# BIOSAMPLE RESEARCH CENTER

## A. Departmental Overview

### Mission and Specific Objectives

The mission of the Biosample Research Center (BRC) is to achieve centralized management at RERF of the human biosamples provided by A-bomb survivors and their children, with five major objectives, as follows:

1. To process and store blood and urine samples newly collected by the Department of Clinical Studies at the Hiroshima and Nagasaki Laboratories.
2. To preserve both newly collected biosamples and archived samples previously collected and preserved by other research departments, such as frozen blood and urine samples and pathological specimens, under optimal conditions.
3. To manage inventories of both newly collected and archived biosamples by creating and updating a comprehensive biosample database.
4. To make the biosample database available to research scientists to facilitate internal and external collaborative research, and to provide biosamples upon request in a timely and efficient manner.
5. To conduct quality assessment and quality control of biosamples by using state-of-the-art analytical technologies and expertise, and to conduct and facilitate internal and external collaborative research using such technologies for "21st century science."

Research projects conducted or facilitated by the BRC are prioritized for effective and productive use of the human biosamples at RERF to identify late medical effects of ionizing radiation in A-bomb survivors and their children, to elucidate molecular mechanisms of the effects, and to develop biomarkers for past radiation exposure and related disease risks, all of which further the mission of RERF.

### Department Resources

As of Nov. 30th 2023, the BRC holds a total of about 2,225,000 tubes of biosamples (1,442,000 tubes stored at the Hiroshima Laboratory, hereinafter referred to as "H," and 783,000 tubes stored in at the Nagasaki Laboratory, hereinafter referred to as "N"), comprising about 1,918,000 tubes of blood samples and about 298,000 tubes of urine samples, including both archival samples and samples processed and stored by the BRC, and 8,149 tubes of DNA samples. These samples were provided by 16,812 AHS participants (H: 11,266, N: 5,546) during a total of about 151,000 visits (H: 94,000, N: 56,000) since 1969, by 12,787 FOCS participants (H: 8,522, N: 4,265) during a total of about 43,000 visits (H: 29,000, N: 14,000) since 2002, and by 4,140 "trio" study subjects (H: 2,224, N: 1,916) since 1985. See Summary Tables (p5) for all the samples managed by the BRC, and samples processed and stored by the BRC this year (Dec. 1, 2022 - Nov. 30, 2023).

The major instrumental resources of the BRC include a quadrupole time-of-flight mass spectrometer (QTOF-MS), TripleTOF 6600+® (SCIEX) used for targeted and non-targeted proteomics and potentially for metabolomics, and an automated high-throughput electrophoresis platform, 4200 TapeStation System® (Agilent Technologies), used for quantitative and size analysis of DNA and RNA samples.

## Internal and External Collaboration

RERF: Department of Clinical Studies, Department of Molecular Biosciences, Department of Information Technology, and Department of Epidemiology

External: RERF projects involving biosamples are often conducted in collaboration with external institutions; these projects are described by the various Departments.

## B. Department Highlights

### FY2023 Departmental Highlights:

#### *1) Biosample Storage and Inventory Management*

- During the past year (Dec. 1st 2022-Nov. 30th 2023), the BRC processed and stored new blood and urine samples from 841 AHS participants (H: 547, N: 294) and 2,532 FOCS participants (H: 1,807, N: 725). A total of 69,287 tubes of blood samples were processed and stored; 49,009 tubes were processed by the Hiroshima BRC, of which 14,340 were transported to Nagasaki for remote backup storage, and 20,278 blood sample tubes were processed by the Nagasaki BRC, of which 6,051 were transported to Hiroshima. The BRC also processed and stored 26,480 tubes of urine samples (H: 18,526, N: 7,954). See Summary Tables (p5) for samples processed and stored by the BRC this year (Dec. 1, 2022 - Nov. 30, 2023), and all the samples managed by the BRC.
- In total, as of Nov. 30th 2023, the BRC holds, about 2,225,000 tubes of samples (stored in H: 1,442,000, in N: 783,000), comprising about 1,918,000 tubes of blood samples (stored in H: 1,240,000, in N: 678,000) and about 298,000 tubes of urine samples (stored in H: 194,000, N: 104,000), including both archival samples and samples processed by the BRC, and 8,149 tubes of DNA samples (stored in H). These samples were provided by 16,812 AHS subjects (H: 11,266, N: 5,546) during a total of about 151,000 visits (H: 94,000, N: 56,000) since 1969, by 12,787 FOCS subjects (H: 8,522, N: 4,265) during a total of about 43,000 visits (H: 29,000, N: 14,000) since 2002, and by 4,140 Trio study subjects (H: 2,224, N: 1,916) since 1985.
- From Jul. 2015 through Nov. 2023, the BRC processed and stored about 550,000 tubes of blood samples (H: 361,000, N: 189,000). Of these blood samples, about 254,000 tubes (H: 155,000, N: 99,000) were provided by 3,609 AHS participants (H: 2,211, N: 1,398) over 9,944 visits (H: 6,057, N: 3,887), while about 297,000 tubes of blood samples (H: 206,000, N: 90,000) were provided by 9,952 FOCS participants (H: 6,836, N: 3,116) over 19,026 visits (H: 13,064, N: 5,962). The BRC has also processed and stored about 141,000 tubes of urine samples (H: 93,000, and at N: 47,000) provided by the AHS and FOCS participants during the same period.
- In preparation for the upcoming relocation of the Hiroshima Laboratory, the BRC has started to retrieve tubes from a robotic deep-freezer biorepository system, BioStore II® (Azenta), at the Hiroshima Lab as RERF has decided to discontinue use of the BioStore II after the relocation. These tubes will be moved to 23 conventional upright freezers. Previously, from 2017 to 2021, the Hiroshima BRC transferred about 592,000 tubes of archival blood and urine samples from conventional upright freezers to the BioStore II after inventorying all transferred samples. The archival sample tubes now occupy 55% of the BioStore II's storage capacity. In addition, about 322,000 tubes of blood and urine samples processed and stored by the BRC since 2015 had also been housed in the BioStore II, occupying up to 8% of its capacity by Mar. 2023.
- In 2020, the BRC designed, optimized, and implemented a laboratory information management system (LIMS), LabVantage® (LabVantage Solutions), to manage biosample workflows such as biosample receipt, processing, storage, and transport; manage biosample inventories and quality information; manage reagent and consumable inventories; and create a comprehensive biosample database. Since 2021, the BRC has

further adapted the LIMS to manage workflows and information related to the provision of biosamples for research use (see below). In addition, the BRC is now customizing the LIMS to manage the inventory of all samples currently stored in the BioStore II, which will be transferred to 23 conventional upright freezers prior to the relocation of the Hiroshima Lab.

The BRC also maintains and manages the following archival samples that have been or will be transferred to the BRC:

- Blood cells donated by Trio Study subjects for genetic studies of transgenerational effects of parental radiation exposure have been transferred to the BRC for centralized management and efficient research use and will be used for the Trio Study beginning in 2024. Historically, these samples have been preserved since 1985 by the Department of Molecular Biosciences (MBS) in liquid nitrogen dewars and freezers. In 2020, the BRC and MBS jointly inventoried about 59,000 tubes of blood samples (about 11,100 tubes of fresh frozen mononuclear cells, about 41,400 tubes of EBV-transformed lymphocytes, and about 6,400 tubes of granulocytes) provided by 4,140 individuals, including 1,004 complete sets of trios comprising AHS subjects, their spouses, and 1,653 children who are $F_1$ study subjects.
- Since 2014, the BRC has inventoried and acquired archival blood and urine samples of AHS and FOCS participants collected and stored by the Department of Clinical Studies and MBS. Currently, the BRC holds archival blood samples of about 1,368,000 tubes (stored at H: 933,000 and at N: 435,000) and archival urine samples of about 157,000 tubes (stored at H: 100,000 and at N: 57,000). These samples were provided by 16,809 AHS subjects (H: 11,264, N: 5,545) during a total of about 141,000 visits (H: 88,000, N: 52,000) since 1969 and by 12,748 FOCS subjects (H: 8,517, N: 4,231) during a total of about 23,700 visits (H: 16,000, N: 7,700) since 2002.
- The BRC also extracted DNA from 8,149 archival blood clot samples provided by 6,127 AHS subjects (H: 3,800, N: 2,327) from 2003 to 2013 using an automated DNA extractor, MagCore® (RBC Bioscience) and maintains the DNA samples.
- To enable effective research use, the BRC has been assisting the pathology laboratory at the Hiroshima Lab with the inventory of archival formalin-fixed paraffin-embedded (FFPE) blocks and glass slides of pathological tissue specimens. The pathology laboratories of the Department of Epidemiology at the Hiroshima and Nagasaki Labs preserve pathological tissue specimens from autopsies of about 8,400 LSS subjects and from surgical procedures of about 12,000 LSS subjects. Inventory of the FFPE blocks is now near completion, and inventory of glass slides is underway. For inventory purposes, the FFPE blocks were indexed, organized, and packaged, and the numbers of available samples and organs of origin were recorded in a database. Similarly, the glass slides are identified, counted, linked to the FFPE blocks, and recorded in the database.

## Summary Tables

**Table 1.** Samples processed and stored by the BRC this year (Dec. 1, 2022 - Nov. 30, 2023)

| Cohort | Laboratory | Partici-pants | Number of Tubes | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Blood | | | | Urine | Grand Total |
| | | | Serum | Plasma | Clot/Cells | Total | | |
| AHS | Hiroshima | 547 | 4,414 | 5,496 | 5,733 | 15,643 | 4,267 | 19,910 |
| | Nagasaki | 294 | 2,132 | 3,145 | 2,861 | 8,138 | 2,240 | 10,378 |
| | Total | 841 | 6,546 | 8,641 | 8,594 | 23,781 | 6,507 | 30,288 |
| FOCS | Hiroshima | 1,807 | 14,117 | 7,039 | 12,210 | 33,366 | 14,259 | 47,625 |
| | Nagasaki | 725 | 5,033 | 2,856 | 4,251 | 12,140 | 5,714 | 17,854 |
| | Total | 2,532 | 19,150 | 9,895 | 16,461 | 45,506 | 19,973 | 65,479 |
| Grand Total | | 3,373 | 25,696 | 18,536 | 25,055 | 69,287 | 26,480 | 95,767 |

**Table 2.** All the samples managed by the BRC (as of Nov. 30, 2023)

| Cohort (Start Year) | Laboratory | Partici-pants | Visits | Number of Tubes | | | |
|---|---|---|---|---|---|---|---|
| | | | | Blood | DNA | Urine | Total |
| AHS (1969) | Hiroshima | 11,266 | 94,461 | 780,912 | 5,822 | 85,400 | 872,134 |
| | Nagasaki | 5,546 | 56,211 | 410,325 | 2,327 | 54,528 | 467,180 |
| | Total | 16,812 | 150,672 | 1,191,237 | 8,149 | 139,928 | 1,339,314 |
| FOCS (2002) | Hiroshima | 8,522 | 28,989 | 461,311 | - | 108,513 | 569,824 |
| | Nagasaki | 4,265 | 13,692 | 206,793 | - | 49,856 | 256,649 |
| | Total | 12,787 | 42,681 | 668,104 | - | 158,369 | 826,473 |
| Trio Study (1985) | Hiroshima | 2,224 | 2,706 | 31,602 | - | - | 31,602 |
| | Nagasaki | 1,916 | 2,335 | 27,377 | - | - | 27,377 |
| | Total | 4,140 | 5,041 | 58,979 | - | - | 58,979 |
| Grand Total | | | | 1,918,320 | 8,149 | 298,297 | 2,224,766 |

### *2) Biosample Provision for Research Use*

- In 2022, the BRC provided blood samples from AHS participants for the research proposal, RP-P2-22, entitled "Preliminary study to determine the applicability for GWAS of DNA extractable blood smears and blood-infiltrated paper discs preserved in the past" (by T. Hayashi at RERF, *et al.*), using the procedures and forms described below. These samples are blood-infiltrated paper discs stored at –80°C for approximately 20 years, provided by 12 AHS participants who re-consented to this study, and will be tested for their applicability to array-based single-nucleotide polymorphism (SNP) typing.
- In 2021, the BRC finalized and implemented detailed sample provision procedures and sample request forms. Since then, in accordance with the procedures and forms, the BRC has adapted and customized the LIMS to manage workflows and information related to the provision of biosamples for research use.
- In 2019, for the first time, the BRC provided blood samples for the research proposal,

RP01-17, entitled "Detection of the onset of hematologic malignancy among atomic bomb survivors" (by Y. Miyazaki at Nagasaki University and M. Imaizumi at RERF, *et al.*). These samples are blood mononuclear cells provided by four AHS participants and processed and stored in liquid nitrogen by the BRC, and are now used to elucidate the clonal dynamics before and after diagnosis of myelodysplastic syndrome by genomic analysis of serial blood samples, and to investigate how clonal dynamics differ by radiation dose.

***3) Biosample Analysis for Quality Control and Future Research***

The quality of stored biosamples is critical to ensure the accuracy and validity of results obtained from any analytical method, including "omics" analyses such as DNA sequencing (whole genome, exome, or targeted sequencing), RNA expression/sequencing analysis, proteomics, and metabolomics. To use preserved blood samples for such analyses with high accuracy, it is necessary to establish standard operating procedures (SOPs) for sample quality assessment to systematically verify the qualitative and quantitative changes in various biomolecules (such as DNA, RNA, proteins, and metabolites) and in various cell types, which are associated with sample processing and storage. Based on such SOPs for quality assessment, it is necessary to establish quality control standards for processing and preserving samples under optimal conditions and to thereby improve SOPs for sample processing and storage.

- Since 2020, the BRC has been developing methods to assess the quality of blood plasma and serum using the quadrupole time-of-f light mass spectrometer (QTOF-MS), TripleTOF 6600+. In 2022, by global non-targeted quantification of enzymatically digested peptides of plasma proteins using hte QTOF-MS, we identified several candidate peptides that could serve as quality markers for past freezing and thawing events. In 2023, also using the QTOF-MS, we developed a protocol to differentially quantify the reduced (SH-Alb) and oxidized (S-Cys-Alb) albumins to determine the ΔS-Cys-Alb value, which represents the oxidizability of albumin, as a quality marker of blood plasma and serum.
- In 2023, using afluorescence-activated cell sorter (FACS), we developed a protocol to identify and isolate human hematopoietic stem/progenitor cells (HSPCs) rfom cryopreserved peripheral blood mononuclear cells (PBMCs). The isolated HSPCs were shown to have both erythroid and myeloid differentiation potential by colony forming assay . These findings may expand the utility of numeroustubes of PBMCs stored in liquid nitrogen dewars at RERF for functional and molecularanalyses of human HSPCs.

**Relevance to the RERF Strategic Goals**

The BRC is central to RERF's strategic goals to use the valuable cohort data and samples to answer key scientific questions using cutting-edge technologies.

- The BRC contributes to the two major RERF clinical cohorts, AHS and FOCS, by processing and storing blood and urine samples provided by study participants with the goal of elucidating radiation effects on the biomedical and health conditions of A-bomb survivors and their children.
- The BRC strives to preserve these valuable samples under optimal conditions based on

sample quality assessment to ensure the applicability of cutting-edge analytical tools to these samples. To accomplish this, the BRC must be equipped with or have routine access to instrumentation to identify genomic, epigenomic, transcriptomic, proteomic, metabolomic, and immunologic features in blood and other biosamples that are caused by radiation exposure. The BRC's expertise in the use of such instrumentation will be expanded and made available to a wider range of RERF investigators to form a collaborative continuum that strengthens RERF research.

- The BRC uses the LIMS to manage workflows and biosample inventories to create and update a comprehensive biosample database, which will be linked with clinical and epidemiological information by the Research Resource Section (RRS) to create an integrated RERF research database. With such a database, researchers will be able to easily locate biosamples available for their research and analyze all relevant data on radiation doses, health effects, and molecular and cellular measurements of biosamples.