cellular, molecular and biochemical levels. The technology to measure one or more of these signatures incorporated into a radiation biodosimetry device constitutes a ''test''. These approaches include assessment of circulating cell depletion kinetics, DNA damage assays (cytogenetics) and various ''omics'' approaches. The kinetics of lymphocyte depletion has been shown to be directly related to the absorbed radiation dose from 0.5 to 10 Gy (*2, 11–12*). DNA damage assessments include the gold standard, dicentric chromosome assay (DCA) (*13*), γ-H2AX assay (*14*), micronucleus assay (*15*), telomere length measurements (*16*), and fluorescence *in situ* hybridization assays (FISH) (*17*). ''Omics'' approaches in radiation biodosimetry include proteomics (*18*), genomics (*19*), metabolomics (*20*), lipidomics (*21*) and transcriptomics (*22*) (Table 1). Other approaches include assays for the detection of cell-free DNA (cfDNA) or RNA (cfRNA) in circulation (*23*), panels of reactive oxygen species (ROS) or reactive nitrogen species (RNS) (*24*), inflammasomes, and the ''cytokine storms'' after radiation exposure (*25*). Furthermore, the ratio of neutrophils to lymphocyte has also been suggested as a means to determine radiation exposure level (*26–28*).

Since 2004, the RNCP has supported radiation biodosimetry-focused research at different stages of development through a variety of funding initiatives, such as pilot programs, standard R01 and Small Business Innovation Research (SBIR) grants, single (U01) and multi-institutional (U19) cooperative agreements, contracts, and Interagency

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Agreements (IAA). Current biodosimetry research funded by the RNCP is listed in Table I. The majority of NIAID support is focused on highly innovative approaches that are early in the development stage. As the technology and research matures, and achieves the specifications outlined by BARDA (described later), the requirements and scope narrows. For instance, early discovery work can be conducted using *in vitro* cell lines or rodent models, with the quality of irradiation, the biokinetics of the target biomolecules, and the technology employed entirely at the discretion of the investigators and test developers. Once the signature is defined and ready for translation, the investigators/test developers need to specify the requirements of the assay to support the intent of use, (e.g., triage, absorbed dose estimation or predictive assay). In an urgent, mass casualty scenario, minimally invasive specimen collections (e.g., whole blood, plasma, or serum from a finger-stick, saliva, urine, hair, tears or sweat, etc.) are preferred over more invasive sampling techniques (e.g., spinal fluid, biopsy, large volumes of blood via venipuncture). When longitudinal sampling is required after acute exposure, even though blood draws are commonly used, repeated collection can pose additional health risks in already fragile patients. These complexities must be translated in the appropriate animal model to develop the specific biodosimetry approach.

Irradiators. Medical outcomes after a radiation incident can be highly unpredictable, given that detonation of an improvised nuclear device will comprise both high-energy photons and neutrons. To simulate a response to a large-scale, radiation public health emergency, most radiation biodosimetry studies typically model exposures using self-shielded gamma irradiators (^{60}Co or ^{137}Cs), X-ray irradiators or linear accelerator (LINAC) systems. These devices can also be combined with small-animal-adapted, micro-computed tomography, image-guided radiation therapy techniques, similar to those intended for diagnostic or therapeutic human use (*29–32*). The most commonly used sources for early biodosimetry studies are either orthovoltage X-ray irradiators that generate photon energies of 16 to 150 KeV (*33*), or gamma rays, mentioned above, that are monoenergetic photons on the order of megavolts (MeV). However, when comparing biological effects from these kinds of irradiators with a source that can produce neutrons (similar to those experienced in Hiroshima at 1–1.5 km from the blast epicenter), investigators demonstrated that urinary metabolites generated in irradiated mice (*34*) were different between neutron and X-ray exposures on days 1 and 7 postirradiation. Therefore, careful thought must go into selection of the appropriate irradiation source, to ensure accuracy and sensitivity when planning advanced development of a radiation biodosimetry test.

Animal models of irradiation. Radiation exposure victims could have variable biological responses due to shielding of the body (e.g., from a building, walls or furniture), resulting in heterogeneous exposures that would likely spare a portion of tissue-regenerating stem and/or progenitor cells in the bone marrow (*35*). However, most radiation biodosimetry studies use TBI with homogenous exposures, resulting in specific biomarker panels. Selection of the appropriate irradiation model for the intended use of the biodosimetry test is extremely important. For example, a TBI model may be appropriate for triage and dose estimation assay in the first few days postirradiation, although there is also interest in understanding how partial-body irradiation (PBI) affects the biodosimetry signature during the early days postirradiation. However, for biodosimetry approaches predicting delayed effects of acute radiation exposure (DEARE), such as pulmonary, cardiac or renal injuries, it may be more appropriate to select a PBI model, to ensure the survival of the animals irradiated at high doses out to 2–4 months postirradiation, and to allow for manifestation of late effects (*36, 37*).

The species used for identification of radiation-induced biomarkers and their measurement are often rodents (mice and rats), minipigs, and nonhuman primates (NHPs). Although most discovery work to identify biodosimetry signatures can be initiated in small animals, their significant biological divergence from the human response presents considerable translational challenges. For instance, of 19 miRNAs identified in irradiated mice, only 7 miRNAs showed significant induction in irradiated NHPs (*38*). For this reason, biomarkers established in rodents should be cross verified in larger mammal models. Ultimately, biomarkers must be explored in those well-established models that may have more synergy with human radiation responses and may be more suited for bridging studies, to allow developers to demonstrate appropriateness of a biodosimetry signature derived from animal data to relevant clinical metrics (*3*).

Technology. Often, technological advances outpace basic research findings; an example of this is the standard method of assessment of DNA strand breaks resulting from exposure to ionizing radiation, DCA (*1*). This assay has several limitations, including loss of sensitivity at higher levels of radiation, time to generate a response, and low throughput. Another standard biodosimetric method is the cytokinesis-block micronucleus (CBMN) assay, a well-established biodosimetry technique for assessing cytogenetic damage (*39*). The CBMN assay quantifies the frequency of micronuclei (MN) in binucleated cells derived from human peripheral lymphocytes. The assay, however, is labor intensive and susceptible to variability, as it typically uses microscopy for manual scoring. Building on these standards, the NIAID-funded Center for High-Throughput, Minimally Invasive Radiation Biodosimetry at Columbia University developed the Rapid Automated Biodosimetry Tool (RABiT), a completely automated, ultra-HT, robotic-based biodosimetry workstation that analyzes blood samples taken from a fingerstick. RABiT biodosimetry assays have been developed for protocols that include the CBMN, DCA and γ-H2AX assays. The next-generation RABiT-II

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system can now also work with commercially available, automated biotech systems that are already in use in the clinical setting (*3, 40*).

Reproducibility. A common cause in the failure to successfully translate biodosimetry approaches is the lack of consistent and reproducible data. In fact, the NIH recently acknowledged issues of poor reproducibility in biomedical research, particularly in preclinical studies using animal models (*41*), and the importance of reproducibility is further emphasized by the FDA (*3*). The key focus of the NIH policy on rigor and reproducibility[7] includes strict application of scientific methods to ensure unbiased and well-controlled experimental design. This also includes methodology, analysis, interpretation and reporting of results, and consideration of relevant biological variables such as sex, age, weight and underlying health conditions. The ultimate aim of these policies is to facilitate the progression of knowledge from the bench side to public health, termed the Biomedical Research Translation Continuum (*42*). In keeping with these recommendations, researchers conducting basic investigations are encouraged to include both sexes in their animal models, while also considering age and pre-existing conditions as crucial variables, and ensuring studies are statistically powered appropriately. In addition to these issues common to all basic studies, radiation biodosimetry research has challenges inherently unique to this field. Results are often complicated by heterogeneity of exposure due to variations in the radiation field (e.g., TBI vs. PBI or radiological contaminations), which can skew biodosimetry signatures (*43–45*). Unlike repurposed drugs for MCM development (*46*), no medical device exists that could potentially be repurposed for biodosimetry triage, dose or predictive purposes.

Due to all the possible considerations that need to be addressed to ensure successful translation from platform inception to final FDA authorization or approval, communication and close collaboration between agency partners, investigator/test developers, and corporate entities are necessary. To this end, the RNCP works closely with BARDA at all stages of biodosimetry development, and has also fostered frequent and productive interactions with the FDA as technologies mature (Fig. 1).

THE BARDA RADIATION BIODOSIMETRY PROGRAM

In 2009, the PHEMCE working group, comprised of representatives from the Centers for Disease Control (CDC), NIAID, the National Cancer Institute (NCI), the Department of Defense (DoD), FDA, and ASPR, convened to establish target product profiles (TPP) for two types of biodosimetry tools (Table 2). These tools were a patient-side, qualitative rapid test that can distinguish low- or no-dose from high-dose absorption, and a quantitative, remote-laboratory, HT absorbed dose test that can predict eventual onset of acute radiation syndrome (ARS) and neutropenia. It was hoped that the quantitative test could better inform therapeutic management, with consequently better allocation of scarce MCM resources. At that time, TPPs for predictive tests of delayed injuries or outcome-related tests were not prioritized for discussion.

The BARDA biodosimetry program seeks to enhance national radiation and nuclear incident preparedness. The goal is for the biodosimetry tests to improve the speed with which medical personnel can identify individuals with clinically significant absorbed doses of radiation, and rapidly assign affected patients to the most appropriate level of care. As a result, there are two categories of tests under development, as shown in Table 2. An initial test would be a POC or patient-side assay. This qualitative test, administered in a field setting, would determine whether an individual has absorbed a $\geq$2 Gy dose. It is estimated that more than a million people will need to be tested if a nuclear detonation occurs in a major city. Therefore, the time to test results needs to be less than 30 min per person and should be easily and accurately performed with little training. The intent of this testing is to separate the individuals who need to be further evaluated from those who do not need immediate MCM treatment. A subsequent biodosimetry test, such as a HT assay, will be administered to determine more quantitatively what level of damage an individual sustained due to irradiation. These HT assays will be performed in pre-established clinical laboratories and are designed to accurately report out absorbed dose estimates across dose ranges of 0.5 to 10 Gy. The turnaround time for the result should be as short as possible, with the goal of 6–8 h as a target. The HT assay is designed to test at least 400,000 individuals within the first week after the detonation. The result from the HT test will be used in triangulation, along with clinical signs and symptoms and the disappearance of lymphocytes and neutrophils, to guide medical staff to better manage irradiated individuals. Development and eventual acquisition of biodosimetry test kits will help the U.S. government assist state, local, and tribal leaders during the immediate aftermath of a nuclear incident.

In 2010, a team of scientists from BARDA contracted with 11 medical device companies to support their development of novel biodosimetry tests. Several of the companies had been previously working with the NIAID to discover biomarkers whose change correlated with absorbed dose. The goal of the contracts was to support development, clinical validation, and FDA clearance, and assist offerors to be prepared to employ them after a nuclear incident. Companies were contracted to demonstrate biomarker proof of concept, develop robust products, integrate tests and their analyzers, verify the performance, validate the tests in the operational settings, and provide plans for the deployment of the diagnostic. The proof-of-concept phase entailed

[7] https://grants.nih.gov/policy/reproducibility/guidance.htm.

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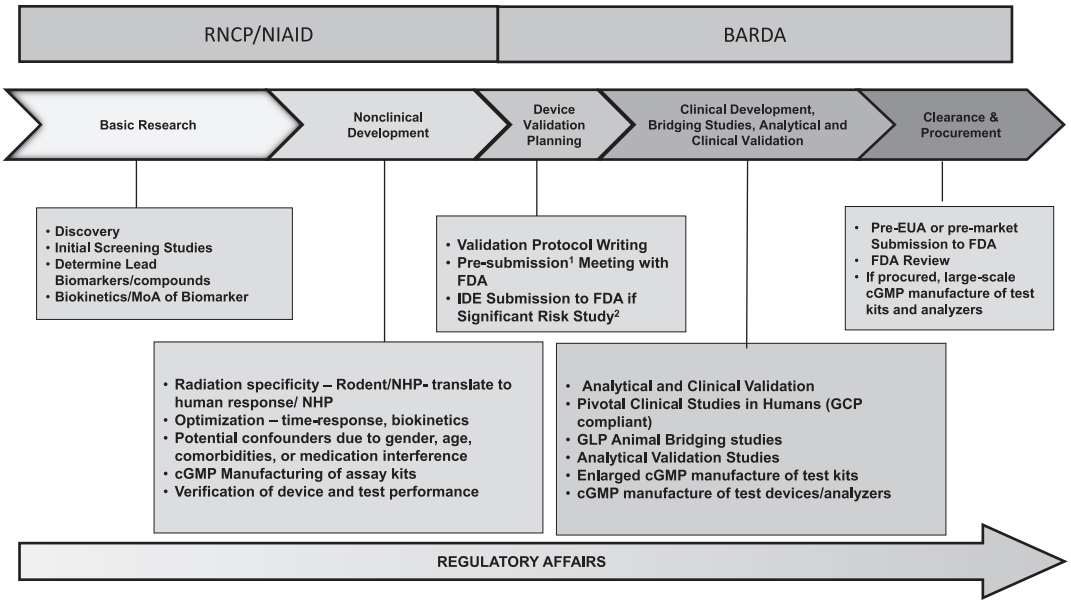


FIG. 1. Discovery and development of radiation biodosimetry tests. Submitting[1] a pre-submission to FDA to obtain regulatory input prior to initiation of validation activities is at the discretion of the sponsor, i.e., is voluntary, although recommended (https://www.fda.gov/media/114034/download). An[2] IDE submission to FDA prior to initiation of a clinical investigation is required for significant risk studies. If the study is non-significant risk or exempt, an IDE is not required to be submitted to FDA prior to initiation of the clinical investigation (https://www.fda.gov/media/71075/download).

demonstrating that the test biomarkers were radiation-specific and changed in a dose-dependent fashion in at least two species, including humans. Product development included: designing testing patterns, controls, and standards; developing algorithms to integrate individual biomarker results into a clinical result; and choosing an appropriate platform for running the test. During the integration phase, biodosimetry product developers used samples from irradiated and nonirradiated humans and animals to find design flaws when freshly obtained specimens were run on the analyzer with the prototype software loaded. They needed to go back in an iterative fashion and revise designs and software, until the outcomes demonstrated the desired accuracy and precision. Once the design of all the biodosimetry test components was locked, developers were ready to start verification and an extensive testing process was implemented. Contractors tested human samples from hundreds of normal, healthy volunteers, along with biofluids from individuals considered to be a part of special populations such as those people with burns, trauma, diabetes, rheumatoid arthritis, and immunodeficiencies, as well as pregnant, and geriatric individuals, to ensure assay specificity. Samples from humans irradiated for therapeutic purposes and samples from NHPs that were irradiated using the same regimen were studied to demonstrate dose responsiveness and interspecies comparability. Test ruggedness was examined by performing the assays in high/low humidity, different temperatures, and altitudes. Varying analysts, laboratories, instrumentation, reagent lots, and testing days allowed the developers to scrutinize test robustness. If the analytical and clinical results of verification testing proved that the locked design features

TABLE 2
Point of Care (POC) and High-throughput (HT) Biodosimetry Test Characteristics

	POC device	HT device
Type of result	Screening/qualitative	Quantitative/semi-quantitative
Concept of operations	Initial triage/sorting	Injury assessment/treatment tool
Exposure level	2 Gy–threshold	Range: 0–10 Gy
Ease of operations	Easy to operate, minimal complexity, requires minimal training, CLIA waived	Laboratory instrument; more labor intensive, requires training
Device characteristics	Integrated components; no separate sample preparation.	May include separate components as needed. High automation desired.
Intended use	Tents, shelters, open settings	Laboratories, hospitals, fixed facilities
No. of patients/event	Up to 1,000,000 within 7 days	Up to 400,000 within 7 days (may need multiple assessments)
Time to result	Rapid but individual sample result (15 to 30 min)	Up to 24 h

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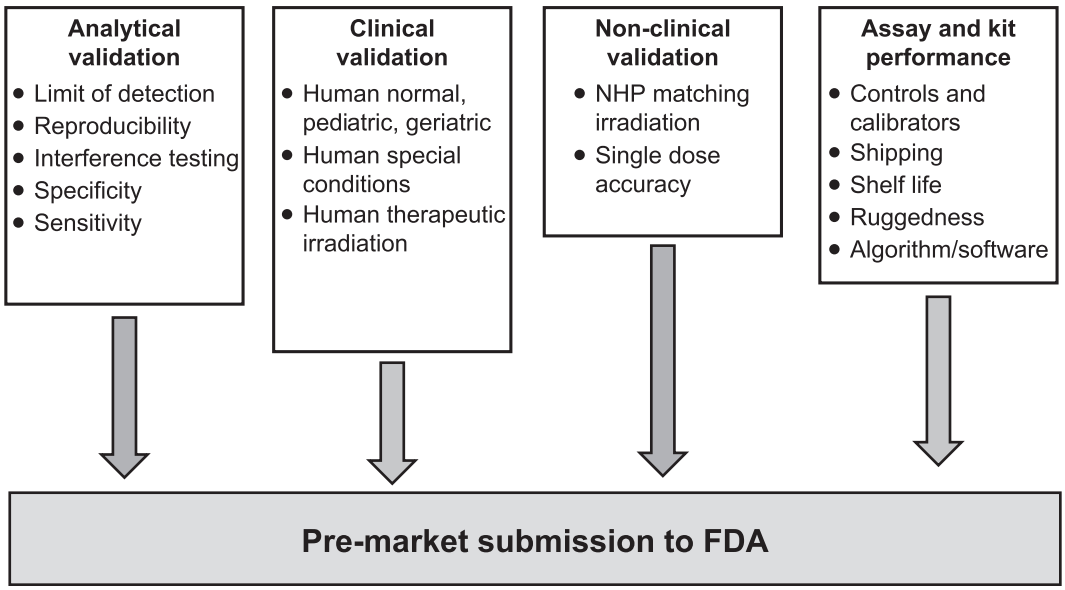


FIG. 2. Outline of validation data for a biodosimetry test pre-market submission to CDRH, FDA.

produced linear, sensitive, precise, accurate, stable results with expected limits of detection and quantitation achieved, the validation process would follow. Validation data include accuracy, limit of detection, dynamic reportable range, sensitivity, measuring internal/expected values, interference testing, reproducibility, specificity, repeatability, controls/calibrators, software, and clinical human and animal bridging data (Fig. 2). A complete description of the requirements and recommendations is found in the FDA guidance document (*3*).

Several pre-submissions will likely need to be submitted to FDA, to obtain feedback on the acceptability of the protocols and studies planned for validation. Data from these studies could be submitted initially in a pre-emergency use authorization (pre-EUA) request (*63*). If the data are adequate to obtain pre-EUA status, the test could be quickly authorized by the FDA if a nuclear incident occurred. If the data obtained in the clinical, animal bridging, and analytical validation studies is sufficiently accurate, linear, sensitive and specific enough to be medically useful, a full marketing application can be submitted for FDA consideration and potential marketing authorization or approval. The U.S. government may elect to acquire one or more of the biodosimetry tests that have undergone FDA scrutiny and place them in the SNS for utilization in an emergency. ASPR, the CDC, and local, state and national authorities will be responsible for the operationalization of these tests. This requirement will necessitate pre-planning and establishment of laboratory networks to successfully employ the tests, when needed.

As mentioned above, there are currently no FDA-authorized, cleared or approved radiation biodosimetry tests to measure absorbed radiation (*28, 47*); however, two types of tests (qualitative and quantitative) are currently under advanced development with support from the HHS. Initial assessments of test accuracy over a range of 0 to 10 Gy are underway, using extensive clinical and non-clinical/analytical validation studies. The minimal TPP of a qualitative biodosimetry test would specify positivity/negativity for radiation exposure based on a clinically relevant absorbed dose threshold(s), ease of use, minimal training, small specimen volume, operable in temporary shelters, and rapid but simple result reporting. One test currently under development with funding support from HHS is the SRI Biodosimetry Diagnostic. SRI International (Menlo Park, CA) has developed a lateral flow immunoassay that uses nitrocellulose strips impregnated with antibodies to three plasma proteins. Circulating levels of these proteins are radiation-responsive in a dose-dependent fashion from 0–10 Gy, beginning at 24 h and continuing to at least 14 days postirradiation. The goal of such a test would be to detect proteins from a finger-stick of blood (*48*), and for the qualitative readout in $<$35 min to accurately distinguish radiation absorption below 2 Gy from $\geq$2 Gy.

In managing the medical aftermath of a nuclear blast, a major concern is the likely need to ration available MCMs due to the limited stockpiles of IV fluids, cytokines, antibiotics and other medical supplies (*49*). Patient location and medical history are not accurate predictors of absorbed dose (*1, 5, 50*), and after initial triage with a qualitative biodosimetry test, there is a gap of several days until the full extent of the severity of ARS appears. Therefore, follow-up evaluation using quantitative, laboratory-based biodosimetry tests would significantly facilitate the prudent dissemination of stockpiled MCMs. For example, administration of a cytokine MCM (e.g., a leukocyte growth factor) to patients with a clinically significant estimated absorbed dose is most effective when administered within the first few days postirradiation (*51–53*). A laboratory-based biodosimetry test must report an accurate absorbed dose

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over a range of 0 to 10 Gy, be performed under Clinical Laboratory Improvement Amendments (CLIA) certification, run on a highly automated device, be incorporated in a network of specifically trained facilities, and deliver a total output of up to 400,000 results per week. There are at least three such tests supported by HHS funding that are currently entering formal device validation phase with the intent to seek full FDA clearance to market.

Two of these tests exploit changes in mRNA levels of radiation-responsive genes. Both use mRNA expression levels calibrated to NHP radiation response patterns after an acute dose (*54*). The ARad test from MRIGlobal (Kansas City, MO) uses quantitative reverse transcription PCR (qRT-PCR) to measure changes in 14 different mRNAs, 13 of which are radiosensitive, and one that is insensitive and therefore, serves as a normalizer. The second test, REDI-Dx® from DxTerity® (Rancho Dominguez, CA), uses a similar number of radioresponsive and nonresponsive mRNAs (15 and 3, respectively), but only two of the RNA species are in common with the panel from the ARad test. The amplification strategy with the REDI-Dx test differs from that of the ARad test, in that the targeted RNAs are converted to specific, amplifiable DNA fragments, not by reverse transcription, but by chemical ligation of two contiguous, hybridizing DNA fragments that are then amplified by qPCR and distinguished by size using capillary electrophoresis. Although they share only two out of the 32 mRNA species, both tests measure a dose-dependent radiation response in Gy from a post-exposure venous blood specimen. The test times are comparable at 6–8 h per run and comparable in daily output, using multiple instruments to achieve 1,000 patient results per 24-h day.

The third HT quantitative test is the CytoRADx™ test (ASELL, Owings Mills, MD), a CBMN assay (*55*). This is a more direct measure of radiation injury because it is based on the abundance of MN generated from radiation-induced breakage of DNA in quiescent lymphocytes. Studies have shown that the number of radiation-induced MN strongly correlates with dose and quality of radiation (*56–58*). The CBMN assay is a direct measure of radiation-induced cytogenetic damage; however, it involves overnight cell culture, resulting in a longer time-to-result than the gene expression assays. FDA authorization/approval of one of these high-throughput tests would permit the assay to be used for patient-specific absorbed dose information to better inform medical staff in designing patient treatment plans.

Current and continued work with federal and industry partners will enable the development, regulatory review, and potential acquisition of radiation biodosimeters within the next few years. Availability of biodosimetry testing will greatly enhance the ability of the federal government to assist state, tribal and local authorities with their response to a large-scale nuclear incident. Second-generation biodosimetry tests may also be desirable to overcome the shortcomings of the initial tests. Some important features to consider in more novel tests would be smaller sample size, greater accuracy, quicker turnaround, and less dependence on animal model testing. The collaboration between corporate laboratories, NIAID, BARDA, the FDA, and other government agencies such as the CDC, NCI, DoD and the National Aeronautics and Space Administration (NASA) will be critical in the development of second-generation biodosimetry tests.

FDA GUIDANCE ON DEVELOPMENT OF RADIATION BIODOSIMETERS

Radiation Biodosimetry Devices

For the purpose of this discussion, radiation biodosimetry devices, or biodosimeters, are defined as *in vitro* diagnostic devices (IVDs) that are intended to assess the absorbed ionizing radiation dose received by a subject by testing of a clinical specimen (e.g., blood, saliva or urine) (*3*).

When used for such casualties, radiation biodosimetry diagnostic devices will contribute to the assessment of absorbed dose to aid clinicians and first responders making triage and treatment decisions.[8] As noted above, biodosimeters may provide quantitative outputs (e.g., estimated absorbed radiation dose in Gy), or qualitative information around a decision-making absorbed dose cut-point or threshold (*5, 28, 47*). Similarly, these devices may be designed for POC or HT clinical laboratory use. Finally, both HT and single sample-type devices could be considered as biodosimeters.

Development of proper radiation biodosimetry tools is a critical unmet public health need, and because it is impossible to obtain samples that accurately reflect the intended use population of biodosimeters in the absence of a large-scale radiological incident, validating the performance of biodosimeters poses significant scientific and regulatory challenges. As such, FDA has worked closely with other government entities, academia and industry to obtain perspectives that would help identify solutions for the scientific challenges associated with radiation biodosimetry device development and validation. This collaboration has provided a regulatory path forward to ensure device safety and effectiveness, and thereby provide significant clinical and public health benefits.

To this end, the FDA sponsored a two-day public meeting on September 27–28, 2012 titled ''Regulatory Science Considerations for Radiation Biodosimetry Devices'' to help inform the FDA's approach and, ultimately, help manufacturers overcome challenges associated with validating the performance of radiation biodosimetry devices for mass exposure scenarios. Based on the input received from the public meeting, as well as continued interactions with stakeholders, the FDA published a final guidance to assist developers with recommendations regarding the validation of a biodosimeter (*8*). Key highlights surrounding

[8] https://www.remm.nlm.gov/PlanningGuidanceNuclearDetonation.pdf.

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the development and marketing of biodosimeters are discussed below, and include human sample availability, the use of animal models, and the FDA regulatory process.

Human Sample Availability Challenges

IVDs generally require evidence that they are fit for purpose by validating both analytical and clinical performance of the test with specimens from the intended use population. A mass exposure scenario would involve people from all demographic categories. Therefore, an ideal biodosimeter would give accurate dose information regardless of patient age, gender, race or health status. For radiation biodosimeters, it is not feasible to obtain clinical samples in the absence of a large-scale radiological incident, therefore, developers of biodosimeters are tasked with obtaining surrogate clinical specimens. Patients exposed to radiation during the course of their treatment for cancer or other diseases, and *ex vivo* irradiation of normal human samples may offer the only alternatives to assess device performance in human samples in the absence of a radiological incident. The use of these samples also poses several challenges, as patients undergoing treatment for cancer or other diseases do not represent the intended use population for the device. Depending on the biomarker measured by the test, the disease state of the patient, or prior and/or current therapeutic regimens may also confound dose estimation results, and thus may not be representative of the results that would be expected when testing the general population that would be potential victims of radiation exposure.

One challenge from using specimens from patients undergoing radiation therapy is that the radiation doses delivered do not reflect the exposure to radiation experienced by victims of an inadvertent exposure or terrorist attack. Cancer therapy radiation dosing is generally fractionated, even in TBI, and is frequently targeted to a specific location in the body.

On the other hand, scenarios developed for radiological terrorism generally indicate that an exposure will be acute and will involve TBI or PBI that is randomly distributed (*59*). Initial exposures will likely occur with very high dose rates, unlike those used to deliver radiotherapy, and involve a mixture of radiation qualities (photons and neutrons) (*8, 60, 61*). Another challenge is that the affected population is generally limited to those cancers, for which radiotherapy is used. The ability to assess representative demographics, as well as additional potential confounders in the product development process, is generally not possible for the intended use subpopulation of exposed subjects, but is possible for the intended subpopulation of non-exposed subjects.

Finally, the volume and number of specimens from patients exposed to radiation may be severely limited, restricting their use in device performance studies. Thus, biodosimetry validation studies that require these types of samples may need to be supplemented with pre-clinical animal studies, to demonstrate the performance of the assay under scenarios that closely mimic the dose and range of exposure as well as expected confounders for the intended use scenario. These challenges pertain to the intended use subpopulation of exposed subjects and may be a potential source of spectrum bias, which occurs when the subjects included in the study do not encompass the complete spectrum of patient characteristics.[9]

The only other possible source of human samples for the evaluation of radiation biodosimetry devices could be samples collected and stored after the accidental or intentional radiation exposure of a population. If cell viability were not required, then these samples would be extremely valuable in verifying device performance. In this instance, sample stability would be the only concern associated with the data generated.

Use of Animal Models

Given the challenges associated with evaluating performance using human samples, unlike for traditional IVDs, animal models may be useful in demonstrating device performance for radiation biodosimeters. The FDA's Center for Drug Evaluation and Research (CDER), and the Center for Biologic Evaluation and Research (CBER) have codified an Animal Rule (21 CFR 314 Subpart I or 601 Subpart H), and developed Guidance for Industry (October 15, 2015: Product Development Under the Animal Rule) as a pathway for therapeutics and biologics that cannot meet the requirements of traditional licensure because human efficacy studies are not possible for ethical reasons or because field studies to assess efficacy are not feasible (*8*). While the Animal Rule does not apply *per se*, animal data can still be considered for medical and radiation biodosimetry devices, as supporting evidence where human, prospective clinical or other samples are not available or limited (*3*).

Because an ''ideal'' sample set does not exist for the evaluation of radiation biodosimetry devices, scientifically justifiable design of both pivotal and device performance studies, using available samples from humans exposed to radiation and supplemented with adequate animal models, is important in demonstrating the safety and effectiveness of biodosimeters. To this end, the Center for Devices for Radiological Health (CDRH), which clears biodosimetry devices, published a final guidance entitled ''Radiation biodosimetry medical countermeasure devices guidance for industry and Food and Drug Administration staff'', on April 18, 2016. The document provides recommendations to developers of biodosimeters regarding the kinds of studies and evidence that will be needed for FDA clearance or approval (*3*). Considerations for sample make-up of such studies should include the technological characteristics of

[9] https://bit.ly/3xys8iC.

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the assay, sample availability and clinical feasibility. The document acknowledges the difficulties in obtaining specimens as outlined above, and proposes how pre-clinical animal testing should supplement data that cannot be readily obtained from clinical testing. The Biodosimetry Guidance Document describes how studies aimed at bridging the animal results and the available human clinical information are important to demonstrate that the performance results obtained using animal samples would be applicable to humans. The FDA pre-submission program offers developers of biodosimeters the opportunity to discuss study designs and implementation prior to the initiation of testing (*61*). Early communication with the FDA regarding the design and make-up of both device evaluation and pivotal studies is highly recommended.

FDA Regulatory Process

As stated above, most biodosimeters fall into the class of medical devices known as IVDs. An IVD refers to those reagents, instruments or systems intended for use in the diagnosis of disease or other conditions, including a determination of a state of health, to cure, mitigate, treat, or prevent disease or its sequelae (*62*). Such products are intended for use in the collection, preparation and examination of specimens taken from the human body. The regulatory requirements for IVDs are dependent upon the classification of the device, the product type, and how it will be used.

Medical devices, including IVDs, are classified as class I, II or III depending on their potential level of risk to patients.

Class I devices require the lowest level of regulation and are subject to general controls. General controls include establishment of registration and medical device listing, good manufacturing practices, submission of pre-market notification, and labeling.

Class II devices require more oversight by the FDA and are subject to both general and special controls. Special controls may include specific labeling requirements, mandatory performance standards and post-market surveillance.

Class III devices have the highest risk. Class III devices are: 1. Devices for which insufficient information exists to determine special controls that in combination with the general controls would provide reasonable assurance of the safety and effectiveness of the device; and 2. Are purported or represented to be used to support or sustain human life, for use in prevention of human health impairment, or present a potential unreasonable risk of illness or injury.

For first-of-kind devices, when there is no legally marketed predicate device, the *de novo* process provides a pathway to class I or class II classification for medical devices. Notwithstanding the special concerns associated with MCMs, radiation biodosimetry devices will be regulated just like every other medical device. The primary regulatory mechanisms that are available for the development and emergency use of medical device MCMs are: 1. Emergency Use Authorization (EUA); 2. Investigational Device Exemption (IDE); and 3. Pre-market submission.

Emergency Use Authorization

An EUA is an alternative to standard regulatory mechanisms that was developed to respond to major public health emergencies. Under an EUA, the FDA grants authorization for products that are not yet fully developed, but have sufficient safety and efficacy data, and have been reviewed by the Agency to be used. Given the recent SARS-CoV2 pandemic, the EUA process is now better understood by many. There are certain criteria for issuing an EUA, which are found in section 564 of the Food, Drug and Cosmetic Act, including that: 1. It should address a specific chemical, biological, radiological, nuclear, or high yield-explosive agent that can cause a serious or life-threatening disease or condition; 2. It is reasonable to believe that the known and potential benefits outweigh the known and potential risks; and 3. There must be no adequate, approved, and available alternatives to the product. An EUA may be granted for an approved product that will be used in a way that is inconsistent with the limitations of approval, or for a product that is not yet approved, but may be permitted to be used for a particular emergency and under certain specified conditions.

An EUA is issued by the FDA after consultation with other government agencies such as the CDC and NIH. Prior to the issuance of an EUA, there must be a declaration of emergency by the HHS Secretary justifying the authorization. Prior to a declaration of emergency, products with sufficient safety information and some evidence of effectiveness may apply for a pre-EUA. Pre-EUAs allow the FDA to review potential radiation biodosimetry devices and establish their potential use prior to a declaration of an emergency. After the declared emergency is over, the product can only be marketed after obtaining FDA 510(k) clearance or pre-market (PMA) approval.

In January 2017, the FDA published a final guidance entitled ‘‘Emergency use authorization of medical products and related authorities’’, which explains general recommendations and procedures applicable to the authorization of the emergency use of certain medical products. This guidance applies to *in vitro* diagnostic devices, under sections 564, 564A, and 564B of the Federal Food, Drug, and Cosmetic Act (FD&C Act) as amended or added by the Pandemic and All-Hazards Preparedness Reauthorization Act of 2013 (PAHPRA) (*63*).

Investigational Device Exemption (IDE)

In most cases, prior to product testing in humans, a new device must be covered by an IDE. The IDE permits device use in clinical studies to collect safety and effectiveness data, while protecting subjects participating in device investigations and assessing risks posed by use of the

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device. Many IVD investigations are exempted from IDE requirements when testing is non-invasive, does not require invasive sampling representing significant risk, does not by design or intention introduce energy into the subject, and is not used as a diagnostic procedure without confirmation by another, medically established diagnostic product or procedure. Labeling requirements, informed consent and Institutional Review Board (IRB) approval still apply.

Pre-market Submission

Because there are currently no regulations guiding the performance validation requirements of this type of device, the traditional 510(k) submission pathway is not appropriate. For first-of-a-kind devices for which the FDA believes special controls and general controls may be appropriate to mitigate the risks, the *de novo* process provides a pathway to class I or class II classification for medical devices. On October 30, 2017, the FDA published a final guidance entitled ''De Novo classification process (evaluation of automatic class III designation): Guidance for industry and food and drug administration staff'', which explains general recommendations and procedures on the process for the submission and review of a De Novo classification request under section 513(f)(2) of the Federal Food, Drug, and Cosmetic Act (the FD&C Act) (*64*). The granting of a De Novo request allows the IVD to be marketed, creating a classification regulation for IVDs of this type, and permits the IVD to serve as a predicate device.

In these regulatory pathways, the FDA assesses the information provided about the IVD, including the directions and conditions for use. FDA reviewers must consider the risk of misdiagnosis and epidemiological misinformation that could be derived from a false-positive or false-negative result. It is also important to consider whether, to assure safety, there must be a restriction or limitation on use of the test system to certain types of laboratories, and whether labeling provides adequate warnings about unsafe use. In general, developers of radiation biodosimeters should consider the claims and capabilities of their biodosimeter as these attributes affect the regulatory path and the resultant data involved.

FDA and CDRH Resources for Biodosimetry Sponsors

As stated above, the FDA uses the pre-submission program (Pre-Sub program) to provide the opportunity for a device developer to obtain FDA feedback prior to intended submission of an IDE or marketing application. The Pre-Sub program can also provide a mechanism for the FDA to provide advice to applicants who are developing protocols for clinical studies, for which an IDE would not be required, such as assessment of non-significant-risk (NSR) devices or for clinical studies conducted outside of the U.S. to support future U.S. marketing applications. A Pre-Sub can be made as a formal written request from an applicant for feedback from FDA, to be provided in the form of a formal written response or, if the manufacturer chooses, a meeting or teleconference, in which the feedback is documented in meeting minutes.

While the Pre-Sub program is one mechanism that the FDA uses to guide and answer industry questions, additional mechanisms to assist manufacturers of radiation biodosimetry devices are listed below:

1. Medical Countermeasures Initiative[10]
2. Office of Counterterrorism and Emerging Threats[11]
3. Device Advice[12]
4. Emergency Use Authorization[13]

These mechanisms, as well as 510(k) summaries or decision summaries for similar legally marketed devices (even if there is not a legally marketed predicate for the proposed device), may be helpful resources, and are available on the FDA website. Furthermore, the FDA lists information regarding all current medical products, including devices that have current EUA status, on the FDA website, which could also be a useful resource to developers of biodosimeters.

CONCLUSIONS

The preparedness of the Nation to respond to a radiological and/or nuclear incident is contingent on availability of suitable tools to triage and treat the effected population. Radiological and nuclear scientific development programs within NIAID, BARDA and FDA have worked closely to develop and implement appropriate biodosimetry tests as a means of advancing medical approaches to counter radiation threats. These putative triage, definitive dose, or predictive assays must be thoroughly tested for reproducibility and validated across laboratories. Ultimately, these tests will help inform treatment decisions by medical professionals to provide critical care to vulnerable populations. All three organizations, along with other government agencies, will continue to work together to achieve the critical public health emergency objectives of safe and effective biodosimetry approaches to respond to a radiation or nuclear incident.

ACKNOWLEDGMENTS

The opinions contained herein are the private views of the authors and are not necessarily those of the NIAID/NIH, BARDA or FDA. Many thanks to RNCP/NIAID colleagues Andrea DiCarlo, Carmen Rios, Lanyn Taliaferro, Tom Winters, Brynn Hollingsworth and David Cassatt for their critical review of the manuscript.

Received: April 5, 2021; accepted: April 28, 2021; published online: June 18, 2021

[10] https://bit.ly/3e3tV7L.
[11] https://bit.ly/3aOO5An.
[12] https://bit.ly/3sXmyD6.
[13] https://bit.ly/3nwDUWc.

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