Research Departments

Departments of Clinical Studies, Hiroshima and Nagasaki

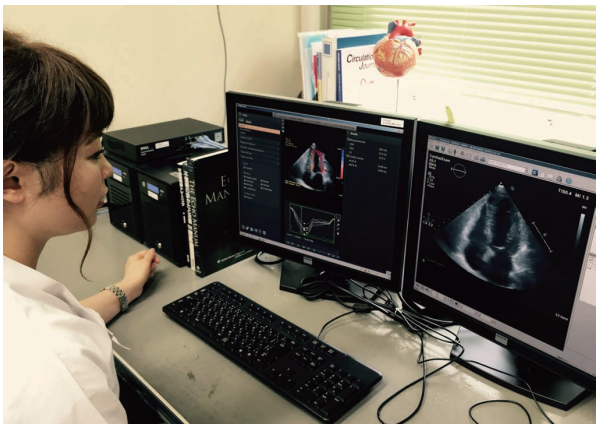
The Department of Clinical Studies conducts health-examination programs of the Adult Health Study (AHS) and the F_1 (children of the atomic bomb survivors) Clinical Study that provide the opportunity for a number of specific investigations to be conducted into a variety of health outcomes.

The AHS biennial health examinations were initiated in 1958 and continue today. The AHS cohort consists of a subcohort of the Life Span Study (LSS) A-bomb survivors of all ages at exposure, also including those exposed *in utero*. These health examinations represent the only point of regular direct contact with the survivors and provide health benefits to that population through early disease detection. Such examinations function as the principal source of biological materials that make possible a wide variety of valuable studies by numerous RERF departments and outside investigators. Sera, blood cells and plasma, and urine have been collected from the AHS participants and stored since 1969, 1990, and 1999, respectively. The AHS program has greatly contributed to RERF's mission of 1) assessing noncancer disease risks from radiation, 2) determining radiation effects on physiological or biochemical abnormalities and correlating this information with other life experiences and modes and patterns of disease, and 3) epidemiologically elucidating mechanisms of radiation effects on cancer and noncancer diseases using stored biosamples and clinical, physiological, and epidemiological information that was obtained through the health examinations.

The AHS continually increases in importance as a result of the accumulation of a large body of clinical data from the 27 rounds of biennial health examinations carried out to date. The data have provided the strongest available evidence of radiation-related increases in morbidity at low-to-moderate doses for noncancer diseases, such as cardiovascular disease (CVD), hyperparathyroidism, thyroid disease, chronic hepatitis B virus infection, and cataract, plus subclinical risk indicators and conditions such as circulatory inflammation.

More than a decade ago the Department of Clinical Studies began the program of F_1 Clinical Study examinations, which were conducted for about 12,000 individuals to analyze the potential heritable effect(s) of A-bomb exposure on polygenic, multifactorial diseases (e.g., diabetes, essential hypertension, coronary heart disease, and stroke) based on prevalence data obtained from 2002 to 2006. However, owing to the young age of the F_1 group (mean age of about 49 years at that time), most of their disease experience was still ahead. Therefore we converted the sample to a cohort for prospective follow-up and started health examinations every four years in November 2010 and have almost completed the second round of examinations.

Research Scientists of Hiroshima Clinical Studies (First row from left) Yoshimi Tatsukawa, Waka Ohishi, Michiko Yamada, (Second row from left) Fukiko Mitsui, Keiko Ueda, Ikuno Takahashi



Xcelera: Analysis software of echocardiographic images
Xcelera is the system that manages and analyzes images obtained from cardiovascular examination. It was introduced to obtain more confident data for cardiac function or structure as well as more detailed assessment of myocardial diastolic dysfunction for the study of heart disease in the Adult Health Study population using echocardiography. The echocardiographic images are quantified using the software included in Xcelera and a database of measurements regarding cardiac function is composed.

Research Scientists of Nagasaki Clinical Studies (From left) Misa Imaizumi, Ayumi Hida, Daisuke Haruta

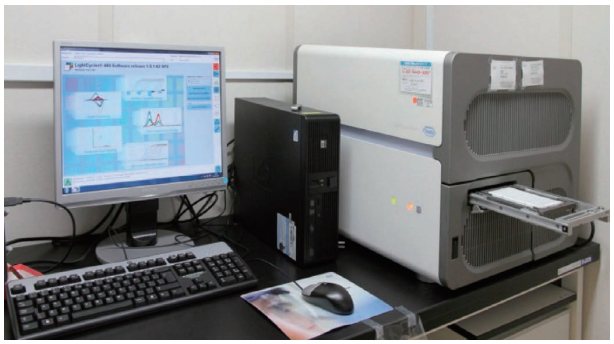
Department of Genetics

The Department of Genetics is divided into two laboratories: the Cytogenetics Laboratory and the Biochemical Genetics Laboratory. The main thrust of the Cytogenetics Laboratory is biodosimetry and mechanistic studies of radiation-induced somatic mutations, while the Biochemical Genetics Laboratory focuses on heritable mutations in animal models and the F_1 generation of survivors of the atomic bombings.

In the Cytogenetics Laboratory, the frequency of stable-type chromosome aberrations (translocations) was examined previously by the solid Giemsa method and is currently being studied using fluorescence *in situ* hybridization (FISH). The results of these FISH studies showed a wide scatter of individual translocation frequencies against physical dose, as was seen in the previous Giemsa study, but a somewhat smaller scatter against another independent biodosimeter, electron spin resonance (ESR) using tooth enamel. We anticipate that such biodosimetric data will provide information on possible random and systematic dose uncertainties in individual doses calculated by DS02 and prove to be valuable for use in cancer risk estimation. Currently, the laboratory has expanded its scope of research to the study of somatic mutations. Specifically, studies are being conducted examining genetic factors in breast and skin cancers and cytogenetic damage of *in utero*-exposed mouse thyroid cells, developing a green fluorescent protein (GFP) mouse model for quantitative measurement of germ-cell mutations, and finding markers for unrepairable DNA radiation damage.

The Biochemical Genetics Laboratory has been examining the frequency and nature of heritable mutations in members of survivor families (mother, father, and offspring). Previous studies were conducted that screened mutations at hyper-variable mini- and micro-satellite loci, and at about 1,000–2,500 loci per genome. None of those studies indicated statistically significant genetic effects of parental exposure to radiation. Recently, a high-density microarray comparative genomic hybridization (CGH) method using over one million probes was introduced to detect relatively large deletion/amplification mutations throughout the genome. This method was first used to estimate the trans-generational effects of radiation in the offspring of model animals and currently in the children of A-bomb survivors. We are also beginning whole genome sequencing-based and whole exome sequencing-based genetic studies using next-generation sequencing technology that will provide the capability to detect smaller mutations.

DNA fragmentation device
DNA fragmentation device has been introduced to the Department of Genetics. DNA can be fragmented to a size of interest with a high reproducibility by stable ultrasonic waves controlled by computer. The amount of DNA required is very small. This device is essential to prepare a DNA library for conducting research using a next generation sequencer.



Real-time PCR device
Real-time PCR device that can analyze 96 samples or 384 samples at one time has been introduced to the Department of Genetics. It has been used in various applications, validation of copy number mutation in genome DNA detected by other methods, and accurate quantification of fragmented genomic DNA libraries for analysis by next generation sequencer.

Research Scientists of Genetics (First row from left) Nori Nakamura (Consultant, Department of Genetics), Asao Noda, Yoshiaki Kodama, Jun-ichi Asakawa, Mieko Kodaira, (Second row from left) Yuko Hirai, Kazumasa Sekihara (temporary), Kanya Hamasaki, Masayuki Hidaka, Yasunari Satoh

Department of Radiobiology/Molecular Epidemiology

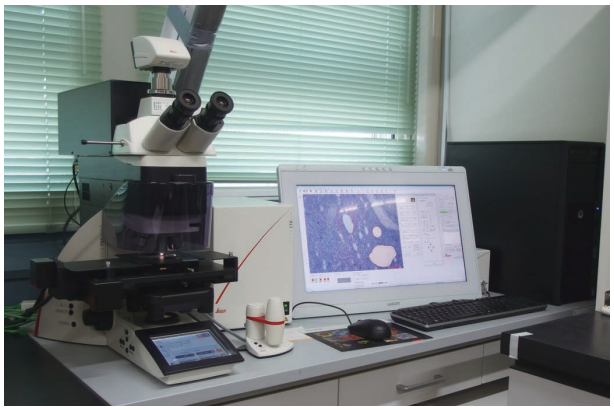
The Department of Radiobiology/Molecular Epidemiology focuses on two research themes: 1) Radiation and Immunologic Effects; and 2) Radiation and Cancer. Immunologic effects are investigated with two approaches: immunobiology and immunogenome studies, which primarily examine profiles of immune cell numbers and function, and inter-individual genetic variations related to disease susceptibility among A-bomb survivors, respectively. Regarding the cancer studies, this department seeks to elucidate the molecular mechanisms underlying radiation-associated increases of selected cancers among A-bomb survivors.

The immunobiology studies hypothesize that radiation accelerates the attenuation of immune function, thereby contributing to the enhanced risks of chronic diseases among A-bomb survivors. To understand the mechanisms of radiation-induced immune dysfunction, we are investigating radiation-related alterations of immune cells and organs (such as the thymus) and radiation effects on responses to influenza vaccines. We also are developing an integrated scoring system for evaluating immunological/inflammatory status of A-bomb survivors to provide a better understanding of the role of immunocompetence in radiation-disease associations.

The immunogenome studies evaluate the genetic basis for inter-individual differences in immune functions and the impact of genetics on susceptibility to radiation-associated diseases. The results obtained from phenotype-genotype association analyses utilizing RERF's extensive cumulative immunology phenotype data have the potential to contribute to fine-grained estimations of individual risks of radiation-associated diseases in A-bomb survivors and other exposed populations.

The cancer studies aim to clarify mechanistic relationships between radiation exposure and cancer development. Toward this end, we are analyzing early molecular events with respect to thyroid, colorectal, and lung cancer development in the Life Span Study (LSS) cohort and are also assessing the carcinogenic potential of altered genes found in these radiation-associated cancers using *in vivo* and *in vitro* experiments. DNA methylation and transcription will be analyzed in normal blood cell subsets from the Adult Health Study (AHS) subjects, with the hypothesis that radiation might cause epigenetic changes that lead to increased risks of selected diseases.

Research Scientists of Radiobiology/Molecular Epidemiology (First row from left) Masataka Taga, Seishi Kyoizumi (NIAID Project Research Scientist), Yoichiro Kusunoki, Tomonori Hayashi, Kei Nakachi (RERF Consultant), (Second row from left) Kengo Yoshida, Yasuharu Niwa, Yiqun Hu, Kiyohiro Hamatani, Junko Kajimura, (upper right) Reiko Ito



A new laser microdissection instrument, LMD7000, for sophisticated molecular analyses of cancer and non-cancer tissue specimens

Laser microdissection (LMD) makes it possible to obtain homogenous, ultrapure samples from heterogenous starting material. A researcher can thereby analyze regions of interest down to single cells, which are selected and excised from the surrounding tissue by a laser. Enormous progress has been made in the development of LMD in recent years, allowing straightforward analysis of various tissue specimens from A-bomb survivors.

Departments of Epidemiology, Hiroshima and Nagasaki

RERF aims to characterize and quantify the late health effects of radiation, based on data of the atomic bomb survivors. The Department of Epidemiology's follow-up of the Life Span Study (LSS) of survivors, the *in utero* cohort, and the F_1 cohort (offspring conceived after the bombing) is crucial to accomplish these purposes. Follow-up outcomes include deaths and causes of death wherever they may occur in Japan, and cancer incidence in Hiroshima and Nagasaki prefectures, where a large percentage of the survivors still dwell. Histological specimens of cancer cases are also collected by tissue registries in collaboration with community pathologists. Around 33% of the LSS cohort members were still alive at the end of 2010, including 82% of those who were less than 10 years old at the time of bombing (ATB). Moreover, 86% of the *in utero* and 89% of the F_1 cohorts are still alive. Therefore, continued follow-up of these young age groups for an additional 20 years or more is clearly essential. Important aims are to investigate consistency between epidemiological evidence and biological mechanisms of radiation effects to epidemiologically evaluate other risk factors for confounding or modification of radiation risks, and to more precisely determine the magnitude of risk for radiosensitive subgroups such as those who were in early childhood or *in utero* at the time of exposure.

International risk assessment groups use the results from these cohorts as the primary basis for radiation-risk estimation, because the data are unparalleled, being a large cohort of all ages who have had a wide range of well characterized doses and a long-term, high-quality disease follow-up. The LSS mortality and incidence data have been periodically analyzed, and a major update to our cancer incidence results will be published in 2015. Major results from our studies are heavily relied upon for the creation of numerous radiation-risk reports, including by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) (2006, 2013), the International Commission on Radiological Protection (ICRP) (2007, 2012), and the National Academy of Sciences Committee on the Biological Effects of Ionizing Radiation (BEIR) VII (2005). Findings by the Epidemiology Department in recent years that have been of particular importance to the radiation protection and risk-assessment community have

Follow-up status (as of the end of 2010)

LSS		
Age at bombing	Initial number	Alive (%)
0–9	23,717	81.6
10–19	25,994	59.0
20–29	15,785	32.6
30–39	17,310	3.7
40–49	18,403	0.1
50+	19,112	0.0
Total	120,321	33.7

In utero exposed survivors		
	Initial number	Alive (%)
Total	3,638	79.9

Children of survivors		
Age at 2010	Initial number	Alive (%)
<40	4,778	96.9
40–49	15,324	95.5
50–59	32,442	89.4
60+	24,272	82.4
Total	76,814	88.9

Atomic bomb survivors who were alive in 1950 (Life Span Study: LSS) have been followed up and the table shows the results as of the end of 2010. Most of those who were 40 years or older at the time of bombings had died while many of those who were less than 10 years at the bombings were alive. Follow-up results since 1945 are shown for *in utero* survivors then the survival was slightly lower than that for survivors who were 0–9 years of age at the time of bombings because of relatively high mortality during the period with bad health conditions before 1950. Distribution of survival is shown by age at 2010 for children of survivors (for deceased people, assuming they were alive) as they were born from 1946 to 1984.

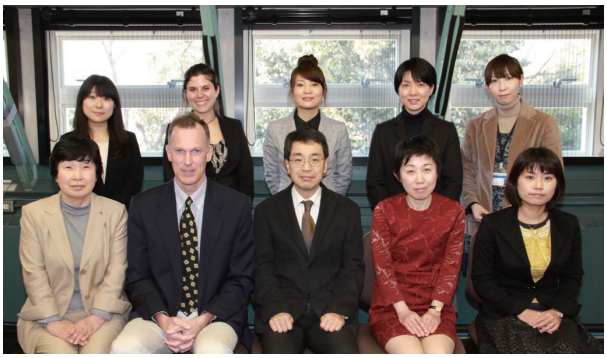
Effects of Exposure to Rain after the Bombings, Mortality, LSS, 1962–2005

Cause of death	Fallout rain status	Hiroshima		Nagasaki	
		No. of deaths	ERR 0 0.2	No. of deaths	ERR 0 0.4
All cause	No	15,997		10,865	
	Yes	6,381		349	
	Unknown	7,941		1,348	
Solid cancer	No	3,573		2,654	
	Yes	1,483		106	
	Unknown	1,892		353	
Leukemia	No	89		65	
	Yes	49		2	
	Unknown	47		13	

Mortality risk (excess relative risk: ERR) of all cause, solid cancer, or leukemia was not different between those reporting rain exposure shortly after the atomic bombing and those reporting no such rain exposure in Hiroshima or Nagasaki based on the interviews conducted in 1949–1961.

Sakata et al., *Radiat Res* 2014

pertained to the magnitude of risk per unit radiation dose for leukemia, total solid cancer, and a variety of solid cancer sites; the shapes of dose-response curves; how cancer risk varies by gender, age at radiation exposure, time since exposure, and age at risk; effect modification—whether radiation effects multiply or only add to disease risks from other risk factors (e.g., smoking); risk of cardiovascular and respiratory disease death from radiation; disease risks among those who received prenatal radiation exposure; and disease risks of the offspring of exposed parents. High-quality cancer-incidence data in Hiroshima and Nagasaki have been published in "Cancer Incidence in Five Continents (CI5)" (by the International Agency for Research on Cancer [IARC]/International Association of Cancer Registries [IACR]), a compilation of worldwide cancer incidence data, and are given the highest rating by that consortium. The data on childhood cancer were also used in the "International Incidence of Childhood Cancer, Volume 3 (IICC-3)" (by IARC/IACR).



Research Scientists of Hiroshima Epidemiology (First row from left) Yukiko Shimizu (Part-time Professional), Eric J. Grant, Kotaro Ozasa, Ritsu Sakata, Hiromi Sugiyama, (Second row from left) Mai Utada, Caitlin M. Milder (Visiting Research Fellow), Ikuno Takahashi (concurrent), Atsuko Sadakane, Ayako Takamori (Visiting Research Fellow)

Research Scientists of Nagasaki Epidemiology (From left) Kotaro Ozasa (concurrent), Midori Soda

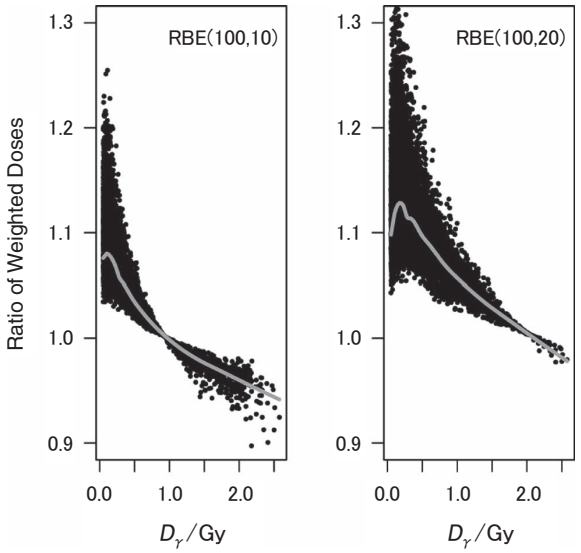
Department of Statistics

The Department of Statistics has historically led in the development of analytical methods for major aspects of the RERF research program of radiation risk estimation, which have also been applied by others to numerous radiation studies of other cohorts. This includes the development of tools and methods to flexibly estimate radiation-associated excess relative and absolute risks of cancers and other adverse health outcomes and to handle various problem areas in the data collected on major RERF cohorts, such as missing data on various covariates, or unknown failures to register incident cancer cases due to undocumented out-migration from cancer tumor registry catchment areas, among many other examples. We also develop or adapt statistical methods to analyze the longitudinal data generated by the Adult Health Study (AHS) and more recently by the F_1 Clinical Study.

We have evaluated or developed special methods of sub-cohort sampling to maximize statistical power when studies cannot measure necessary covariates on the entire cohort, and to address special issues related to variables that modify the radiation risk per unit dose or are intermediate steps on a causal pathway between radiation and a health outcome under study. We also develop many special methods to meet the requirements of RERF's basic-science research in genetics, immunology, radiation biology, and molecular epidemiology, notably applying new methods to analyze their high-dimensional data.

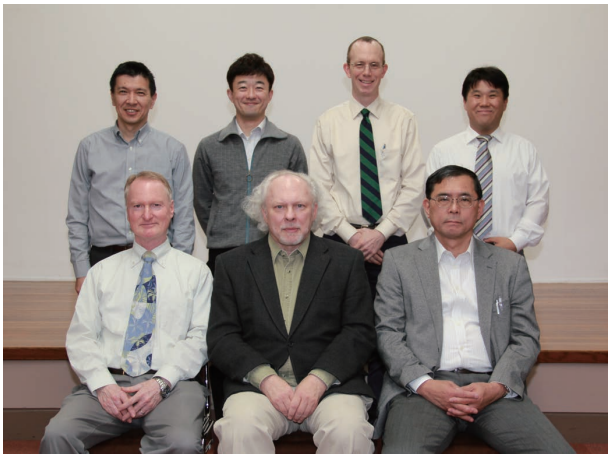
Unlike other departments, we engage in both consulting and research and devote a majority of staff efforts to our consulting role. Given the crucial importance of sound statistical advice in the design, analysis, and conduct of studies, the Department of Statistics seeks to adopt a proactive approach to consulting, beginning with early involvement in study design, and estimation of the statistical power of potential studies. This is critical information in evaluating how effectively a given project will be able to address its proposed scientific question, which in turn impacts its value to the RERF mission. Our second major responsibility is to provide sound statistical analyses of data gathered by investigators in all the research departments.

Work in dosimetry, including investigation into dose uncertainty, is by definition central to the RERF mission of evaluating the effects of ionizing radiation on human health, as such efforts provide individual dose information for measures of dose-response. The department implements RERF dosimetry systems and maintains a database of survivor dose estimates, presently using the DS02 system developed by a combined external and internal scientific working group, and provides key statistical and dosimetric support to RERF projects in biodosimetry. The Statistics Department in collaboration with the Epidemiology Department played a major role in the recent revision of survivor input data, including a much-improved implementation of individuals' terrain shielding, and will organize and lead a new working group of external scientists to provide an improved organ-dosimetry capability for DS02. The department does not undertake dosimetry-related research for its own purposes unless it stands to measurably improve the RERF dosimetry.



The ratio of total weighted doses computed with variable RBEs to the weighted doses obtained with the constant R = 10.
The points represent the values obtained with variable RBEs having a maximum at lowest doses of 100 and values at 1 Gy of 10 (left panel) and 20 (right panel). The gray lines are based on the median neutron doses.

(Cullings et al., *Radiat Res* 2014; 182(6):587–98)



Research Scientists of Statistics (First row from left) John B. Cologne, Harry M. Cullings, Eiji Nakashima, (Second row from left) Kyoji Furukawa, Munechika Misumi, Reid D. Landes, Young Min Kim

Department of Information Technology

Research Scientist of Information Technology, Hiroaki Katayama

The Department of Information Technology (ITD) consists of the Systems Technology Section and the Library and Archives Section, both of which are engaged in support activities for RERF's research.

The Systems Technology Section engages in the maintenance of network and hardware environments, including personal computers, the construction of a variety of databases for analysis (e.g., databases for epidemiological Life Span Study [LSS] research, resource management, Adult Health Study [AHS], F_1 epidemiologic and clinical studies, etc.), and the development of relevant application software. The databases are managed in a technologically advanced manner with the aim of allowing RERF's researchers to effectively and rapidly access essential archives for research and administrative purposes. With the aim of helping research scientists understand those databases with their complex structure, the section has also been involved in such research-support activities as the creation of a data dictionary and a document-management database. Among the section's recent focuses are the prevention of illegal attacks on the RERF network and infections with computer viruses, and the addition of new functions for personal-information management of the data on study subjects at RERF in line with Japan's Personal Information Protection Law.

The Library and Archives Section is composed of the Library Unit and the Archives Unit, with the former handling the procedures for purchase and storage of scientific journals, management and preservation of books, handling requests from RERF's research scientists for copies of papers, and assistance in electronic searches. The Archives Unit is responsible for the storage, indexing, digitizing, and distribution of historical archival material, and the distribution of RERF publications.

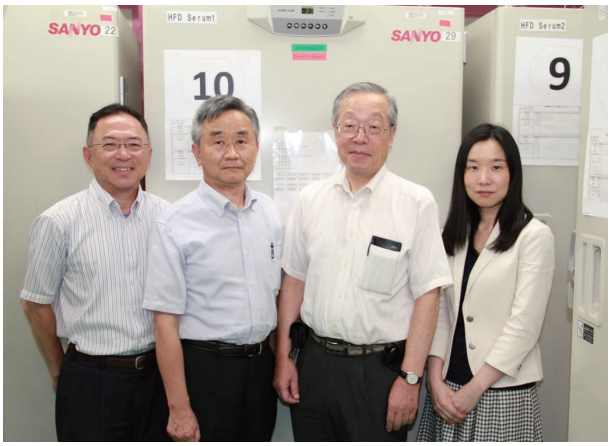
The researcher of ITD participates in a variety of collaborative projects with outside research organizations. Research and collaborative activities are: participation in the World Health Organization (WHO) Radiation Emergency Medical Preparedness and Network (REMPAN); cooperative technical expertise for Hiroshima University as one of western Japan's tertiary medical institutes for radiation overexposures; creation of an epidemiological research database devoted to the Ministry of Education, Culture, Sports, Science and Technology (MEXT) Grant-in-Aid for Scientific Research project on low-dose radiation effects from nuclear tests conducted in Semipalatinsk, Republic of Kazakhstan by the former Soviet Union; and joining the SEMI-NUC project (prospective cohort study of residents near the Semipalatinsk nuclear test site—feasibility assessment) by the International Agency for Research on Cancer (IARC) as a member of the external advisory board.

Biosample Center

Procedures for storing biosamples and managing biosample information at ABCC-RERF have, until now, been largely dependent on individual research departments. To preserve these precious biosamples, which include human blood, urine, pathological specimens, and teeth, in good condition over the long term, and to promote further research utilizing such samples, it was crucial to centralize their management and create a database for sample information. In April 2013, the Biosample Center (hereinafter referred to as "the Center") was established to undertake this work. With the goal of clarifying radiation effects on disease and on biological and molecular changes among A-bomb survivors and their children, the Center is centralizing sample management, arranging appropriate storage for quality control, and ensuring effective use of this invaluable material, donated by A-bomb survivors, their children, and spouses. To achieve these objectives, biosamples and sample data previously stored in various departments will be moved to the Center, and samples collected in the future will also be handled and stored there with newly manualized preparation methods. In addition, sample information will be stored in an RERF database for centralized management.

The 59 deep freezers and 29 liquid nitrogen tanks used for storage of biosamples are currently installed in Unit G of the Hiroshima Laboratory. Because they had been filled to capacity, securing space for the biosamples became a task of the highest priority. To solve the space issue, we decided to introduce a robotic deep-freezer biorepository system to accommodate and effectively manage future samples, in addition to the 780,000 existing samples. In September 2014, RERF contracted with Brooks Japan K.K. to install the system at the Center, scheduled for completion in September 2015. In FY2014, in preparation for the installation, the floor level of Rooms 106 and 107 on the first floor of Unit G was lowered, and the interior was renovated. At the same time, we held periodic preparatory meetings with contractors to review various issues related to the specifications of the robotic biorepository system. We also conducted frequent liaison meetings to establish an internal storage system tailored to storage tubes with two-dimensional barcodes designed for the robotic biorepository system.

In preparation for transition of biosample storage operations to the Center, we also improved the storage environment, including purchase of safety cabinets and standardization of sample preparation methods. Remodeling of Unit G is planned for next fiscal year (FY2015) to create a preparation room, laboratory, office, and storage repository to facilitate full operation of the Center. Completion of the work is scheduled for summer 2015.



Research Scientists of Biosample Center (From left) Tomonori Hayashi, Yoshiaki Kodama, Kazunori Kodama, Misa Imaizumi

Radioisotope Facility

The Radioisotope (RI) Facility is a laboratory where the radiation effects for either germ cells or somatic cells are examined by using unsealed radioisotopes. Use of seven types of radioisotopes, including ^{32}P, ^{3}H, ^{51}Cr, and ^{125}I, is permitted. Recently, ^{32}P has been most frequently used for DNA analyses.

All of RERF's research departments share the RI facility. Three persons from the Department of Genetics and nine from the Department of Radiobiology/Molecular Epidemiology hold the appropriate registration for authorized use of the RI facility, and these registered staff receive education and training once a year and health examinations twice a year. Two persons are facility administrators responsible for safe conduct of experiments.

Legal regulations concerning the facility's safe management are strictly followed. Upon receipt of notices from the Nuclear Regulation Authority, Japan's official supervisory authority for such laboratories, we inspect our facility for any inadequacies and make efforts to improve our operations accordingly.

2470 Wizard^2 Gamma Counter
3.0 inch well-type NaI(Tl) gamma counter. This device provides improved counting efficiency, extended energy range, and better energy resolutions for gamma emitting radionuclides such as ^{125}I and ^{51}Cr. It is used for protein labelling in biomedical research and also for monitoring of environmental contamination.

Staff of Radioisotope Facility (From left) Yuji Yoneyama, Asao Noda