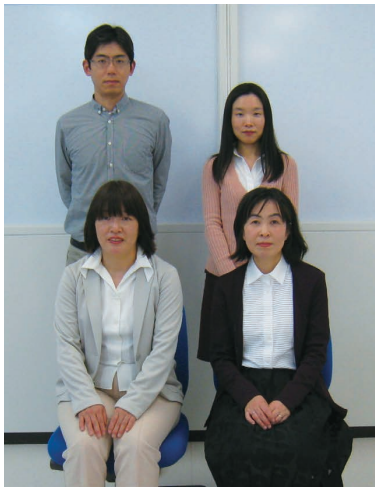
Research Departments

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 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 risk 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 were obtained through the health examinations.



Research Scientists of Nagasaki Clinical Studies (First row from left) Nobuko Sera, Ayumi Hida, (Second row from left) Daisuke Haruta, Misa Imaizumi

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

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) 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 is still ahead, so we converted the sample to a cohort for prospective follow-up and are now partway through the first round of examinations of a longitudinal study.

An automated biochemical analyzer was introduced to the Division of Clinical Laboratories, Department of Clinical Studies, Nagasaki. With this equipment, enzymes related to liver and kidney function, as well as glucose, lipids, and so on, are measured simultaneously. Because this new machine requires smaller amounts of reagents than previous units, operating costs are less expensive. When there is a problem, the equipment is better equipped to search for the cause. Furthermore, because there is a representative office in Nagasaki, maintenance and repairs can be done more quickly.

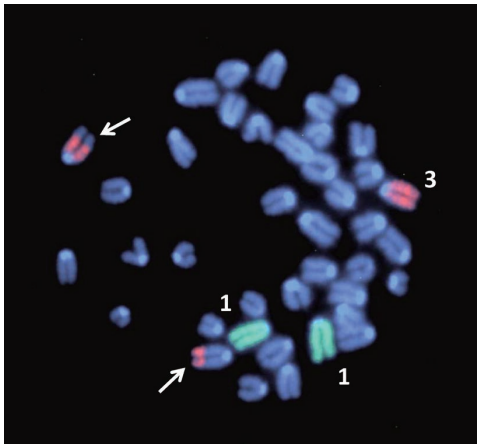
Department of Genetics

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 somatic mutations and biodosimetry, while the Biochemical Genetics Laboratory focuses on heritable mutations in the F_1 generation.

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 and is also studying genetic factors in breast and skin cancers, conducting a cytogenetic study on *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 collecting blood samples and establishing Epstein-Barr virus (EBV)-transformed cell lines from members of survivor families (mother, father, and offspring) for molecular studies. We also are developing competence in more advanced DNA-based assays. Several past 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, high-density microarray comparative genomic hybridization (CGH) methods using over one million probes have been 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 genetic studies using next-generation sequencing technology that will provide the capability to detect smaller mutations.

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



Translocation frequencies were investigated by fluorescent *in situ* hybridization (FISH) in mouse thyroid cells following fetal X-ray irradiation. Chromosomes 1 and 3 were stained with FITC (green) and Cy3 (red), respectively. All other chromosomes were counterstained with DAPI (blue). Translocations are shown as bi-color chromosomes (arrows). Introduction of the FISH method to mouse chromosomal research has allowed us to quickly and accurately examine the translocation frequency in tissues such as the thyroid from which it is difficult to obtain a large number of metaphases in primary culture.

Department of Radiobiology/Molecular Epidemiology

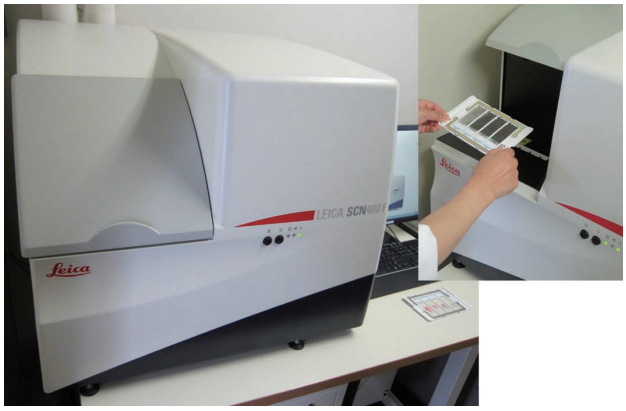
The Department of Radiobiology/Molecular Epidemiology focuses on two research themes: 1) Radiation and Immunologic Effects; and 2) Radiation and Cancer. Immunologic effects are investigated with two approaches: immunobiology and immunogenome studies, which primarily examine profiles of immune cell numbers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

The immunobiology studies hypothesize that radiation accelerates immune attenuation, partially resulting in 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) that are potentially involved. The effects of past radiation exposure on influenza vaccine responses are being evaluated as a direct health-related outcome. We also are developing an integrated scoring system for evaluating immunological/inflammatory status of A-bomb survivors to provide the key to understand the role of immunocompetence in radiation-disease associations.

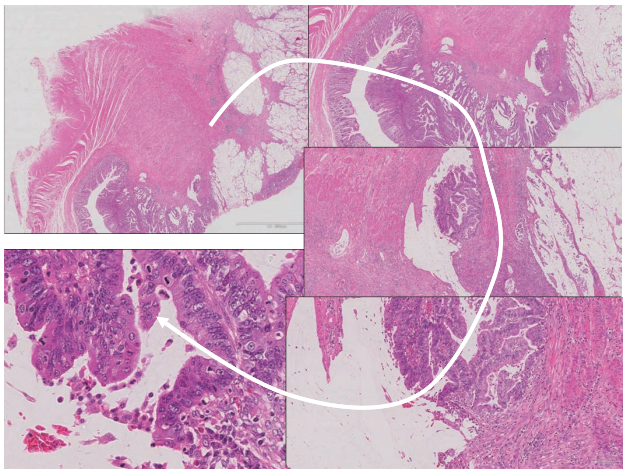
The immunogenome studies evaluate the genetic basis for inter-individual differences in immune functions and the impact of genetics on susceptibility to radiation-associated diseases. The results obtained from phenotype-genotype association analyses utilizing cumulative immunology data will have the potential to contribute to the individualized prevention 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 among A-bomb survivors. Toward this end, we have been analyzing early molecular events in the development of thyroid, colorectal, and lung cancers in the Life Span Study (LSS) cohort. We are also beginning to evaluate epigenetic alterations (i.e., modification of genetic functions by means other than through changing the DNA) in normal blood cell subsets from the Adult Health Study (AHS) subjects, based on the hypothesis that radiation might cause epigenetic changes in, for example, DNA transcription or methylation that lead to increased risks of selected diseases.

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



Using a virtual slide system (Leica), which was introduced to the Department of Radiobiology/Molecular Epidemiology this fiscal year, high-quality images of tissue specimens can be analyzed on a PC monitor through high-speed scanning of stained specimens on glass slides. In this system, a single scanning of one slide can replicate a number of ×1– ×400 magnitude images with high resolution and contrast.



These images indicate that we can observe a colon cancer specimen with sequential ×1– ×400 magnification (clockwise). This system facilitates detailed and rapid histological analyses of pathological specimens obtained from A-bomb survivors.

Departments of Epidemiology, Hiroshima and Nagasaki

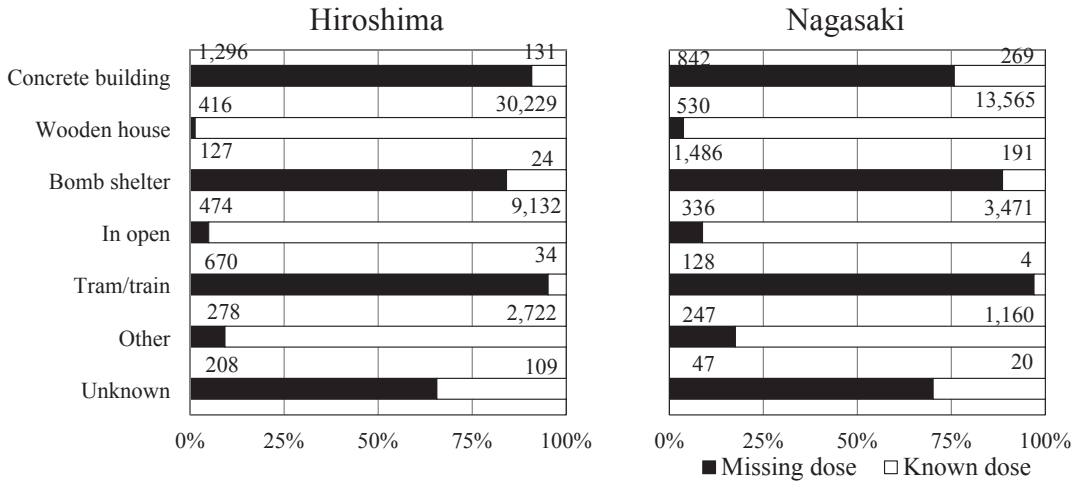
RERF aims to characterize and quantify the late health effects of radiation, based on data of the atomic bomb survivors. The Department of Epidemiology's follow-up of the Life Span Study (LSS) of survivors, the *in utero* cohort, and the F_1 cohort is crucial to accomplish these purposes. Follow-up outcomes 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 2009, including 82% of those who were less than 10 years old at the time of bombing (ATB). Moreover, 87% 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 those results are heavily relied upon for the creation of numerous radiation-risk reports,

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

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

Proportions and counts of proximal (<3 km) Life Span Study cohort members with known and unknown DS02 doses by the 1949 Radiation Census responses



The proportions of subjects with missing doses are large for those who were in "concrete buildings," "bomb shelters," and "Tram/Train" due to heavy shielding. The proportions with missing doses are small among those exposed in wooden houses or "in open" where shielding parameters were more homogeneous and could be estimated. These data show that more than 99% of proximal LSS cohort members had data documenting their location and general shielding prior to the start date of October 1, 1950, and that unknown doses are not primarily due to mortality of proximal survivors in the years 1950–1963 when more sophisticated questionnaires were in progress.
(Ozasa K, Grant EJ, et al., *Am J Epidemiol* 2013; 177:569–73)

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 pertained to the magnitude of risk per unit radiation dose for leukemia, total solid cancer, and a variety of solid 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 exposure; and disease risks of the offspring of exposed parents.

High-quality cancer-incidence data in Hiroshima and Nagasaki have also been contributing to the "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).

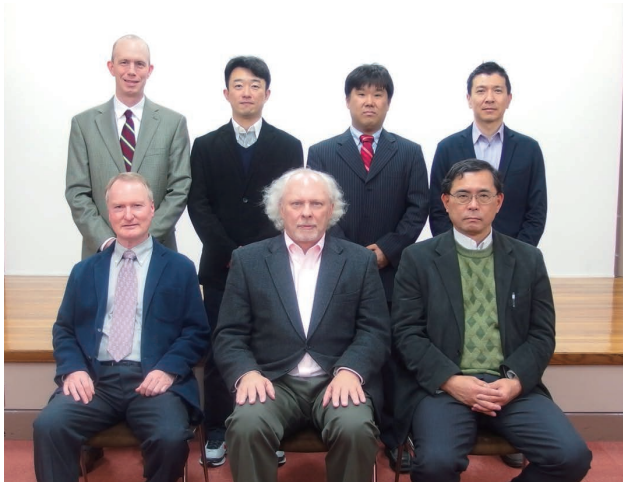
Department of Statistics

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 additive risk and to handle various problem areas in the longitudinal data of major RERF cohorts, such as missing data on various covariates, or failure to register incident cancer cases due to undocumented out-migration from cancer tumor registry catchment areas, among many other examples.

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.

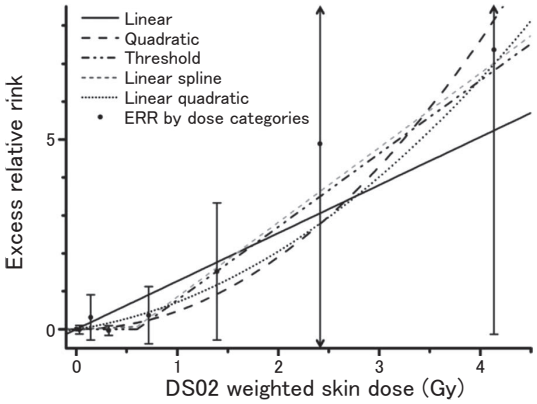
Unlike other departments, we engage in both consulting and research and devote a majority of staff efforts to our consulting role. Given the crucial importance of sound statistical advice in the design, analysis, and conduct of studies, the Department of Statistics seeks to adopt a proactive approach 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,



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

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 department does not undertake dosimetry-related research for its own purposes unless it stands to measurably improve the RERF dosimetry.



Basal cell carcinoma radiation dose-response curves for various excess relative models; Atomic bomb survivors (Japan) diagnosed between 1958 and 1996. The excess relative models include variables; gender, period at diagnosis, and log age 70 as the background parameters, and age at the time of bombing as the effect modifier. The 95% Wald confidence bounds were also estimated.
(Sugiyama H et al., *Radiat Res* 2014; 181(5):531–9)

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.

ITD participates in a variety of collaborative projects with outside research organizations. For example, the department is providing technical expertise to the Osaka Medical Center for Cancer and Cardiovascular Diseases in constructing a comprehensive cancer control system in Osaka prefecture. Among ITD's 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

Storage procedures and information management regarding biological samples stored at ABCC-RERF such as human blood, urine, pathological specimens, and teeth have been largely dependent until now on each research department. To preserve the precious biosamples in good condition over the long term and to further promote research utilizing such samples, centralized management of the biosamples and establishment of a database containing the sample data was crucial. In April 2013, the Biosample Center (hereinafter referred to as "the Center") was established to undertake such work. With the objective of contributing to the clarification of radiation effects on disease and biological and molecular changes among A-bomb survivors and their children, the Center is working toward centralized management, appropriate storage for quality control, and effective use of the invaluable biosamples donated by A-bomb survivors and their children (and spouses). To achieve these objectives, the biosamples and sample data previously stored at each department will be moved to the Center, and samples to be collected in the future will be handled and stored there in accordance with new procedural standards. In addition, sample data will be stored in an RERF database for centralized management of that information.

Since the 59 deep freezers and 26 liquid nitrogen tanks currently installed in Unit G of the Hiroshima Laboratory are anticipated to reach their capacity by the end of fiscal year 2013, securing space for the biosamples is a task of the highest priority. Partial renovation and electrical and piping work in Unit G were completed by the end of March 2014 before the end of the first fiscal year of the Center's operations. As a fundamental solution to the space issue, introduction of a robotic deep-freezer biorepository system is planned to accommodate, as well as effectively arrange and manage, samples to be collected in the future in addition to the existing 710,000 samples. Efforts toward arrangement of the Center include compilation of a database of sample management information and installation of a new clean bench used for the preparation process for sample storage. Moreover, a standardized manual for biosample quality control has been prepared for the Hiroshima and Nagasaki laboratories and standardized procedures for such work as blood sample collection and dispensation have been established. To respond to disasters and other contingencies, a temperature monitoring and notification system has been introduced in all the deep freezers with biosamples, and the backup power supply system has been enhanced. In addition, efforts are being made to store the samples in duplicate in both Hiroshima and Nagasaki as a risk management measure. Remodeling of the first floor of Unit G is planned for next fiscal year (FY2014) to create a preparatory room, laboratory, office, and storage repository with the aim of achieving full operations of the Center.

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

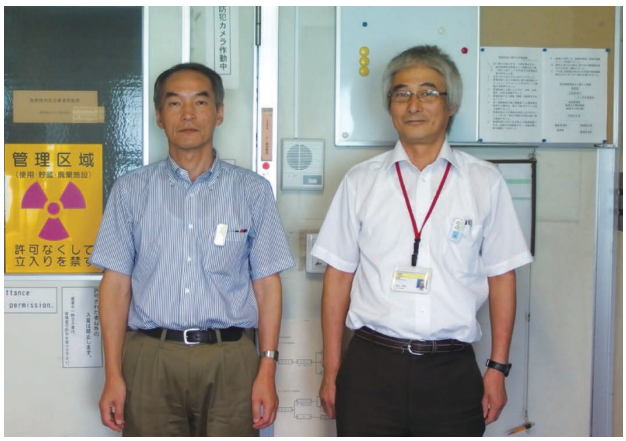
Radioisotope Facility

The Radioisotope (RI) Facility is a laboratory where the radiation effects for either germ cells or somatic cells are examined by using liquid 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. Five persons from the Department of Genetics and ten 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.

This liquid scintillation counter (LSC-7200, Hitachi-Aloka Medical) is used for measuring radioactivity when performing metabolic tracer and uptake experiments of beta-emitting nuclide-labelled compounds into cells and genes. Measurement can be made effectively by mixing samples with a scintillator. The device is effective in measuring low-energy beta-emitting nuclides or low-level radioactivity for routine contamination tests in the RI Facility (radioactivity monitoring), among other times.



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