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 and spouses, 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 departments, such as frozen blood and urine samples and pathological tissue samples, 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."

The research projects conducted or facilitated by the BRC are prioritized to ensure the effective and productive utilization of human biosamples at RERF. These projects aim to identify the late medical effects of ionizing radiation in A-bomb survivors and their children, elucidate the molecular mechanisms underlying these effects, and develop biomarkers for past radiation exposure, disease risks, and disease progression. These efforts are integral to advancing RERF's mission. The BRC plays a central role in achieving RERF's strategic goals, as outlined below:

Continue: 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.

Create: 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.

Collaborate: 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.

Collate: The BRC uses a laboratory information management system (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 locate biosamples available for their research and analyze all relevant data, including radiation doses, health effects, and molecular and cellular measurements of biosamples.

Department Resources

As of November 30, 2024, the BRC held a total of about 2,310,000 tubes of biosamples (1,493,000 tubes stored at the Hiroshima Laboratory, hereinafter referred to as "H," and 817,000 tubes stored in at the Nagasaki Laboratory, hereinafter referred to as "N"), comprising about 1,979,000 tubes of blood samples and about 322,000 tubes of urine samples, including both archival samples and samples processed and stored by the BRC, and 8,149 tubes of DNA samples. Those samples were provided by 16,812 AHS participants (H: 11,266, N: 5,546) during a total of about 151,000 visits (H: 95,000, N: 56,000) since 1969, by 12,634 FOCS participants (H: 8,522, N: 4,112) during a total of about 45,000 visits (H: 30,000, N: 14,000) since 2002, and by 4,140 Trio Study subjects (H: 2,224, N: 1,916) since 1985. See Summary Tables (p7) for all the samples managed by the BRC, and samples processed and stored by the BRC this year (Dec. 1, 2023 – Nov. 30, 2024).

The BRC's major instrumental resources include a quadrupole time-of-flight mass spectrometer (QTOF-MS), and the TripleTOF 6600+® (SCIEX), which is utilized for both targeted and non-targeted proteomics, as well as potentially for metabolomics. Additionally, the center employs an automated high-throughput electrophoresis platform, the 4200 TapeStation System® (Agilent Technologies), for the quantitative and size analysis of DNA and RNA.

Internal and External Collaboration

RERF: Departments of Clinical Studies, Molecular Biosciences, Information Technology, Epidemiology, and Statistics.
External: RERF projects involving biosamples are frequently conducted in collaboration with external institutions, with details of these projects provided by other departments.

Summary of Response to 2024 SAC Recommendations

Recommendation: Are there duplicate samples at two facilities? What is the backup plan for these samples since they are so valuable?

Response: Since 2015, half of the aliquots of all newly collected serum and plasma samples have been transported to the other city (*i.e.*, from Hiroshima to Nagasaki and vice versa) for backup storage of duplicate samples. Even before 2015, some blood serum (since 1987) and plasma (since 1990) samples from AHS and FOCS examinees were stored in the other city for backup. Overall, 33% and 22% of serum and plasma samples, respectively, and 5% of AHS blood cell samples are stored in the other city for backup.

Recommendation: The group might want to consider miRNA studies since miRNAs are known to remain intact even in formalin-fixed paraffin-embedded (FFPE) tissues.

Response: We agree to consider miRNAs as molecules of interest for quality assessment of various biosamples, especially pathological tissue samples due to the long-term stability of miRNAs in FFPE samples, for future mechanistic or biomarker studies on radiation effects. We know that cell-free miRNAs in blood and urine have been shown in many previous studies to be useful biomarkers to evaluate physiological conditions and to detect or monitor various preclinical and clinical disease states such as cancer and cardiovascular disease. Therefore, in addition to our current analysis of proteins, DNA and blood cells, we would like to perform miRNA analysis for quality assessment of such biosamples, if the BRC were

able to employ additional research scientists.

Recommendation: How has assessment of sample quality been done in the past? Can standardized operation protocols (SOPs) be developed that rely on past information? Who will develop the SOPs and how will they be checked for accuracy by the group? A plan for updating these SOPs is also needed.

Response: The research scientists and technical staff at the BRC have developed SOPs for sample quality assessment. These SOPs will be regularly reviewed and updated to ensure accuracy, based on established guidelines, scientific literature, sample quality assessment results, and input or requests from researchers. We appreciate the suggestion to develop SOPs based on past information. In the past, investigators often conducted pilot studies to assess sample quality using a small portion of samples prior to full-scale studies. For example, in preparation for SNP array analysis using aged blood smears, a pilot study is being conducted to assess the quality of DNA samples extracted from blood smears and amplified *in vitro* (RP-P2-22 by Hayashi T). We will plan to develop SOPs for sample quality assessment based on such previous studies, as suggested by the SAC.

Recommendation: It is noted that to prepare for the development of a Research Resource Center the samples will be catalogued. How are they currently catalogued? Why is the movement to the Research Resource Center (RRC) important for the work and its progression?

Response: Blood and urine samples from AHS, FOCS, and the Trio Study subjects are currently catalogued using a biosample database developed in-house by the Information Technology Department of RERF and a LIMS optimized and operated by the BRC. A comprehensive biosample database will be generated by the LIMS and made available to the RRC in the future for integration with radiation dose, clinical, and epidemiologic information to promote biosample research.

Recommendation: We find in the write-ups that the robotic freezer will no longer be in use after relocating to the Hiroshima University campus because of financial reasons. For the current Trio Genome Study and future GWAS studies, it is crucial to maximally reduce the risk of human errors. From this point of view, it is recommended that RERF should build up stream-lined automated systems for molecular biology, and the robotic freezers should be kept as a part of the whole robotized.

Response: We are committed to developing streamlined, automated systems, including the implementation of a robotic freezer, to minimize the risk of human error. While the financial situation remains uncertain due to the rising costs associated with constructing the new building for the Hiroshima Laboratory, we have ensured that the building's design can accommodate a robotic freezer. We are currently seeking additional funding to facilitate its installation.

Recommendation: It seems that sharing "lessons learned" from the RERF experience could be useful to the general scientific community. In particular, information on discerning the quality of the paraffin samples would be very useful to the broad community as these are not likely to be selectively useful only for human samples.

Response: We agree with the recommendation to publish the "lessons learned" at RERF in order to share the experience. In fact, detailed meeting proceedings on the mission and activities of the BRC were published this year (*J Hiroshima Med Assoc*). We will initiate

quality assessment of pathological tissue samples when these samples are transferred from the Department of Epidemiology to the BRC after completion of the inventory.

B. Department Highlights

FY2024 Departmental Highlights:

1) Biosample Storage and Inventory Management

Processing and Storage of Blood and Urine Samples

- *This Year*: During the past year (Dec. 1, 2023 – Nov. 30, 2024), the BRC processed and stored new blood and urine samples from 772 AHS participants (H: 524, N: 248) and 2,299 FOCS participants (H: 1,511, N: 788). A total of 61,219 tubes of blood samples were processed and stored; 41,070 tubes were processed by the Hiroshima BRC, of which 11,909 were transported to Nagasaki for remote backup storage, and 20,149 blood sample tubes were processed by the Nagasaki BRC, of which 5,988 were transported to Hiroshima. The BRC also processed and stored 24,025 tubes of urine samples (H: 15,953, N: 8,072). Please refer to the summary tables (p7) for details on the samples processed and stored by the BRC this year, as well as all samples maintained and managed by the BRC.
- *Overall*: In total, as of Nov. 30, 2024, the BRC held about 2,310,000 tubes of samples (stored in H: 1,493,000, in N: 817,000), comprising about 1,979,000 tubes of blood samples (stored in H: 1,275,000, in N: 704,000) and about 322,000 tubes of urine samples (stored in H: 210,000, N: 112,000), 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: 95,000, N: 56,000) since 1969, by 12,634 FOCS subjects (H: 8,522, N: 4,112) during a total of about 45,000 visits (H: 30,000, N: 14,000) since 2002, and by 4,140 Trio Study subjects (H: 2,224, N: 1,916) since 1985. The breakdown of these total samples is as follows:

(1) Samples processed by the BRC since 2015:

From Jul. 2015 through Nov. 2024, the BRC processed and stored about 612,000 tubes of blood samples (H: 402,000, N: 209,000). Of these blood samples, about 275,000 tubes (H: 169,000, N: 106,000) were provided by 3,615 AHS participants (H: 2,215, N: 1,400) over 10,716 visits (H: 6,581, N: 4,135), while about 337,000 tubes of blood samples (H: 233,000, N: 104,000) were provided by 9,999 FOCS participants (H: 6,856, N: 3,143) over 21,325 visits (H: 14,575, N: 6,750). The BRC has also processed and stored about 165,000 tubes of urine samples (H: 109,000, and at N: 56,000) provided by the AHS and FOCS participants during the same period.

(2) Archival samples transferred from other departments:

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 the Department of Molecular Biosciences (MBS). Currently, the BRC holds archival blood samples of about 1,368,000 tubes (stored in H: 933,000 and in N: 435,000) and archival urine samples of about 157,000 tubes (stored in H: 100,000 and in 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 12,595 FOCS subjects (H: 8,517, N: 4,078) during a total of about 23,500 visits (H: 16,000, N: 7,600) since 2002. The BRC extracted DNA from 8,149 archival blood clot samples provided by 6,127 AHS subjects (H: 3,800, N: 2,327) during the period 2003–2013 using an automated DNA extractor, MagCore® (RBC Bioscience), and maintains the DNA samples.

(3) Trio Study samples:

Blood cells donated by Trio Study subjects for genetic studies of the 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 Genome Study, which was initiated in 2024. Historically, post-1985, these samples were collected and preserved by 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 F1 study subjects.

Maintenance and Management of Blood and Urine Samples

- 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 Laboratory. All tubes of blood and urine samples processed and stored by the BRC since 2015 will be removed from the BioStore II and transferred to six conventional upright freezers by Mar. 2025. However, we still have the 592,000 tubes of archival blood and urine samples that were previously moved from conventional upright freezers to the BioStore II from 2017 to 2021, and these archival sample tubes now occupy 55% of the BioStore II's storage capacity. We have applied for funding for a robotic freezer for the new facility, and hope to move as many specimens as possible directly into the new freezer.
- In 2020, the BRC designed, optimized, and implemented a 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 samples currently stored in the BioStore II, which may need to be transferred to conventional upright freezers prior to the relocation of the Hiroshima Laboratory.

Maintenance and Management of Pathological Tissue Samples

- To enable effective research use, the BRC has been assisting the pathology lab at the Hiroshima Laboratory with the inventory of archival FFPE blocks and glass slides of pathological tissue samples. The pathology laboratories of the Department of Epidemiology at the Hiroshima and Nagasaki Laboratories preserve autopsy tissue samples of FFPE blocks from about 5,600 Life Span Study (LSS) subjects and glass slides from about 6,100 LSS subjects, as well as surgical samples of FFPE blocks from about 6,100 LSS subjects and glass slides from about 10,900 LSS subjects.
- Inventory of the FFPE blocks has been completed, and inventory of glass slides is approximately 80% complete. For inventory purposes, the FFPE blocks were indexed, organized, and packaged, and the numbers of available samples and their 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, 2023 – Nov. 30, 2024)

Cohort	Laboratory	Partici-pants	Number of tubes					
			Blood				Urine	Grand total
			Serum	Plasma	Clot/Cells	Total		
AHS	Hiroshima	524	3,849	5,328	5,360	14,537	4,083	18,620
	Nagasaki	248	1,738	2,691	2,412	6,841	1,880	8,721
	Total	772	5,587	8,019	7,772	21,378	5,963	27,341
FOCS	Hiroshima	1,511	10,652	6,162	9,719	26,533	11,870	38,403
	Nagasaki	788	5,363	3,267	4,678	13,308	6,192	19,500
	Total	2,299	16,015	9,429	14,397	39,841	18,062	57,903
Grand total		3,071	21,602	17,448	22,169	61,219	24,025	85,244

Table 2. All the samples managed by the BRC (as of Nov. 30, 2024)

Cohort (start year)	Laboratory	Partici-pants	Visits	Number of tubes			
				Blood	DNA	Urine	Total
AHS (1969)	Hiroshima	11,266	94,973	795,418	5,822	89,483	890,723
	Nagasaki	5,546	56,441	417,129	2,327	56,408	475,864
	Total	16,812	151,414	1,212,547	8,149	145,891	1,366,587
FOCS (2002)	Hiroshima	8,522	30,498	487,843	-	120,381	608,224
	Nagasaki	4,112	14,327	219,949	-	56,048	275,997
	Total	12,634	44,825	707,792	-	176,429	884,221
Trio Study (1985)	Hiroshima	2,224	2,706	31,585	-	-	31,585
	Nagasaki	1,916	2,335	27,359	-	-	27,359
	Total	4,140	5,041	58,944	-	-	58,944
Grand total				1,979,283	8,149	322,320	2,309,752

The average number of blood sample tubes per participant is 72.1, 56.0, and 14.2 for AHS, FOCS, and the Trio Study, respectively. Similarly, the average number of urine sample tubes per participant is 20.6 and 14.1 for AHS and FOCS, respectively. The average number of DNA sample tubes per AHS participant is 1.3.

2) Biosample Provision for Research Use

- In 2024, the BRC began providing blood samples for the Trio Genome Study, RP3-23, titled "Study of the association between parental radiation exposure and occurrence of *de novo* germline mutations in their offspring" (by A. Uchimura at RERF, *et al.*). A total of 35 tubes of blood samples (19 tubes of mononuclear cells and 16 tubes of granulocyte-rich fractions) from 19 study subjects were provided for the study.
- In 2022, the BRC provided blood samples from AHS participants for the research proposal RP-P2-22 titled "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 that have been stored at –80°C for about 20 years, provided by 12 AHS participants who re-consented to this study, and will be tested for their applicability to single-nucleotide polymorphism (SNP) array 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 titled "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 (bulk or single-cell analysis), proteomics, and metabolomics. To use preserved blood samples for such analyses with high accuracy, it is necessary to establish 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 QTOF-MS, TripleTOF 6600+. In 2022–2024, through global non-targeted quantification of enzymatically digested peptides from plasma and serum proteins using the 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 2023–2024, using a fluorescence-activated cell sorter (FACS), we developed a protocol to identify and isolate human hematopoietic stem/progenitor cells (HSPCs) from 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 could expand the utility of numerous tubes of PBMCs stored in liquid nitrogen dewars at RERF for functional and molecular analyses of human HSPCs.