SWAN REPOSITORY
STUDY OF WOMEN'S HEALTH ACROSS THE NATION

MANUAL OF OPERATIONS

Version 032316

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TABLE OF ABBREVIATIONS USED IN THE DOCUMENT

Abbreviation	Meaning
ACD	Acid Citrate Dexrose (anticoagulant)
CC	Coordinating Center
CLASS	Central Laboratory, University of Michigan
CSAP	Concept Sheet and Analysis Plan (SWAN)
dbGaP	Database of Genotypes and Phenotypes (from National Center for Genomes & Phenotypes)
DHS	Daily Hormone Study
DNA	Deoxyribose Nucleic Acid
DTA	Data Transfer Agreement
DUA	Data Use Agreement
EBV	Epstein-Barr Virus
EDTA	Ethylene-diamine Tetracetic Acid (anticoagulant)
EID	Encrypted ID
FMP	Final Menstrual Period
ICPSR	Inter-university Consortium for Political and Social Research
IMS	Inventory Management Services
IRB	Institutional Review Board
ISBER	International Society for Biological & Environmental Repositories
IT	Information Technology
LOU	Letter of Understanding
MRL	Medical Research Laboratories, Highland Heights, KY
MTA	Material Transfer Agreement
MOO	Manual of Operations
NIA	National Institute on Aging
NIH	National Institutes of Health
NINR	National Institute of Nursing Research
ORWH	Office of Research in Women's Health
OTT	Office of Technology Transfer
PBMC	Phosphate-buffered Mononuclear Cells
PD	Project Directors
PHI	Protected Health Information
SAS	Statistical Analysis System
SC	SWAN Steering Committee
SCR	Specimen Collection Record (forms)
SNP	Single Nucleotide Polymorphism
SRAG	SWAN Repository Advisory Group

SRO	SWAN Repository Organization
SWAN	Study of Women's health Across the Nation
UC	University of California
UM	University of Michigan

1.0 INTRODUCTION AND OVERVIEW OF THE SWAN REPOSITORY

1.1 OVERVIEW OF THE SWAN STUDY AND THE SWAN COHORT

The Study of Women's Health Across the Nation (SWAN) is the parent study of the SWAN Repository. SWAN was initiated in 1994, with funding from the National Institute on Aging (NIA) and support from the National Institute of Nursing Research (NINR) and the Office of Research on Women's Health (ORWH), at a time when scientific inquiry about the menopause was in its infancy. SWAN addresses the fundamental question: "What is menopause and how does it affect women's health during and after the menopausal transition?" This national, longitudinal, cohort study of 3302 African-American, Chinese, Hispanic, Japanese, and White women has made major contributions to the scientific understanding of reproductive aging and the changing health profile during midlife as women transition through menopause and into post-menopause. These 3302 women are the sole contributors of all SWAN Repository biospecimens.

The SWAN initiative is comprised of a Coordinating Center (CC), a central laboratory (CLASS), a Repository, and seven clinical sites – Boston, Chicago, Davis (California), Detroit, Los Angeles, New Jersey, and Pittsburgh. In 1996 the SWAN clinics launched baseline examinations of SWAN participants. The 3302 women making up the SWAN longitudinal cohort were initially premenopausal, with at least one ovary intact, and between the ages of 42-52. Over the 14 subsequent follow up visits, SWAN Clinics have gathered vast amounts of information about age-related chronic diseases, such as diabetes, cardiovascular disease, depression, bone loss and osteoporosis, as well as descriptions of the participants' physical and cognitive functioning. The clinics also collected from participants samples of blood (serum and plasma) and urine on an annual basis, plus an additional onetime DNA sample.

SWAN is now in its fifth and final funding cycle, and is seeing the participants for a final follow up visit (Visit 15). SWAN V will complete characterization of the menopausal transition through the late post-menopause and bridge scientific knowledge of women's health from the midlife through the seventh decade (as of 2015, the start of SWAN V, the mean age of SWAN participants is 65 years). Until SWAN, no comprehensive study of sufficient duration had been undertaken among diverse populations to define normal and abnormal hormonal alterations during midlife with links to change in important health outcomes.

1.2 THE SWAN REPOSITORY

1.2.1. The Initialization and Aims of the Repository

Under the leadership of the original Michigan SWAN site PI, MaryFran Sowers, the SWAN Repository was officially established in 2000. The Repository received separate funding from NIA as the biologic specimen arm of the SWAN Study. This funding provided for a dedicated team of administrators, managers, programmers and a housing facility, allowing SWAN investigators and scientific investigators worldwide the opportunity to utilize the extensive collection of biological specimens to address ongoing questions related to the health of women. Aims of the newly-established SWAN Repository were:

- To establish a research resource of serum, plasma, and urine, with accompanying data, for SWAN investigators and other members of the scientific community
- To establish a Repository of DNA
- To establish mechanisms to store and provide access to samples
- To establish logistic arrangements to link data collected in SWAN to Repository specimens
- To develop the mechanisms for timely processing, review and approval (or disapproval) of requests to access Repository materials
- To develop infrastructure and maintenance mechanisms for the Repository that will remain viable for the scientific community upon the completion of the parent SWAN study.

The Repository team has successfully established a valuable and accessible research resource in the SWAN biospecimen collections, along with the mechanisms necessary for opening and marketing the collections to the appropriate scientific communities and for careful consideration of approval and release. The team has also created: helpful online tools to organize and search the vast SWAN phenotypic data which accompanies the samples; online application and review systems; protocols to return Repository-generated data; and cost-recovery mechanisms.

The Repository continues to provide for the maintenance and utilization of the biological specimen collections, and continues to maintain and develop all supportive tools and mechanisms.

1.2.2. Overview of Available Resources

The SWAN Repository resources available to the scientific community are comprised of three SWAN Study collections: a.) SWAN Core samples, including serum, plasma and urine (see section 3.1); b.) Daily Hormone Study (DHS) samples, including serum and daily urine samples (section 3.2); and c.) Genetic Study samples, including extracted DNA and EBV-transformed cell lines (see section 3.3) – as summarized below:

a. SWAN Core samples available:

Material Type	Vial Volumes (Average)	Number of Women with this resource available (at baseline)
Serum	0.5 mL	3269
Plasma, EDTA	1.0 mL	3159
Plasma, Citrate	0.5 mL	3167
Urine	0.5 mL	2375

b. SWAN Daily Hormone Study (DHS) samples available:

Material Type	Vial Volumes (Average)	Number of Women with this resource available (at baseline)
Urine	5 mL/0.5 mL	884
Serum	0.5 mL	884

c. SWAN Genetic samples available:

Material Type	Vial Volumes (Average)	Number of Women with this resource available
Extracted DNA	5 µg total volume	1538
EBV-Transformed Cell lines	10 mL of cells	1538

2.0. ORGANIZATIONAL STRUCTURE AND FUNCTIONS

2.1. REPOSITORY ADMINISTRATION AT THE UNIVERSITY OF MICHIGAN

2.1.1. Purpose and Activities

Primary responsibility for Repository administration and management is located at the University of Michigan (UM), although no specimens are stored there (see 2.6 Repository Physical Facilities). At UM, current Repository PI Siobán Harlow, Co-I Daniel McConnell, Repository manager Steffenie Merillat, administrator Charlotte Carlson, and web developer Robin Wylie work together, along with an analyst as needed for sample design strategies, to provide ethical and productive stewardship for Repository resources. The UM team is specifically responsible for:

- Securing and administering fiscal resources for the Repository;
- Overseeing the physical storage (subcontracted to Precision Bioservices) and inventory database system (subcontracted to IMS, Inc.);
- Managing all activities related to the selection, distribution, and recovery of resources, including Material Transfer and Data Transfer Agreements (MTA, DTA) including data recovery;
- Managing the Repository and its resources in an ethical manner consistent with Institutional Review Board and NIH data-sharing and resource distribution requirements;

- Assuring activities are consistent with repository best practices (codified by the International Society of Biological and Experimental Repositories (ISBER));
- Administering the online inquiry and application submission and review process;
- Optimizing accessibility to resources for the applicant including making data resources available in user-friendly formats and preparing documentation;
- Promoting awareness about Repository resources in the scientific community; and
- Interfacing with the SWAN Coordinating Center (CC), the SWAN Steering Committee (SC) and the NIA.

The SWAN Repository has an effective collaborative structure that actively interfaces with the SWAN CC and Steering Committee (SC) and the CLASS Lab. The inter-linked administrative infrastructure ensures smooth Repository functioning while providing the platform for formation of a SWAN Legacy Organization. SWAN CC staff and SWAN investigators serve on the key operational and advisory bodies of the Repository.

2.1.2. Key Personnel & Contact Information, UM Repository Team

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2.2. THE SWAN REPOSITORY ORGANIZATION (SRO)

2.2.1. SRO Purpose and Activities

The SWAN Repository Organization (SRO) is a committee which provides oversight and guidance on Repository ethical and operational issues and reviews all applications for Repository resources. The SRO is responsible for three major activities:

- Performing the initial review and approval of Repository applications, specifically reviewing proposals for: sample size and adequate power; negotiating reasonable sample requests with respect to number and volume of samples to ensure lowest necessary impact on inventory, especially to scarce samples; ensuring acceptable lab procedures; and performing New Studies functions (checking for overlap).
- Monitoring Repository operations, reviewing quarterly reports from the Repository's physical facility (Precision Bioservices), and participating in the oversight and direction of activities such as inventory investigations, molecular work, aliquoting, and culling.
- Providing expertise and input to help shape Repository policies and procedures, including policies such as the cost-recovery program, data-sharing, value of specimens and

guidelines for approving release of high-value specimens, and promotional programs to increase utilization of Repository resources.

2.2.2. SRO Membership

The SRO is comprised of Repository investigators, SWAN investigators including the New Studies Chair and CC representatives and statisticians, outside experts in genetics and biobanking, and an NIA representative. The SRO meets at least quarterly by conference call.

Current SRO Members include:

Siobán Harlow, Repository PI, UM
Daniel McConnell, Repository Co-I, CLASS laboratory, University of Michigan
Steffenie Merillat, Repository Manager, UM, ex-officio
Sybil Crawford, SWAN CC statistician, University of Massachusetts, Worcester
Sherri-Ann Burnett-Bowie, SWAN New Studies Chair, Massachusetts General Hospital
Deborah Martin, SWAN CC data coordinator
Sharon Kardia, statistical geneticist, UM
Janet Sinsheimer, statistical geneticist, University of California Los Angeles
Elaine Gunter, biorepository consultant, Specimen Solutions, LLC
Chhanda Dutta, SWAN Project Officer, NIA
Maria Mori Brooks, SWAN CC PI and statistician, University of Pittsburgh, *ad hoc*

2.3. THE SWAN REPOSITORY ADVISORY GROUP (SRAG)

2.3.1. SRAG Purpose and Activities

The SWAN Repository Advisory Group (SRAG) is a committee which meets quarterly by conference call to provide recommendations on major policy issues and Repository direction, to review Repository applications requesting genetics specimens or any samples deemed rare or "reserved" (see section 7.1.3). SRAG members are also responsible for reviewing the quality of the science in any Repository application not receiving external peer review (i.e., through NIH). In the past, SRAG provided guidance in the development of the Repository DNA resources.

SRAG review is conducted when any of the following conditions are true of applications:

- Applications are requesting Genetic specimens;
- Applications are requesting Reserved specimens; or
- No scientific peer review will be done on the proposal.

2.3.2. SRAG Membership

The SRAG committee includes representative SWAN investigators; scientists outside of SWAN with expertise in epidemiology, genetics, geriatrics, and ethics; and Ad Hoc scientists for review of applications with subject areas outside the expertise of current SRAG members. Current SRAG Members include:

Siobán Harlow, Repository PI, UM – ex-officio
Gail Greendale, SWAN investigator and geriatrician, University of California Los Angeles
Howard Kravitz, SWAN investigator, Rush University
Ann Boyd, Ethicist, Hood College
Jeffrey Williamson, geriatrician, Wake Forest Baptist Medical Center
Sharon Kardia, statistical geneticist, UM
Janet Sinsheimer, statistical geneticist, University of California Los Angeles

2.4. THE ROLE OF THE SWAN STEERING COMMITTEE (SC)

The SWAN Steering Committee (SC) provides final approval on all applications for specimen release recommended through the Repository review process. The SC includes SWAN PIs from each clinical site. Approval is obtained after discussion on regular SC calls, as needed, and voting through email or a confidential online poll. Email communication with the SC goes through the SWAN Coordinating Center, typically Vicky Palombizio (palombiziov@edc.pitt.edu) and/or Joyce Monroe (MonroeJ@edc.pitt.edu).

2.5. THE ROLE OF THE NATIONAL INSTITUTE OF AGING (NIA)

NIA approval is required on all requests for Reserved and/or Restricted Repository samples. NIA approval comes from Chhanda Dutta (DuttaC@nia.nih.gov), Program officer for the SWAN Repository, via email from Dr. Harlow, Repository PI.

2.6. REPOSITORY'S PHYSICAL FACILITY – PRECISION BIOSERVICES

2.6.1. Purpose and Activities

Physical specimen storage and maintenance of SWAN Repository specimens are contracted to Precision Bioservices (formerly SeraCare), located in Frederick, MD (http://www.precisionbioservices.com/).

Precision is responsible for the receipt of frozen specimens from clinical sites, completing thorough inventory processes, maintaining and documenting appropriate storage conditions (both mechanical and liquid nitrogen freezers), retrieving specimens for distribution based on specifications provided by the Repository, and shipping designated specimens as requested. Precision Bioservices Cell Biology division also performs specimen services such as aliquotting, immortalization of B-lymphocytes, and DNA extraction.

Precision Bioservices is accredited by the College of American Pathologists (CAP) Biorepository Accreditation Program (BAP) and is ISO9001 and ISO 13485 certified. Precision performs tasks under Standard Operating Procedures, and follows ISBER and NCI Best Practices for Biorepositories and applicable IATA shipping procedures.

The Precision Bioservices Standard Operating Procedures (SOPs) relevant to SWAN are listed in section 7.5.1.

2.6.2. Key Personnel & Contact Information

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2.6.3. History and Changes of Physical Facilities since SWAN Study Inception

Transition from CLASS to McKesson: When the SWAN Study began collecting its baseline visit samples from participants in 1996, no physical facility was designated as a repository. Instead, clinic sites collected extra specimens with long term storage in mind and shipped them to SWAN's central laboratory, CLASS. The CLASS lab stored these samples until 2000, when the SWAN Repository was initialized and funded as a separate entity. This funding provided for the storage and maintenance of the SWAN Repository samples in a specialized biobank facility. Chosen for this role was McKesson Bioservices located in Rockville, MD. All samples designated as Repository samples were then shipped from the CLASS Lab at UM to McKesson in 2000.

Transition from McKesson to SeraCare: In 2005, at the time of the Repository's grant renewal, the subcontract for Repository storage services was re-bid and SeraCare Bioservices took over the task of housing SWAN Repository specimens. Samples were shipped by truck from McKesson to SeraCare in November, 2005. At the same time, new database structures were built to fit the specific needs of the SWAN Repository. Inventory Management Services, Inc. (IMS) provided this work (see section below).

NOTE: BBI=SeraCare=Precision. SeraCare Bioservices was named BBI Biotech at the time of the initial subcontract. Soon after, they were acquired and renamed SeraCare Life Sciences. Later in April, 2012, SeraCare Life Sciences was acquired by Linden, LLC and officially changed its name to Precision Bioservices in January 2013. (McKesson is now Fisher BioServices.)

2.7. INVENTORY MANAGEMENT SERVICES

2.7.1. IMS Purpose and Activities

The SWAN Repository subcontracts inventory management services to Information Management Services (IMS), who work closely with Precision Bioservices. IMS is responsible for building and maintaining databases and electronic resources necessary for tracking and managing the SWAN Repository specimen inventories. IMS is the developer of Biological Systems Inventory (BSI-II), one of the major repository inventory management and control systems which fully meet Federal IT requirements. IMS provides updated inventory files monthly, with an automated data transfer to UM occurring at the first of each month.

2.7.2. Key Personnel & Contact Information

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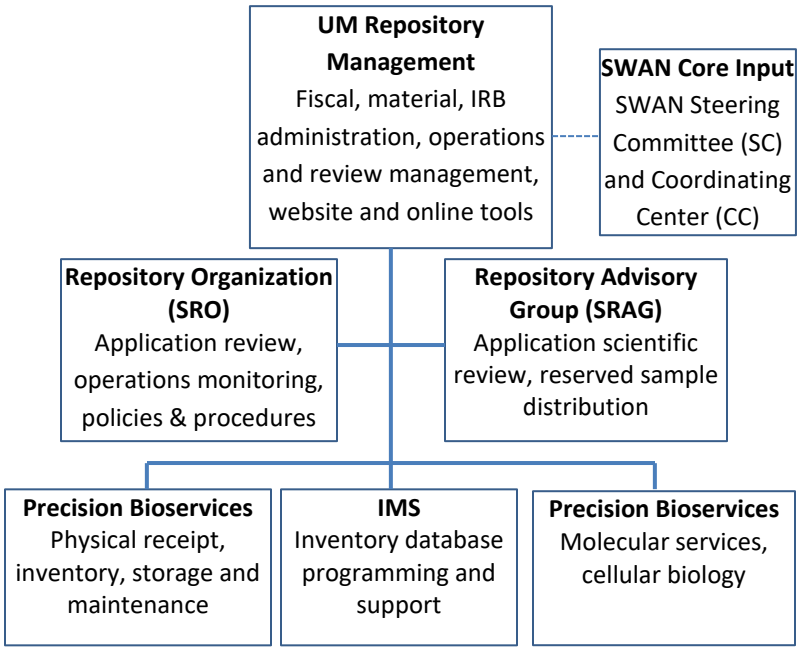
2.8. DIAGRAM OF SWAN REPOSITORY ORGANIZATIONS AND FUNCTIONS



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3.0. SWAN STUDIES [GIVING RISE TO REPOSITORY SPECIMENS] AND INFORMED CONSENT

3.1. SWAN CORE STUDY

3.1.1. Brief Study Background

SWAN's core study was designed as a prospective, multicenter, multiethnic, multidisciplinary study of the natural history of the menopausal transition in women. An initial cross-sectional survey was administered to 16,065 women aged 40-55 women across the seven clinical facilities. From that group, eligible women were identified to continue in the longitudinal study, resulting in a longitudinal cohort of 3302 women. Clinic visits included questionnaire administration, physical functioning measurements, and additional protocols which varied between sites. An early morning blood draw and urine collection were also part of each visit. A portion of these specimens were collected for the purpose of being stored by the SWAN Repository for future use.

3.1.2. Consent for Core Specimens in the Repository

All women participating in the SWAN Core study went through an informed consent process. Participants were given options to participate in the Core studies (which include the procedures done at all 7 SWAN clinic sites); Sub-core studies (done at more than 1 but less than 7 clinic sites, such as the Bone study); and Site-Specific studies (done at one particular site).

The procedures in SWAN are classified as "minimal risk". All participants signed a detailed consent document explaining the purpose, risks and benefits of the proposed research, which is approved by the local SWAN site IRB for each study visit. Each site also was directed to include

specific language regarding the contribution of blood and urine specimens to the Repository for future use.

3.1.2.1. Core IRB/PHI issue for Repository specimens (2014)

In 2014, a series of special meetings were held to review whether information recorded on the vial labels of specimens stored in the Repository was considered PHI (protected health information) and whether further actions were necessary to comply with IRB regulations at any of the 7 SWAN clinic sites. Specifically, in addition to a unique sample barcode, the vial labels included participant initials and date of collection, which had been part of the specimen collection protocol to help resolve potential discrepancies if tubes were found without accompanying paperwork and vice versa.

Each site raised the issue with their local IRB after review of their consent documents. IRBs at five sites classified the stored specimens as research samples based on the current operational structure of the SWAN Repository. IRBs at two sites, UC Davis and Chicago, determined that because of affiliations with health systems, the initials on the vials constituted PHI and required removal prior to redistribution.

UC Davis participants were recontacted, and 283 women (as of 2/24/2016) gave special consent to store and distribute their samples as labeled. In total for UC Davis, samples at the Repository from 293 women (including the 283 reconsented women, plus 10 deactivated) will not require relabeling, however samples from the remaining 168 UCDavis women will be relabeled at the time of distribution. For Chicago, only the Core samples are affected, as consent forms for the DHS stored samples had specific language addressing their storage for future use. Protocols were established to ensure this relabeling requirement is met for the non-consenting UC Davis samples and for all Chicago Core samples before leaving the Repository.

DNA samples were not affected as the vials and labels were not created at the sites, and do not contain initials or dates.

3.2. DAILY HORMONE STUDY (DHS)

3.2.1. Brief Study Background

The Daily Hormone Study (DHS) is a sub-study of SWAN, developed to provide a more complete understanding of the variation in hormone concentrations throughout menstrual cycles of the peri-menopausal transition and to characterize changes of within-cycle events. A subset of 900 SWAN participants, coming from all sites and ethnic groups, participated in the DHS sub study by completing daily diaries about symptoms and feelings, and collecting urine samples every morning for one complete menstrual cycle or for up to 50 days, each year until the participants became post-menopausal. Samples from these daily urine collections, along with a serum specimen, are available through the Repository.

3.2.2. Consent for DHS Samples in the Repository

A separate form was used to obtain informed consent from the DHS participants. Each of the seven SWAN sites consented participants with their own IRB-approved forms. Specific language was included about Repository storage and future use. (See also 3.1.2.1, UC Davis samples require relabeling prior to redistribution.)

3.3. SWAN DNA STUDY

3.3.1. Brief Study Background

As one of the Repository's original aims, a SWAN DNA Collection was established. A one-time draw of whole blood was obtained from a subset of the SWAN participants. In addition, a mouthwash sample was collected to obtain buccal cells. This special collection took place in 2002-2003, spanning Follow-up visits 04-06.

DNA was extracted from blood cells of the whole blood sample, and used by the Repository to genotype several genes in the sex steroid hormone pathway. These genotype results are stored and available via the Repository application process, along with the DNA and immortalized cell lines which were created through EBV (Epstein-Barr virus) transformation. (For details on DNA processing see section 4.3.)

Although SWAN DNA samples were collected by all 7 Study sites, the SWAN New Jersey site subsequently requested that all genetic specimens obtained from their participants be destroyed (2006).

3.3.2. Consent for DNA samples in the Repository

All SWAN participants were given the option to participate in the DNA Study. The final collection includes 1538 women who consented to DNA and contributed a sample which successfully went through EBV (Epstein Barr Virus) transformation. Each site consented their participants using their own IRB-approved forms. Consent forms included general methodology of obtaining and processing the specimens, as well as the right of the participant to request information that becomes available as a result of any research. Participants were given the option to agree to each item below individually:

- To provide a whole blood sample;
- To provide a buccal cell sample;
- To have cell lines created from their samples.

The participants completed 3 copies of the consent form. One copy stayed at the site, one copy remains with the participant and a third copy was sent to the Repository. To ensure participant confidentiality, the copy that was sent to the Repository did not include the participant's signature or initials, but instead had a label that linked the consent form with the specimens which were provided by the participant. (The Repository copy of the informed consent was later destroyed at McKesson at the time the collection moved to SeraCare Bioservices.) The consent form was included with the specimens when shipped to the Repository. Specimens were

not processed if they were received without the Informed Consent Form. There was a seven-day reconciliation period in which any discrepancies could be resolved before the sample was destroyed. *Copies of the DNA Consent Forms can be found in the Section 3 Appendices.*

3.3.2.1. Returning Genetic Results to SWAN Participants

The DNA Informed Consent explicitly states that SWAN is not allowed to contact the participants to convey genetic results discovered through any Repository studies. However, participants can initiate contact with SWAN if they choose to learn if results have been obtained from their samples. Therefore, investigators using SWAN DNA and genetic samples are obligated to be prepared to provide individual results if a SWAN participant contacts her SWAN clinic site or the Repository inquiring about DNA test results. Investigators also need to be prepared to provide Genetic Counseling to affected SWAN participants if appropriate.

3.3.3. Certificate of Confidentiality adds Privacy Protection

The SWAN Repository obtained a Certificate of Confidentiality (MH-AG-01-02) from the Department of Health and Human Services, which, as provided in section 301(d) of the Public Health Service Act 42 U.S.C. 241(d), states:

> *"Persons so authorized to protect the privacy of such individuals may not be compelled in any Federal, State, or local civil, criminal, administrative, legislative, or other proceedings to identify such individuals."*

The Certificate of Confidentiality mitigates the risk of involuntary release of genetic information for both the Principal Investigators and the participants. The protection afforded by the Certificate is permanent with respect to subjects who participate in the research during the time the Certificate is in effect (1/9/2002 through 5/21/2005). All DNA samples were collected within this timeframe. *A copy of the Repository's Certificate of Confidentiality can be found in the Appendix, Section 3.*

3.3.4. Review of IRB Restrictions for DNA

In January 2012 a group consisting of S Sherman, S Harlow, Dan McConnell, G Greendale, C Crandall, S Kardia, D Evans, and S Merillat met to review the sites IRB documentation for DNA collection; to discuss the consequent limitations and required procedures for DNA-related research; to discuss potential Bone and GWAS proposals and enhanced genome-wide exome resequencing; and strategies and priorities for generating additional genetic proposals.

The committee set forth the following guidelines after reviewing Repository IRB and informed consents from all six sites contributing DNA:

"It is our considered opinion that the IRB and consent documents require that:

1) The SWAN REPOSITORY and SWAN must have a high level of involvement in oversight of stored DNA samples, including review of the science by the SRO and the appropriate scientific advisory committee.

2) All data generated from Repository samples, including DNA data, must be returned to the SWAN Repository and incorporated into the databank accessible to SWAN researchers. Current policy, per agreements which all Repository material grantees must sign, is that investigators will return their result data sets to the Repository within 3 years of the end of the grant funding period.

3) DNA-related data generated from DNA samples must be anonymized. Thus,
    a. SWAN data provided to investigators conducting DNA studies contain new encrypted IDs, known only to specified staff of the Repository, as the Trusted Broker.
    b. DNA results requested for subsequent analyses by SWAN investigators also require encryption. Requests for this data must be made to the SWAN Repository via an Application, and individual datasets for these analyses will be generated by the Repository.
    c. At the time that SWAN and the SWAN Repository is terminated, all DNA material and identifying information linking DNA results to the SWAN data must be destroyed.
    d. SWAN can place genetic results in dbGaP (the Database of Genotypes & Phenotypes, from the National Center for Genomes & Phenotypes), as these data are anonymized and the process for utilization of the data requires application, scientific review, IRB approval, a defined timeline for utilization and annual reports. When required as a condition of funding, only the specific genetic results and a limited amount of phenotypic information are required to be posted. Anonymization of the data is the standard in the field."

SECTION 4.0 – SPECIMEN COLLECTION, PROCESSING, ONSITE STORAGE & SHIPPING

4.0 SPECIMEN COLLECTION, PROCESSING, ONSITE STORAGE & SHIPPING

4.1. SWAN CORE SPECIMENS (Annual Visits)

A common specimen collection protocol was developed in SWAN to ensure quality and consistency among the blood and urine samples collected at all seven clinic sites, beginning at the baseline visit. The detailed protocol outlines steps involved in the blood draw and urine collection, specimen processing and aliquotting, temporary storage at site clinics, and shipping to the Repository. Standardized collection kits, developed at the CLASS lab, contained the appropriate number and type of Vacutainers®, cryovials, and a sterile urine collection cup along with preprinted barcode labels and Specimen Collection Record (SCR) paperwork for all Core SWAN participants. Project Directors (PDs) and other key clinic personnel at all sites participate in hands-on training and certification on an annual basis.

4.1.1. Annual Visit Serum & Plasma Collection, Processing, and Onsite Storage Overview

Repository blood samples were obtained at each follow up visit, at all seven clinic sites, between days two and five of the participant's menstrual cycle for regularly cycling women who are not using birth control pills or HRT. For women who were no longer cycling regularly or post-menopausal, blood was drawn at their clinic visit, scheduled to be on the anniversary of their previous visit. Samples were collected from patients who had been fasting with nothing but water as intake for previous 12 hours, and between 8:00-10:00 AM. Any deviations from the protocol were noted on the SCR.

Additional details below; also found in the SWAN Manual of Operation (MOO) section 6.1.2

4.1.1.1. Blood Collection Supply Kit

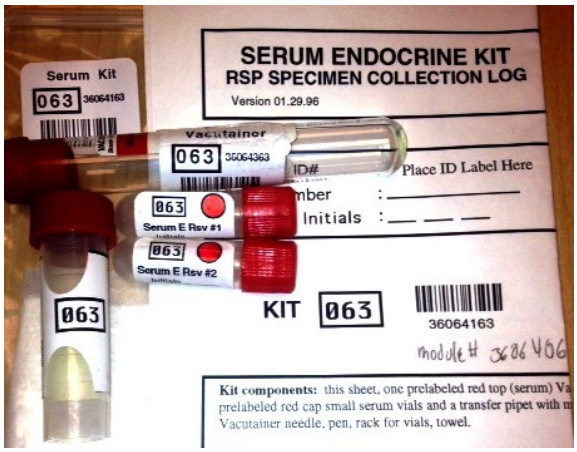
Specimen collection supplies needed for Repository-designated and other SWAN samples were provided to study sites by the CLASS Lab, as one module/packet per subject. The content of each module/packet included the following Repository blood-related supplies:

- Specimen Collection Log /Specimen Collection Record
- Blood Drawing Kit:

- Two (2) pre-labeled 10-mL red-top (serum) Vacutainer tubes (for Repository samples, as well as for measuring endocrine measures, glucose, insulin, bone markers)
- One (1) pre-labeled 7-mL red-top (serum) Vacutainer tube (for Repository samples)
- One (1) pre-labeled 5-mL blue-top (citrate plasma) Vacutainer tube (for Repository samples as well as for measuring clotting factors)
- One (1) Vacutainer Eclipse™ multi-sample needle with safety lock
- One (1) single-use Vacutainer holder

- Blood Aliquoting Kit:
  - Seventeen (17) pre-labeled red-cap small serum cryovials (Repository)
  - Three (3) pre-labeled blue cap small plasma cryovials (Repository)
  - Three (3) disposable absorbent pads

CLASS Central Laboratory staff pre-labeled all Vacutainer and transfer vials using labels containing a unique bar code and highly visible three-digit number common to all materials in the kit (see example below).



4.1.1.2. Blood Collection

SWAN sites followed the protocol below to uniformly schedule visits and collect Repository serum and plasma from all participants:

(a) Scheduling of Blood Draws

1. For a Respondent with regular bleeding (predictable within a week) and no current use of prescription HRT or birth control pills, a fasting blood specimen should be obtained before 10:00 AM., and preferably between 8:00-10:00 AM., during days 2 - 5 of her menstrual cycle window. For Respondents with current use of prescription HRT/BC pills or those who have had a hysterectomy or no bleeding in past 3 months, a fasting blood sample should be drawn between 8:00-10:00 AM within the data collection window.

2. Within the first 60 days of initiation of follow-up data collection, the site will attempt to obtain a fasting blood sample drawn during days 2-5 of the Respondent's menstrual cycle. If the site determines during a blood draw attempt that the Respondent is not fasting or is not in days 2-5 of her menstrual cycle, then the blood sample should not be taken at that time and another attempt made at a later date. Depending on a woman's menstrual regularity, there may be one or more opportunities for a blood draw in the menstrual window during the first 60 days.
3. After 60 days have elapsed, the site staff will attempt to schedule a respondent's blood draw whenever it is convenient and feasible, without regard to day of menstrual cycle. On this last attempt, sites should try to get a fasting blood sample if at all possible, but if this is not possible because the respondent has eaten, the blood sample still should be drawn and the reason for this protocol variation documented.

(b) Blood Collection Protocol

1. Subjects must be fasting for ALL specimen collections. Fasting is defined as nothing to eat or drink except water for at least 12 hours.
2. Have the Subject seated for at least five minutes prior to blood collection. Complete the blood collection preferably between 8:00-10:00 AM. Note on the Specimen Collection Log in what position the Subject is during specimen collection (i.e., sitting, lying down) and use the same position used for drawing blood at baseline and each annual follow-up visit. This information will be printed on the Respondent's Contact Record.
3. Using a Vacutainer multi-sample needle, collect the blood in the following order. After each tube is filled, remove it, replace it with the next tube, and mix each tube 8-10 times gently by inversion

    #1: 10-mL pre-labeled red-top Vacutainer (CLASS, Repository)
    #2: 10-mL pre-labeled red-top Vacutainer (CLASS, Repository)
    #3: 7-mL pre-labeled red-top Vacutainer (Repository)
    #4: 5-mL pre-labeled blue-top (Citrate) Vacutainer (CLASS, Repository)
    #5: 7 mL pre-labeled purple-top (EDTA) Vacutainer (CLASS, Repository)

    Collect a total of 39 mL of blood in 5 Vacutainers. Use a butterfly needle (21 g) and/or 5-mL (pediatric) Vacutainer sizes on women with smaller veins. Collect the samples in this order to prevent the carry-over of Vacutainer additives/ anticoagulants into the red-top (serum) tube.

4.1.1.3. Onsite Processing of Blood Samples (Serum & Plasma)

Overview

Plasma: Store the blue-top and purple-top tubes in a 4-8°C refrigerator (or in a cooler on wet or blue ice) for a maximum of two hours prior to centrifugation. Immediately centrifuge if possible.

Serum: Allow the red-top tubes to sit at room temperature for a minimum of 30 minutes to a maximum (preferred) of 60 minutes to allow the clot to form. Then, refrigerate the tubes at (4-8 °C) for a minimum of 30 to a maximum (preferred) of 60 minutes before centrifugation.

See details below, or in the SWAN Manual of Operation (MOO) section 6.1.2

Protocol

a. Before Centrifugation

1.) Serum: Allow red-top tubes to sit at room temperature from 30 to (preferred) 60 minutes to allow the clot to form; then refrigerate the tubes at 4-8 °C for 30 to (preferred) 60 minutes before centrifugation.

2.) Plasma: Centrifuge blue and purple-top tubes immediately after collection. If this is not possible, place them in a cooler on blue ice or wet ice, or in a refrigerator (4-8 °C), for no more than 2 hours, then centrifuge them.

b. Centrifuging Blood Samples

Within 2 hours of collection, centrifuge all tubes:

- in a refrigerated centrifuge (4°C)
- at a minimum force of 1300 x g
- for 20 minutes

c. Aliquotting Blood Samples

For the SWAN Study, it is preferable to have fewer aliquots at a given volume than more aliquots at a reduced volume. Do not aliquot less than a 0.5-mL volume into any vial. To avoid unnecessary freeze/thaws and assure optimal use of these specimens, all Repository aliquots are to contain a standard volume of 0.5 mL.

Use the Finnpipette™ pipettor (provided to each site by CLASS) and the plastic, disposable Finnpipette tips to transfer the serum and plasma, changing to a new Finnpipette tip for each new blood tube.

- From red-top Vacutainer #1, pipet 2.0 mL serum to the large (S1), 1.0 mL to the smaller vial (S2) and **0.5 mL serum into each of the four (4) Repository cryovials (S3-6)**. Close vial caps tightly.
- From red-top Vacutainer #2:
  Non-Bone Turnover study sites: Pipet 1.0 mL serum to the larger vial (S7), **and 0.5 mL serum into each of the six (6) smaller cryovials (S9-S14)**. Close vial caps tightly. Note: S8 is not collected.

Bone Turnover study sites: From red-top Vacutainer #2, Pipet 1.0 mL serum into each of the two larger vials (S7, S8) and **0.5 mL serum into each of the six (6) smaller cryovials (S9-S14)**. Close vial caps tightly.

- From red-top Vacutainer #3, **Pipet 0.5 mL serum to each of the other seven (7) smaller cryovials (S15-S21)**. Close vial caps tightly.
- From the blue-top Vacutainer, transfer 1.0 mL plasma to the larger blue-cap tube (P1) and **0.5 mL into each of the three (3) blue-cap Repository cryovials (P2 - P4).** Take care to aspirate NO cells. Close vial caps tightly.
- From the purple-top Vacutainer, transfer three (3) 1.0-mL plasma aliquots to the three (3) larger purple-cap tubes (E1, E2, and E3). Close vial caps tightly.

If cells or platelets are accidentally aspirated in the plasma aliquoting process, centrifuge the tubes a second time and transfer the contents cleanly to prevent the carryover of cells or platelets to the plasma sample.

Carefully match ID labels on the collection tubes with ID labels on corresponding aliquot tubes from the same kit. Write the Subject's three initials and the date of collection on the label of each tube. Attach the subject ID label to the Specimen Collection Record. Record the subject initials onto the Shipping Record. Complete the Specimen Collection Record, checking that ID labels are correctly affixed and noting any problems such as insufficient volume, spills, etc.

4.1.1.4. Temporary Onsite Storage of Blood Samples

Follow the steps below to ensure proper handling and storage of Repository serum and plasma samples before shipment to the Repository facility.

- Check that all aliquot tube caps are securely closed; Check that all labels are securely placed on the tubes.
- Return all aliquots to the appropriate shipping bags, seal bags securely and place the bags with tubes upright in a -20 °C (or lower) non-self-defrosting freezer. Carefully monitor and record the freezer temperature daily in a log.
- Store frozen specimens locally for no more than 30 days, and at least overnight before shipping to the Repository. Distributing specimens among the designated shipping bags prior to freezing will eliminate the need to open frozen bags later, and will avoid partial thawing of specimens when preparing them for shipping.

4.1.2. Annual Visit Urine Collection, Processing, and Onsite Storage

*NOTE: Urine sample collection took place only at the **five SWAN sites** studying bone health – Michigan, MGH, UC Davis, UCLA, and Pittsburgh. The Chicago and NJ sites do not have Repository urine samples.*

Overview

An early morning (before 9:00 AM) sample of urine for measurement of bone markers and Repository was collected from participants at the time of, or immediately prior to, their visit. *See details below, or in the SWAN Manual of Operation (MOO) section 6.1.2*

4.1.2.1. Urine Specimen Collection Supply Kit

In addition to the blood collection supplies described in section 4.1.1.1. above, each supply module/packet from the CLASS Lab included the following urine collection supplies:

- One (1) 50-mL sterile urine collection cup
- One (1) pre-labeled yellow-cap large urine vial
- Eight (8) pre-labeled yellow-cap small urine vials

4.1.2.2. Urine Specimen Collection

At the time of, or immediately prior to, her study visit, ask the Subject to collect an early morning sample of urine. This sample should be collected before 9:00 AM, but should not be the first voided urine, and brought to the clinic study visit. Record the estimated time of urination.

4.1.2.3. Urine Specimen Onsite Processing

a. Handling: While the urine need not be refrigerated between the time of collection and delivery to the site that same morning, it should be protected from direct sunlight by wrapping in aluminum foil or placing in an envelope. Immediately aliquot the urine and place into freezer storage. If not aliquoting the specimen immediately, refrigerate (4-8 °C) the urine upon receiving it from the subject, or place it in a cooler on blue ice for not more than 4 hours.

b. Aliquoting: Using the Finnpipette pipettor and plastic tips, transfer 0.5 mL of urine into each of the eight pre-labeled (Repository) yellow-capped vials. Close vial caps securely. Write the Subject's three initials and the date of draw on the label of each tube.

4.1.2.4. Urine Specimen Temporary Onsite Storage

Freeze the vials at -20 °C (or lower) until the monthly shipment to the Repository, when they are packaged with dry ice (8 x 0.5 mL vials). Write in the notes section on the Specimen Collection Record form any comments such as problems with the urine collection, insufficient volume, spills, etc.

4.1.3. Shipping Annual Visit Specimens to the Repository

Ship all Core Annual Visit specimens to the Repository on a monthly on dry ice and according to all IATA shipping regulations. Attach a copy of the Repository Shipping Record to

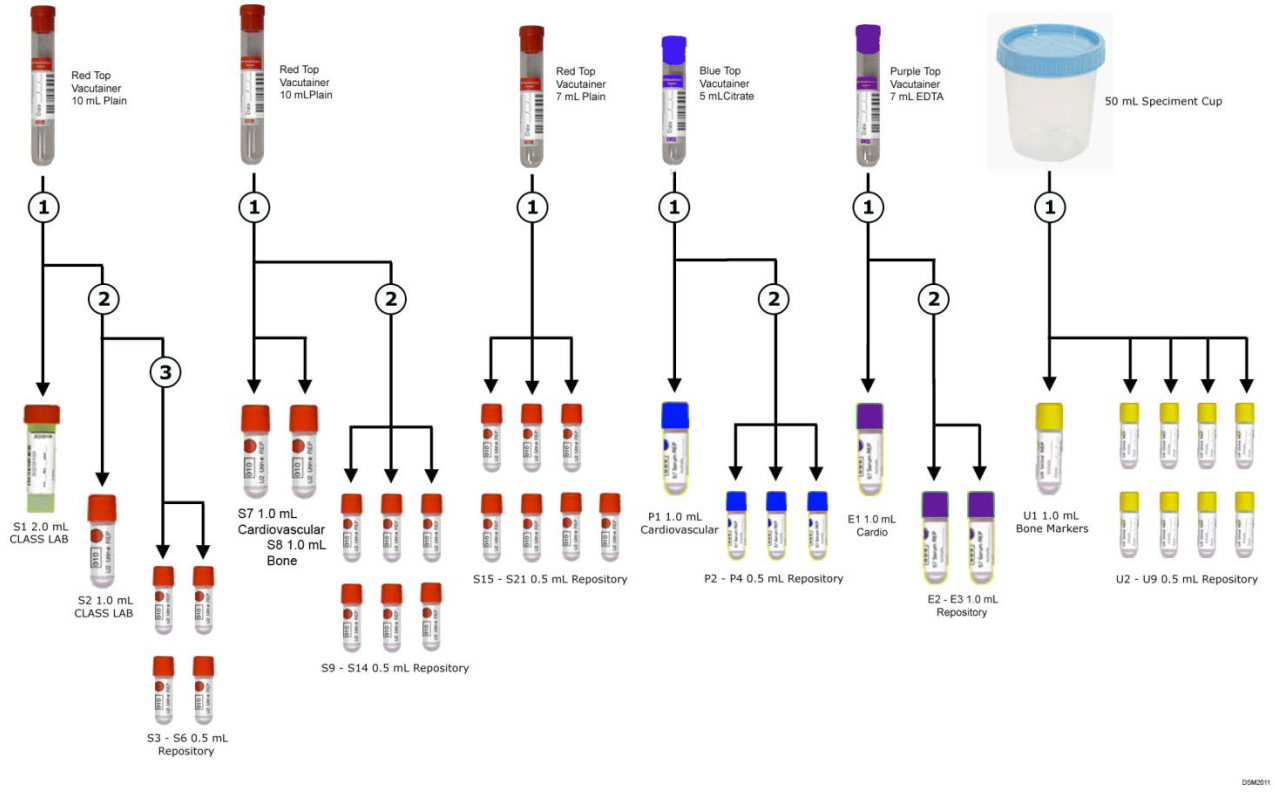
copies of the specimen collection records, and enclose with the shipment. Keep a copy of the completed Repository Shipping Record at the site.

4.1.4. Summary of Annual Visit Blood and Urine Samples Collected for Repository

The schema below, Figure 6.3 from the SWAN MOO, shows the blood and urine collection protocol for Core SWAN Study participants. This protocol was followed for Visit 01 to Visit 13. A change in collection, decreasing serum and increasing plasma, took effect for the final SWAN visit, V15 in SWAN V (see next section, 4.1.5).

In cases of short draws, alternate priorities were outlined consistently for all sites. Specific protocols covered a number of short draw scenarios, most often eliminating the purple-top tube and shifting vials to go to the assays of highest priority. *(see Figures 6.9a – 6.9d, short draw schemas, Section 4 Appendices)*

SWAN MOO Figure 6.3, Full Sampling Schema



The table below shows the number and volume of vials collected for the Repository, for each specimen type between Baseline (V00) and Visit 13, along with tube top colors, vial names and applicable SCR version numbers.

Repository Specimen Collection Protocols, SWAN I-SWAN IV (Visit 00-13)

			SWAN I **Baseline Visit 00** SCR 03/15/1996		SWAN I **Visit 01(A)** SCR 02/01/1997		SWAN I-III **Visits 01(B)–10** SCR 09/01/97 SCR 09/01/00		SWAN IV **Visits 12-13** SCR 05/01/2009	
Specimen Type	**Tube cap color**	**Vial Names** (Visit1b-13)	**# Vials**	**Volume (mL)**	**# Vials**	**Volume (mL)**	**# Vials**	**Volume (mL)**	**# Vials**	**Volume (mL)**
Serum	Red-top	S3-S6 S9-S21	5	1.0	4	1.0	17	0.5	17	0.5
Plasma, Citrate	Blue-top	P2, P3, P4	1	1.0	4	1.0	3	0.5	3	0.5
Plasma, EDTA	Lavender -top	E2, E3	1	1.0	-	-	-	-	2	1.0
Urine	Yellow-top	U2-U9	-	-	4	1.0	8	0.5	8	0.5

Several items to NOTE about Repository specimen collections:
Visits 11 and 14: Follow up Visits 11 and 14 were interim visits with no Repository sample collection.

Visit 01: The SWAN specimen collection protocol changed in the middle of the Visit 01 cycle, and two versions of the Specimen Collection Record (version 02/01/1997 and version 09/01/1997) were used. The number of aliquot tubes collected and the volume collected within each tube differs between the two versions. The collections included the following:
02/01/1997 – 2 red-top tubes, 2 blue-top tubes, and 1 purple-top tube were collected
09/01/1997 – 3 red-top tubes, 1 blue-top tube, and 1 purple-top tube were collected.

EDTA Plasma: EDTA plasma was not collected for Repository in Visits 01-10; however, many of the vials sent to the MRL laboratory during this period was recovered in 2007 and added into available Repository inventory. In Visits 12 and 13, while EDTA plasma was being collected for the Repository, it was excluded in cases of a short-blood draw. In these situations, the E1, E2 and E3 vials were processed as serum (not plasma) and sent to CLASS. These exceptions were documented in the specimen logs.

4.1.5. Visit 15 (SWAN V) Updates to Core Annual Visit Specimen Collection

Some changes were made to the specimen collection and processing protocols for Visit 15, the final SWAN clinic visit. These changes are summarized here.

4.1.5.1. V15 Repository Specimens Collected

The number of vials collected for each material type was adjusted to better match Repository utilization and demand. The number of serum vials collected was

reduced, EDTA plasma increased, and urine was collected in fewer but larger vials, as seen in table below.

			SWAN V Visit 15 SCR 04/26/2015	
Specimen Type	**Tube color**	**Vial Names**	**# Vials**	**Volume (mL)**
Serum	Red-top	S3-S6, S10-S13	8	0.5
Plasma, Citrate	Blue-top	P2-P4	3	0.5
Plasma, EDTA	Lavender-top	E4-E10	7	0.5
Urine	Yellow-top	U1-U4	4	2.0

4.1.5.2. Updated Repository Shipping Address and Personnel

All V 15 Repository sample shipments and related questions should be sent to:
SWAN REPOSITORY - PRECISION BIOSERVICES (Frederick, MD)
Shipping Address:
Precision for Medicine
8425 Progress Drive, Suite M
Frederick, Maryland 21701

Contact Person:
Misti Dowell
Project Manager
Phone: 240-306-4104
Fax: 301-668-3416
Misti.Dowell@precisionformedicine.com

4.1.5.3. New Bone Turnover/Urine Collection Sites

Four SWAN sites will participate in the bone turnover protocols in SWAN V and collect Repository urine samples: Michigan, MGH, UCLA and Pittsburgh. (Previously, the UC Davis site also participated as a bone site, but will not in V15.)

4.1.5.4. Specimen Boxes Used to Store and Ship Repository Samples

After collection, Repository samples in Visit 15 are stored locally and shipped in 2” (9x9) cardboard specimen boxes rather than the reclosable plastic bags. This change was made to keep the vials upright during the initial freezing process, provide better protection during shipping, and make the task of inventorying quicker and easier.

4.1.5.5. Electronic Manifests

The Visit 15 Repository samples collected will be entered into an online database by each site. The Coordinating Center will manage and track the samples as they are shipped to CLASS and the Repository. Access to electronic shipping manifests via a secure online site (EDC CloudShare) is available to the Repository personnel responsible for inventorying the incoming shipments. Previously the Repository entered the data from the paper SCR forms which were (and still are) included in each shipment. The electronic forms will eliminate the need for this data entry step at the Repository.

Table 6.1
Integrated Summary of Specimen Shipping for Visit 15

	Purpose	Vol	Medium	Label	Cap	Store	Ship To	Freq	Packing
CLASS Specimens:									
1	Hormones, insulin	1 x 2 mL	Serum S1	CLASS	Red	<-20C	CLASS	Monthly	Dry Ice
2	Endo Mass Spec	1 x 1 mL	Serum S2	CLASS	Red	<-20C	CLASS	Monthly	Dry Ice
3	Glucose; Lipids	1 x 1 mL	Serum S7	CLASS	Red	<-20C	CLASS	Monthly	Dry Ice
4	Inflamm Markers	1 x 1 mL	Serum S8	CLASS	Red	<-20C	CLASS	Monthly	Dry Ice
5	Adipokines	1 x 1 mL	Serum S9	CLASS	Red	<-20C	CLASS	Monthly	Dry Ice
6	Clotting factors	1 x 1 mL	Plasma P1	CLASS	Blue	<-20C	CLASS	Monthly	Dry Ice
7	CV Markers	1 x 1 mL	Plasma E1	CLASS	Purple	<-20C	CLASS	Monthly	Dry Ice
8	Inflamm Markers	1 x 1 mL	Plasma E2-3	CLASS	Purple	<-20C	CLASS	Monthly	Dry Ice
Repository Specimens (Precision Bioservices):									
9	Repository (Serum)	8 x 0.5 mL	Serum S3-6; S10-13	Repository	Red	<-20C	Repository	Monthly	Dry Ice
10	Repository (Citrate)	3 x 0.5 mL	Plasma P2-4	Repository	Blue	<-20C	Repository	Monthly	Dry Ice
11	Repository (EDTA)	7 x 0.5 mL	Plasma E4-10	Repository	Purple	<-20C	Repository	Monthly	Dry Ice
12	Repository (Urine)	4 x 2.0 mL	Urine U1-4	Repository	Yellow	<-20C	Repository	Monthly	Dry Ice

For Visit 15 sampling schema diagrams, see Appendix.

4.2. DAILY HORMONE STUDY (DHS) SAMPLES

4.2.1. DHS Urine Kit, Collection, Processing, and Onsite Storage

4.2.1.1. DHS Urine Collection Kit

Specimen collection supplies for the DHS urine collection were provided to all SWAN study sites by the CLASS laboratory. Supplies were set up in modules, in which one packet included all supplies necessary for the collection of DHS urine for one participant.

Four boxes were provided. Two of the boxes (marked with a red dot to indicate non-frozen storage) contained 50 labeled yellow-cap vials (25 pairs), with each pair arranged in sequence, marked "A" and "B" as indicated by numbers on the inside of the box and on their labels. The other two boxes (marked with a blue dot to indicate that they should be placed in the freezer to receive the samples for frozen storage) contained dividers with spaces for 50 vials, a log sheet, two permanent markers, 50 paper cups and 2 plastic urine collection cups. The log sheet was to be retained by the site and used at the time of kit retrieval. The subject ID number was to be placed on the log sheet immediately upon issuing a kit.

CLASS Urine Collection Kit Contents List:

- 100 pre-labeled Urine Vials each containing 200uL of 50% glycerol
- Two (2) permanent markers
- Two (2) plastic Urine Specimen collection cups
- Fifty (50) paper Urine Specimen collection cups
- Four (4) boxes with dividers
- Urine Collection Log Sheet (to be kept at site)
- A Daily Diary for the participant to fill out each day of collection

4.2.1.2. DHS Urine Specimen Collection

Daily samples of urine for measurements of endocrine markers were obtained each morning throughout a woman's menstrual cycle. Samples were collected from the first day of the cycle until the first day of the next cycle, or for up to 50 days.

If the participant went to sleep before midnight and woke up with her period having started, she collected her first day's urine upon awakening that morning; if her period began during the day, she began collecting urine the next morning upon awakening. (Daily urine samples were all to be obtained from the first urination in the morning upon awakening.) Collection of urine every morning continued until the first day of her next menstrual period, or through the fiftieth consecutive day, whichever occurred first. The participants were informed to call the site on the last day of urine collection to schedule phlebotomy and specimen retrieval.

After collection, the urine vials were promptly (within an hour) placed in the box in the freezer to match the numbers on the bottom of the box. If the participant

collected her urine specimen somewhere other than her home one morning, the specimen was to be refrigerated within 3 hours and frozen within 24 hours. The woman was to write on the Daily Diary, "Not Cold".

At the time of kit pick-up, kits were placed in a styrofoam or plastic insulated box (like an igloo), containing 2 pieces of previously frozen blue ice. The box was taped (if foam) or snapped (if plastic) shut. The transportation time was not to exceed 1 hour. If participants refused a home visit to retrieve the kits, they could transport the specimens to the site in the styrofoam box or a cooler packed with at least 2 trays of ice cubes or 2 pieces of previously frozen blue ice. Again, transportation time was not to exceed 1 hour.

4.2.1.3. DHS Urine Specimen Processing & the Urine Collection Log

Site clinicians kept the samples on ice in the container in which they were received while they filled out the Urine Collection Log. Recorded on the form was: participant ID number, visit number, participant initials, and site. Each tube was visually inspected for fill level and integrity of the tube. The date was written in the Urine Collection Log box to the left if the specimen was present, and the corresponding date was checked in the box for the "B" series of tubes as long as its date matched the "A" tube. If the date on the "B" tube did not match, the correct date on the "B" tube was recorded in the box on the Urine Collection Log. Comments were added to the right of the tube number and letter. Missing tubes, uncapped tubes with urine in them, partially filled tubes or other tube integrity problems with the specimen were recorded in the comments box. *NOTE:* If a tube was missing or empty, staff recorded the date as '–9/-9/-9-9' and recorded 'EMPTY' in the related comments field.

DHS URINE PROCESSING NOTE: In 2007, the majority of the DHS urine samples from Visits H1-H6 were aliquoted down from 5-mL tubes to 0.5-mL tubes, creating 10 aliquots of each daily sample. This activity was performed at SeraCare (now Precision Bioservices) following a detailed protocol outlined in SeraCare's SOP25347.

4.2.1.4. DHS Urine Temporary Storage at Sites

As soon as the Urine Collection Log was completed, the urine vials were maintained in a freezer at -20°C or below until they were shipped to the Repository. Repository DHS shipments occurred monthly.

4.2.2. DHS Serum Kit, Collection, Processing, and Onsite Storage

4.2.2.1. DHS Serum Collection Kit

DHS serum specimen collection supplies were also provided to study sites by CLASS as part of a module. Each module included all supplies for the collection of serum specimens for endocrine markers and Repository samples.

The content of each module was as follows:

One (1) pre-labeled red-top (serum) Vacutainer tube
One (1) pre-labeled red cap large serum cryovial (CLASS)
Six (6) pre-labeled red cap small serum cryovials (Repository)
One (1) transfer pipet with markers at 0.5 mL and 1.0 mL
Two (2) absorbent pads
Two (2) shipping bags (one each for CLASS and Repository)

4.2.2.2. DHS Serum Specimen Collection

a. Specimen Labeling/Logging Procedures

The following steps were followed when preparing for DHS serum collection:

1. Remove the pre-labeled CLASS tubes.
2. Enter the date of the collection and three Subject initials on all the tube labels, corresponding to the first, middle and last name. Use a hyphen if no middle name is given. Print the initial letters clearly in plain block letters.
3. The draw date must correspond with the date of the visit. Therefore, **DO NOT** enter draw dates ahead of time.
4. **DO NOT** use a vial (i.e., 20B) intended for another collection (i.e., 25B) and manually change the vial number. This manual change will cause an error when entering the barcode data into the Repository database.

b. Blood Collection Protocol:

Using a Vacutainer multi-sample needle, collect one 10 mL red-top Vacutainer tube of blood, and immediately invert it 8-10 times for gentle mixing after collection.

A butterfly needle (21 g) and/or 5-mL ("pediatric") Vacutainer may be used on women with smaller veins.

4.2.2.3. DHS Serum Specimen Processing at Sites

Sites followed a standard protocol for processing DHS serum onsite, as follows:

a. Before Centrifugation

Allow red-top tubes to sit at room temperature for 30 to 60 minutes maximum (preferred) to allow optimal clot formation, then refrigerate the tubes (4-8 °C) for 30 to 60 minutes (preferred) before centrifugation. If collection is occurring at a field site, the 30 to 60 minutes of transport time (in the car) will count towards the 30-60 minutes at room temperature for clotting. If more than 1

hour transportation time is needed, put the tube on ice after 60 minutes (if possible).

b. Centrifugation

Within 2 hours of collection, centrifuge the red-top tube:

- in a refrigerated centrifuge (4 -8 °C)
- at a minimum force of 1300 X g
- for 20 minutes.

c. Aliquotting

Using the plastic, disposable pipet provided in the kits, transfer the serum as follows:

- From the red-top Vacutainer, pipet 2.0 mL serum to the large vial (D1) and pipet remaining serum into the smaller vials (D2-D7) in 0.5-mL aliquots. If insufficient serum exists to make all aliquots 0.5-mL, distribute the serum evenly across the tubes (D2-D7).
- Should cells or platelets be aspirated in the aliquoting process, centrifuge the tube a second time to prevent the carryover of cells or platelets to the next sample vial.

d. Complete the Specimen Collection Record

- Check that ID labels are correctly affixed to the Specimen Collection Record.
- Note any problems such as insufficient volume, spills, etc. on the Specimen Collection Record.

4.2.2.4. DHS Serum Temporary Storage at Sites

After checking that all aliquot tube caps are secure, and that all labels are secure on the tubes, separate the samples into two shipping bags: 1) the original bag that held the Serum Specimen collection supplies (for CLASS) and 2) a second bag provided in the module (for Repository). Seal the bags securely and place them upright in a -20 °C (or lower) non-self-defrosting freezer. Monitor and record freezer temperature daily and record in freezer temperature log. Store frozen specimens locally at least overnight and for no more than 30 days. Separate specimens into the designated shipping bags prior to freezing to eliminate the need to open frozen bags later and to avoid partial thawing of specimens when preparing them for shipment to the laboratory or Repository.

4.2.3. Shipping DHS Specimens to the Repository

DHS Samples were shipped to the Repository on a monthly basis, and included:

- DHS URINE – Up to 50 yellow-capped urine vials marked "B" (01B – 50B). Each vial contained 5 mL of urine and a small amount of cryoprotectant (glycerol).
- DHS SERUM – 6 red-capped serum vials (D2 - D7) , vials at 0.5mL in volume

The Repository Shipping Record was included in each shipment, along with copies of the entire DHS Specimen Collection Record and the entire DHS Urine Collection Log for each ID/Visit included in the shipment. A copy was filed as part of the site records.

Complete shipping instructions for the DHS Repository samples are as follows:

Place the yellow-capped "B" urine vials and six red-capped serum vials (D2 - D7) into an empty box (or reclosable plastic bag). Place pre-affixed Dri Mop™ absorbent pads in each box (bag). Assemble all boxes awaiting shipment, with only one box (bag) per subject in a shipment, complete the Repository Shipping Record form, and transfer boxes (bags) to a reusable Styrofoam shipping container (e.g. Thermosafe™ #399). Add approximately 10-15 lb dry ice, close the Styrofoam lid securely, then close and seal the outer container. Remember to include the Repository Shipping Record and copies of the DHS Specimen Collection Record and the DHS Urine Collection Log for each ID/Visit included in the shipment. Attach to the shipping box the red and black biohazard label and a shipping label addressed to:

Address:
McKesson BioServices
685 Lofstrand Lane
Rockville, MD 20850
Email for sites: swan@mckesson.com

Contact Person:
Christine Bravo
(301) 424-7658
(301) 340-3275 Fax
Christine.Bravo@mckesson.com

Affix a Dry Ice Label (9, International Goods) reading: "Dangerous goods, shipper's declaration not required. Dry Ice 9, UN 1845, 1 x 6.6 kg" and add your name and address as sender.

Fax the Repository Shipping Record to McKesson (prior to sending the shipment). McKesson BioServices will confirm receipt of shipment notification messages and inform sites whether it is okay to proceed with shipment.

Ship prepaid (by Repository) for overnight delivery by the shipper (FedEx).

Attach the shipper's copy of the air bill to a copy of the completed Repository Shipping Record and file the forms locally.

McKesson BioServices will confirm receipt of shipment by e-mail.

4.3. DNA SAMPLES

In 2002-2003, spanning Visits 04-06, SWAN participants had the option of donating blood and buccal cells for DNA extraction and B-cell immortalization. Participation was voluntary and the participants signed an Informed Consent Form (see section 3.3).

A one-time collection of blood served as the substrate for the preparation of genetic and cellular materials for the DNA Repository. This collection typically took place at the time of the follow up clinic visit. Two 10-mL ACD Vacutainers of whole blood were drawn for this purpose. Additionally, a mouthwash/buccal cell sample was collected. Participants did NOT have to meet requirements for fasting or time of month cycling for these collections. Specific collection and storage protocols were as follows:

4.3.1. DNA Specimen Collection and Onsite Activities

4.3.1.1. DNA Specimen Collection Supplies Kit

DNA specimen collection supplies were provided to study sites in modules created by McKesson BioServices. Each packet included all supplies for collection of specimens for the DNA Repository. For each subject, McKesson provided the following:

- Two (2) 10-mL Vacutainer tubes with ACD preservative
- Labels for the Vacutainer tubes
- Scope™ Mouthwash (Original Mint flavor) - 1.5 fl. oz.
- Bitran™ reclosable plastic bag - 6" x 6"
- Reclosable plastic bag - 6" x 9" bag with outer pouch
- Nalgene™ sterile 15-mL cryovial
- Shipping instructions
- Temperature Tracker datalogger

Specimen collection records were also provided. The subject ID number was placed on the specimen collection record immediately upon issuing a kit. The site retained one copy, while the other copy was shipped with the biological specimens.

4.3.1.2. DNA Specimen Collection

DNA samples were to be drawn, whenever possible, Monday through Friday for shipping no later than the following day to ensure sample quality and integrity.

<u>Blood Collection protocol:</u>

Using a Vacutainer multi-sample needle, collect two tubes of blood:

Two (2) – 10-mL ACD Vacutainer tubes. Immediately after collection, invert each tube 8-10 times for gentle mixing.

<u>Mouthwash/spit Collection Instructions for Participants:</u>

- Do not eat, drink, or rinse your mouth for one hour before collecting the saliva sample.
- Open the collection container and fill it half full of mouthwash (to the red fill line).
- Swish the mouthwash from the container around in your mouth vigorously for 45 seconds. We'll watch the clock while you do this. Do not gargle or clear your throat.
- Holding the container close to your mouth, spit all of the mouthwash back into the container. Replace the top on the container, and screw it on tightly.

4.3.1.3. DNA Collection Record/Log and Specimen Processing at Sites

Because the DNA study only required the collection of 3 specimens (2 blood tubes and 1 mouthwash/saliva specimen), the Specimen Collection Record and Specimen Collection Log were combined into one form. The technician filled out this form at the clinic site. The form was completed with information including date, participant ID and date of birth, and the corresponding specimen labels were affixed to each specimen collected. Each tube was inspected. Missing tubes, partially filled tubes, or other problems with the specimen were recorded on the Log.
NOTE: If a tube was missing or empty, staff recorded the date as '–9/-9/-9-9' and 'EMPTY' in the related comments field.

While the sites were responsible for effective collection, as listed above, no specimen processing for these samples took place at study sites. All processing occurred at McKesson.

4.3.1.4. DNA Specimen Temporary Storage at Sites

DNA specimens required NO REFRIGERATION OR FREEZING at the sites.

Clinic personnel were to: visually inspect each tube for fill level and vial integrity; check that all ACD tube caps are secure; and check that all labels are secure on the tubes.

DNA Specimens were to be retained at the site for no more than 24 hours prior to shipment to the Repository. (If a Saturday draw could not be avoided, it was retained until the following Monday.)

4.3.1.5. Shipping of DNA Specimens to the Repository

To package the shipment, these instructions were followed:

Place Vacutainer tubes and mouthwash/saliva collection containers into the appropriate sections of the DNA shipping container (up to 4 sets/participants). Place both sections into the reclosable plastic bag. Remove all air from the bag and seal it. Place both specimen sections back into the styrofoam shipping kit.
Take the temperature tracker and hold down the start button until a star appears in the window. Once the star appears, the tracker is activated and should be placed between

the ACD tubes and the buccal cell collection, to monitor temperature in the shipper during shipment, until it is opened for processing.

Complete the Repository Shipping Record. Remember to include the Repository Shipping Record and copies of the DNA Specimen Collection Record and the Informed Consent Form for each Participant included in the shipment.

No blue ice or dry ice is added. Close the Styrofoam lid securely and then close and seal the outer container. Attach to the shipping box the shipping label addressed to:

Address:
McKesson BioServices
685 Lofstrand Lane
Rockville, MD 20850
Email for sites: swan@mckesson.com

Contact Person:
Christine Bravo
(301) 424-7658
(301) 340-3275 Fax
Christine.Bravo@mckesson.com

Ship via FedEx WITHOUT BLUE ICE OR DRY ICE, prepaid (by Repository) for overnight delivery by the shipper. Attach the shipper's copy of the airbill to a copy of the completed Repository Shipping Record and file the forms locally at the site. Confirmation of receipt of samples will be sent by McKesson by e-mail.

4.3.2. DNA Specimen Activity at McKesson

SWAN clinic sites shipped specimens for DNA preparation and B-cell transformation to McKesson BioServices within 24 hours of specimen collection. At McKesson, any necessary discrepancy reconciliation between samples and consent forms received was completed. Also, the buccal cell samples and one of the 10ml whole blood samples were processed into DNA Pellets and stored for future use. The temperature tracker information was downloaded and stored in the study processing data at McKesson.

4.3.2.1. Consent – Sample Discrepancy Reconciliation

Upon arrival at McKesson BioServices, the receiving technician matched the participant's Informed Consent Form with the blood tubes and buccal cell collection cups. In the event that the specimens did not match the information on the Informed Consent Forms, the specimens were still processed and stored for a period of seven days and an Inconsistency Form was sent to the site for clarification. The possible specimen dispositions are listed below:

Disposition	Specimen	Processing Lab
DNA from Buccal Cells	1 collection cup	McKesson BioServices
DNA from Blood	1 Vacutainer	McKesson BioServices
B-cell Immortalization	1 Vacutainer	BBI Biotech

If the Informed Consent Form did not specify a process, but the specimen was received in the shipment, the specimen was processed using the following guidelines.

A.	Problem:	DNA from Buccal Cell not specified
	Temporary solution:	Specimen processed into a pellet and frozen.
		No DNA extraction until problem resolved.
B.	Problem:	DNA from Blood and B-cell Immortalization not specified
	Temporary solution:	Vacutainer #1 processed up to the buffy coat stage and frozen.
		No DNA extraction until problem resolved
		Vacutainer #2 transferred to BBI Biotech for processing.
		B-cells not transferred to repository until problem resolved.
C.	Problem:	B-cell Immortalization and DNA from Blood specified.
		Only one vacutainer received.
	Temporary solution:	Specimen processed following blood DNA extraction procedure.

The SWAN DNA Repository Inconsistency Form was faxed to the SWAN site for resolution. Possible resolutions included: Missing specimen(s) shipped, or missing Informed Consent Form that matched received samples sent.

If the problem could not be resolved, the specimen was destroyed, and the comment documented. McKesson BioServices removed the specimens from the freezer and destroyed them by autoclaving. If the specimens were being processed for B-cell Transformation, BBI Biotech was notified that the specimens were to be destroyed. The SWAN McKesson DNA Repository Inventory Database was updated, showing that the specimens were destroyed.

4.3.2.2. Buccal Cell Sample Processed and Stored as DNA Pellet

Buccal cell samples were spun for 15 minutes at 2700 rpm. The supernatant was removed, leaving only the buccal cell pellet, which was then resuspended in 1.0 mL of Tris EDTA pH 8.0, transferred to a 2.0-mL cryovial, and stored at –80 ^{o}C.

4.3.2.3. One Whole Blood Sample Processed and Stored as DNA Pellet

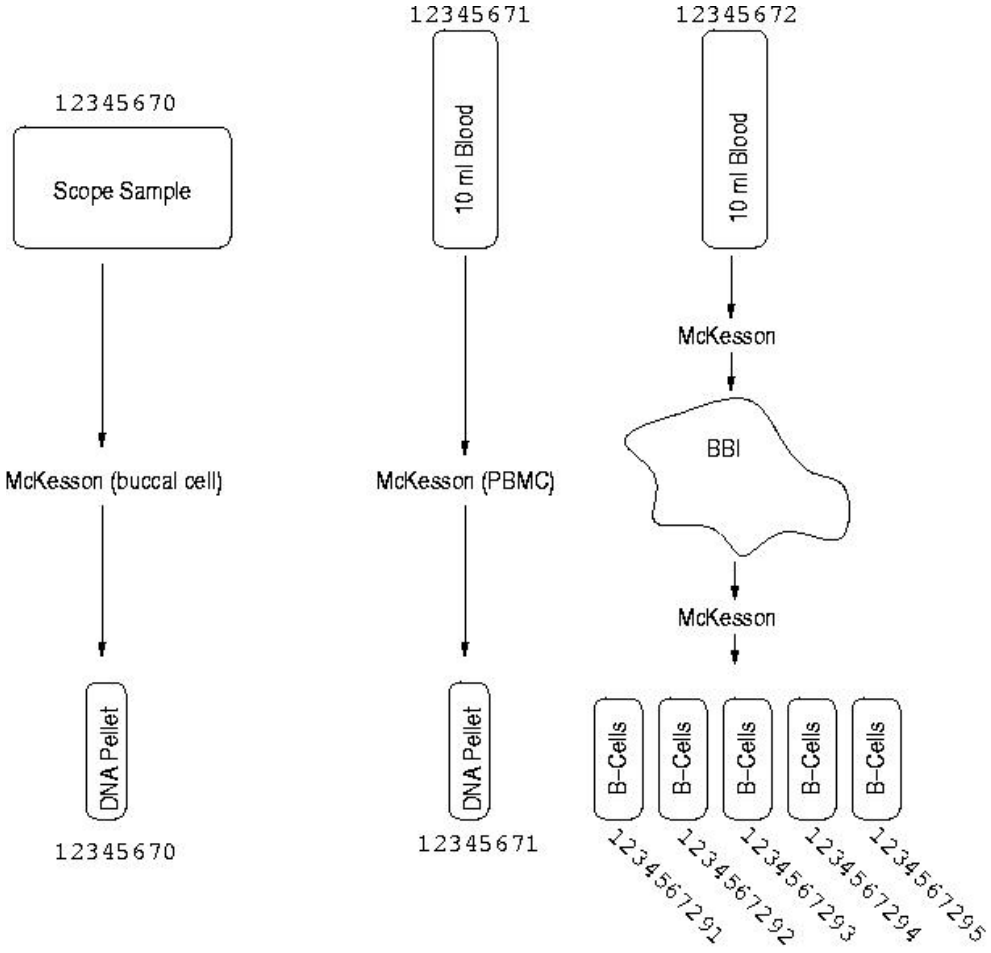
One whole blood was processed to a pellet state. The contents of the tube were pipetted into a Lymphoprep™ tube and the empty tube washed with Hank's balanced salt solution at a volume equal to that of the whole blood contents. The Hank's solution was then pipetted into the Lymphoprep tube, and the contents centrifuged for 10 minutes at 2500 rpm. The white blood cells were carefully aspirated,

transferred to a conical centrifuge tube and spun for an additional 10 minutes at 1200 rpm. Then, these steps were repeated. After the final washing, the cell pellet was resuspended in 1.0-mL of Freeze Medium™ (80% DMEM, 10% Fetal Bovine Serum, 10% DMSO) and aliquoted into a 2.0-mL cryovial labeled "PBMC", leaving at least 50 µL of resuspended cells in the 15-mL centrifuge tube. This 50 µL of cells in the 15-mL centrifuge tube was transferred to a microcentrifuge tube to determine cell concentration by adding 50 µL of Trypan Blue dye and counting live and dead cells with a hemocytometer. The sample was frozen and stored in the vapor phase of liquid nitrogen (VP-LN_2, -150 to -180 °C).

4.3.2.4. Second whole blood sample sent to BBI Biotech

The second whole blood sample was shipped to BBI Biotech (now Precision Bioservices) for EBV Transformation (immortalization), see section below.

4.3.2.5. DNA Specimen Processing Overview



4.3.3. BBI Biotech – EBV Transformation

The second whole blood specimen was shipped to BBI Biotech (now Precision Bioservices). There, the whole blood samples were transformed to an immortalized state utilizing the Epstein-Barr virus, using the protocol as follows:

Phosphate-buffered mononuclear cells (PBMCs) were extracted from the second whole blood sample and centrifuged to pellet form. The PBMCs were washed, counted, isolated and frozen, with $5x10^6$ viable cells set aside for Epstein-Barr virus (EBV) transformation. PBMCs were resuspended in 2.5 mL of Cyclosporin-Containing Transformation Medium (CS) and transferred to a slant tube. 1.0 mL of frozen EBV-containing supernatant was then quickly thawed in a 37°-water bath. The PBMC pellet was inoculated with the EBV-containing supernatant and placed in a dual incubation chamber ($37^0C/5\%$ CO_2).

Seven days (+/-2 days) following innoculation, 1.0 mL of culture fluid was removed from each slant tube. 1.0 mL of EBV-containing supernatant was acquired and thawed in a 37 ^0C water bath. For each mL of EBV supernate, 10 µL of 100X Cyclosporin-A stock was added and mixed. The slant tube culture was then inoculated with 1.0 mL of the EBV+Cyclosporin-A mixture. Any pellet growth and bacterial or fungal contamination was recorded. After four weeks (+/-3 days), if the slant tube culture showed pellet growth, medium yellowing, and absence of bacterial or fungal contamination, the culture was transferred to a T25 flask and 6.5 mL of warmed culture medium was distributed to each T25 flask. The pellet was resuspended in a slant tube, transferred to the appropriate T25 flask, and cultures returned to a 37 $^0C/5\%$ CO_2 incubator.

When the T25 culture showed significant cell cluster formation, medium yellowing and absence of bacterial or fungal contamination, it was passed to a T75 flask along with 20 mL of the warmed culture medium. The new T75 flask cultures were placed into a 37 $^0C/5\%$ CO_2 incubator. This process was repeated and incorporated the placement of 60 mL of warmed culture medium into the existing T75 culture flask. Afterward, 45 mL was recollected and transferred to a new, paired flask. Prior to harvesting, a hemocytometer count was performed on all paired T75 flasks suspected of having more than $60x10^6$ viable cells. If the flasks contained the required number of viable cells, they were repooled into a single flask and centrifuged at 1500 rpm (450xG) for 15 minutes, and the supernatant was discarded. For each culture harvest, the remaining cell pellet was re-suspended in 5.0 mL of freezing medium and **dispensed into ten pre-labeled vials** and frozen. *Mycoplasma* testing was done later. Transformed cells were aliquoted into individual cryovials holding approximately 1 million cells each and frozen in VP-LN_2.

4.3.4. University of Pittsburgh Genomics and Proteomics Center – DNA Extraction & Genotyping

One of the10 aliquots of transformed cells was shipped from BBI Biotech to the University of Pittsburgh Genomics and Proteomics Center for extraction, purification, dilution and genotyping. Because of the high cell density, the Puregene™ system was used. In the first

1020 samples, the average yield was more than 210 μ/g with an average purity (260/280 nm ratio) of 1.87.

Single nucleotide polymorphisms (SNPs) associated with six genes related to reproductive hormones were selected for evaluation. The genes included CYP19 (aromatase) located on 15q21.1, 17α-HSD, two cytochrome P450 enzymes related to estrogen catabolism from estrone to hydroxylated estrone urinary excretion products (CYP1A1 and CYP1B1), and two estrogen receptors (ER-alpha and ER-beta receptor). There were 4-5 SNPs selected per gene. SNPs were selected based on a review of the literature and evaluation of information from the National Center for Biotechnology SNP database (www.ncbi.nlm.nih.gov/SNP) and the Celera database (www.celera.com). The specific SNPs and their associated genes are listed in the Section 4 Appendices.

Genotyping was performed through TaqMan™ technology, a high-throughput approach that uses two fluorogenic probes with a "minorgroove binder" that is matched for the SNPs being assayed. When a SNP match was achieved, the fluorescent probe was cleaved, producing a signal. In the absence of a match, displacement caused the quencher to remain in proximity to the fluorophor, and there was no fluorescence signal. Reactions were read on an ABI 7900HT™ and the allele discrimination module software on the ABI SDS™.

NOTE: Currently, Precision Bioservices holds all SWAN genetic samples – pellets, extracted DNA, immortalized cells and buccal cells.

5.0. DATA AND INVENTORY MANAGEMENT

5.1. REPOSITORY RECEIPT AND INVENTORY OF NEW SPECIMENS

5.1.1. SWAN Clinic Sites Ship Frozen Samples to the Repository

According to the standard protocol (see Section 4), all seven SWAN clinic sites ship newly collected serum, plasma and urine samples to the Repository (located at Precision Bioservices) on dry ice using FedEx overnight services.

5.1.2. Repository Facility Receives and Inventories New Specimens

The Repository facility, Precision Bioservices, receives the shipments and follows procedures found in proprietary Standard Operating Procedures (SOPs) of Precision, specifically SOP25220 (Receipt of Incoming Frozen Samples) and SOP25330 (SWAN Study Specific Information).

Generally, shipments are received and placed in temporary frozen storage (-80 °C) and a confirmation email is sent to the shipping site. In SWAN I-IV, the Specimen Collection Record (SCR) forms were sent as paper copies along with the samples, serving as the shipping manifest, and were entered by the Repository into a Forms database using double data entry. In SWAN V (Visit 15), the SCR data is provided electronically to approved Precision personnel

after the clinic sites entered the data into a secure database hosted by the SWAN Coordinating Center. This is typically done within 5 business days of receipt of a shipment.

The SCR data serves as the manifest for the Inventory Scan. The frozen samples, shipped in bags and boxes, are scanned into the Inventory database (the BSI-II), examined visually (for color, vial integrity, volume confirmation), assigned a location, and placed into long-term storage boxes.

Any discrepancies found in this process are included in a Discrepancy Report and sent to the clinic site. Examples of common discrepancies include: samples in the shipment with no forms; missing samples; empty vials; samples received which are intended for assay at the CLASS lab; vials cracked or frozen sideways or upside down.

5.1.3. Samples are Committed into Repository Inventory

When all discrepancies are resolved and all corrections entered into the database, samples are placed into long-term storage. Finally, a 5% location verification is performed for QC purposes.

5.2. INVENTORY MANAGEMENT ACTIVITIES

5.2.1. Inventory Investigations

5.2.1.1. Purpose and Scope

Beginning in 2009 and concluding in 2013, inventory investigations were conducted on stored Repository specimen collections from Baseline through Visit 10. The purpose of these investigations was to examine the accuracy of recorded material types and vial volumes. While the time and financial burden involved in these vial-by-vial investigations was considerable, the SRO and NIA agreed the efforts were warranted to have a firm understanding of available inventory and to be able to accurately promote the specimens to the scientific community.

The extensiveness of each visit year's investigation ranged from 100% of specimens from earlier visits to 5% from later visits, as clinic and repository protocols became more standardized and regulated, and fewer errors were found. The table below shows the percentage of each visit years' collections which were investigated.

Visit	% Inventoried
V00	100%
V01	100%
V02	100%
V03	100%
V04	100%
V05	10%
V06	5%
V07	5%
V08	5%
V09	5%

V10	5%

5.2.1.2. Results

This 5-year investigation resulted in reliably-recorded material types in the inventory database. It also highlighted specific visits, and sites, where vial volumes were consistently accurate, and areas which will require additional volume verification before release. With this knowledge, the Repository ended the collection-wide investigations and updated protocols to perform any additional volume verification work only on vials being requested and pulled for an approved study. This effort shifts the burden of 100% volume accuracy to the end-user investigators, and related costs can be included in Repository recharge (i.e., cost-recovery) rates, in line with industry standards.

5.2.2. DNA Renewal Activities

5.2.2.1. Project and Purpose

A molecular project to renew the SWAN Repository's DNA collection was approved and funded in the Repository III renewal/supplement. The goals of this molecular work were to:

- Renew and replenish the Repository's supply of extracted DNA, a frequently requested specimen type;
- Document/assure that the immortalized lymphocyte cell lines remain viable (frozen in LN2 for 7+ years);
- Document/assure that all immortalized lymphocyte cell lines are free of cross-contamination; and
- Expand the pool of cell lines in anticipation of increasingly more demand (proteomics, metabolomics, and selected gene expression studies).

5.2.2.2. Tasks Involved

The major tasks involved in the project were expanding currently frozen immortalized (EBV-transformed) cell lines, extracting DNA, and performing SNP analysis on 13 previously genotyped SNPs. All tasks were completed at Precision Bioservices.

The project was divided into two phases: Phase 1 included 828 of the 1538 women (roughly half) with DNA available. These 828 were chosen based on menopausal status - having "clean" FMPs at the time Phase 1 began (early 2012). Phase 2 began after Phase 1 showed successful results, and included 403 women who were not in Phase 1 and had since been classified as 'clean post" OR had 2 or fewer vials of extracted DNA remaining in stock. A total of 1231 cells (80%) were included in the project.

5.2.2.3. Results

Precision Bioservices attempted the expansion of all Phase 1 (828) and Phase 2 (403) previously frozen EBV transformed cell lines, with a 97% success rate.

Cell Line Expansion Results: Approximately 97% of cell lines were successfully expanded.

Total Phase 1 and 2 Cells to undergo expansion	**1231**	
EBV Transformed cell lines successfully expanded	1192	97%
Number of EBV cell lines that failed	39	3%

All vials were screened for *Mycoplasma* contamination and sterility, and found to be clean. For each cell line, a portion of the newly expanded cells was used to create new stock of frozen material with the remainder being used for DNA extraction, QC testing of the DNA, and aliquoting.

Following DNA extraction, one sample from each woman was genotyped using 13 off-the-shelf SNPs which had been previously analyzed. Match rates for re-genotyped SNPs from the sex steroid pathway were 94%-100% for the 13 SNPs analyzed.

New Repository inventory/yields:

- 3 Dry cell pellets (each 10 million cells/mL/vial)
    - 1 of these 3 pellets was immediately used to:
        - Create 3 new working stock DNA vials (ready to distribute);
        - Create 1 master stock ; and
        - Genotype the 13 SNPs.
    - 2 remaining pellets stored for future DNA extraction.
- AND 2 vials of Viable Cells (also 10 million cells/ml/vial) – to be used for future expansions.

5.3. REPOSITORY DATA MANAGEMENT AND REPORTING

5.3.1. IMS Services

The SWAN Repository subcontracts inventory management services to Information Management Services (IMS), who work closely with Precision Bioservices. IMS is the developer of Biological Systems Inventory (BSI-II), one of the major repository inventory management and control systems which fully meet Federal IT requirements. IMS was initially responsible for building a custom inventory database, using the BSI-II, to transfer and house the SWAN Repository collections. IMS maintains and updates the database and all electronic resources necessary for tracking and managing the SWAN Repository specimen inventories. IMS provides updated inventory files monthly, via an automated transfer to UM, occurring on the first of each month.

For a list of IMS key personnel and contact information, see Section 2 of this manual.

5.3.2. Inventory Management at UM

The monthly inventory files, transferred from the BSI-II database, are received by email to the Repository Manager (Merillat) on the 1st of each month. The text files (txt.gz format) contain the entire SWAN Inventory, divided into tables by material type: Serum, Plasma, Urine, DNA, and Other (containing genetic materials other than DNA). These inventory tables are used, as needed, to replace existing tables when new inquiries require sample size estimates, embargo or pull lists need to be created.

5.3.3. Quarterly Reports

5.3.3.1. Quarterly Reports from Precision to UM

Precision provides a quarterly report to the UM Repository team, reporting on all activities performed, including incoming shipments received, vial investigations or database updates performed, outgoing shipments released, and other special project tasks. Equipment maintenance and repair performed on the SWAN freezers is also reported and any equipment recommendations are given. An electronic table of new specimens received that quarter accompanies the report.

5.3.3.2. Quarterly Reports from UM to SWAN Sites

The UM Repository Manager produces a quarterly report for the 7 SWAN Clinic Sites in times of new specimen collection. This report shows all shipments received from the sites, plus any deficiencies or problems with the shipments, and looks at the number and volume of vials collected at each clinic, helping to correct any collection or shipping issues in a timely fashion.

5.4. SWAN STUDY DATA MANAGEMENT AT THE REPOSITORY

The SWAN Repository receives copies of clean, frozen datasets from each SWAN study visit, as well as analyses datasets, from the Coordinating Center (CC). The variables in these datasets are indexed in the publicly-available SWAN Data Warehouse (see section 8.1.2). Limited, encrypted datasets are individually created from these data for each approved Repository study (see section 6.3.3).

SECTION 6.0 – REPOSITORY PROCEDURES: APPLICATION, REVIEW, AND RELEASE

6.0. REPOSITORY APPLICATIONS AND REVIEW

6.1. REPOSITORY APPLICATION PROCESS

6.1.1. Independent Online Review of Available Resources

Applicants are encouraged to begin by independently reviewing online descriptions and summaries of available resources to determine whether to proceed with a formal request to obtain resources. Online descriptions and summaries include:

- SWAN Data summarized through Data Warehouse keywords and searches (swanrepository.com)
- SWAN Repository Samples summarized on Biospecimens Page (swanrepository.com)
    - Specimen types available
    - Summary of specimens by visit year
    - Volumes
    - Specimen collection info
    - By Race/Ethnicity

6.1.2. Step One: Inquiry Checklist Submission

6.1.2.1. Inquiry Checklist content and submission

The Inquiry Checklist is the first submission in the process of obtaining Repository resources and is found on the website (swanrepository.com). This brief initial assessment provides information describing the proposed study and the resources necessary to complete it, including which specimen types investigators may be interested in, volumes, and time points of interest, and inclusion/exclusion criteria of the proposed sample set and data set. *The Inquiry Checklist form can be found in the Section 6 Appendices.*

6.1.2.2. Define sample request and sample size

The Inquiry Checklist is submitted electronically to UM Repository staff, who use the information to confirm the availability of data and/or specimens and to provide investigators with sample size estimates. In many cases, sample requests are adjusted and refined based on information provided in this step. An estimate of total fees (section 7.2) for the sample set is provided to the applicant investigator.

6.1.2.3. Timing

This step can typically be completed with 10 business days.

6.1.3. Step Two: Full Repository Application Submission and Review

6.1.3.1. Repository Application content and submission

With adequate sample sizes, applicants proceed with the Full Application. This form is similar to NIH applications, including the major sections: Introduction, Specific

Aims and Hypotheses; Background & Significance; Preliminary Studies; and Methods & Materials. The full Application form is available to complete and submit online, via the Repository website (swanrepository.com). A preview of the Application (pdf document) can also be found there for applicants to preview questions and develop content before beginning their submissions. *The Repository Application form can be found in the Section 6 Appendices.*

6.1.3.2. SWAN Sponsor: Inclusion of a SWAN Investigator

There must be assurances that a SWAN investigator will join the approved investigation to facilitate the appropriate and effective utilization of the information resources provided to the grantee and to provide assistance to the investigators toward the successful completion of the project. This Repository project investigator is expected to have full status accorded a co-investigator on any research endeavor.

If the lead investigator of a Repository application is not a SWAN investigator, a SWAN investigator knowledgeable in the area of the proposed work must be included in the study. This person will be known as the SWAN Sponsor.

6.1.3.3. SRO Review

All submitted Applications are reviewed by the SRO (described in section 2.2). Completed applications are sent to three SRO members including a statistician for review. The SRO primary reviewers evaluate the project's specific goals and hypotheses, the research approach, sample size and power estimates and quality of the laboratory and analytical methods, ensuring that proposed scientific aims can be addressed with Repository resources, that the study has adequate power to address the stated aims, and that no overlap exists with SWAN core or ancillary protocols or with approved Repository protocols. Questions or concerns may be sent back to applicant investigators to respond to. Reviews and reviewer recommendations are sent to the full SRO for discussion and a vote. Possible outcomes are: Approve without revision; Approve with minor revision; Reconsider after more extensive revision; Reject current proposal.

6.1.3.4. SRAG Review

A SRAG review is conducted when any of the following conditions are true of applications:

- Genetic specimens are requested;
- Reserved specimens are requested; or
- No scientific peer review will be done on the proposal.

In these cases, the application and SRO recommendation are sent to the SRAG committee (section 2.3), and SRAG will review the quality of the science and evaluate the ethical use of the requested biologic materials. Their recommendation, along with that from the SRO, will be sent to SC for approval.

6.1.3.5. SC Approval

SWAN Steering Committee approval is required of all applications. There are 11 voting SC members, including the 7 clinic sites plus the Lab, the CC, the SC Chair, and NIA. This vote is conducted via confidential online polls or through the SWAN CC by email vote (from Vicky Palombizio), where votes are emailed from all voting members of the SC back to the CC (Vicky) and results forwarded to the Repository.

Site PIs abstain from voting on Repository applications that they or their SWAN co-investigators are contributing to.

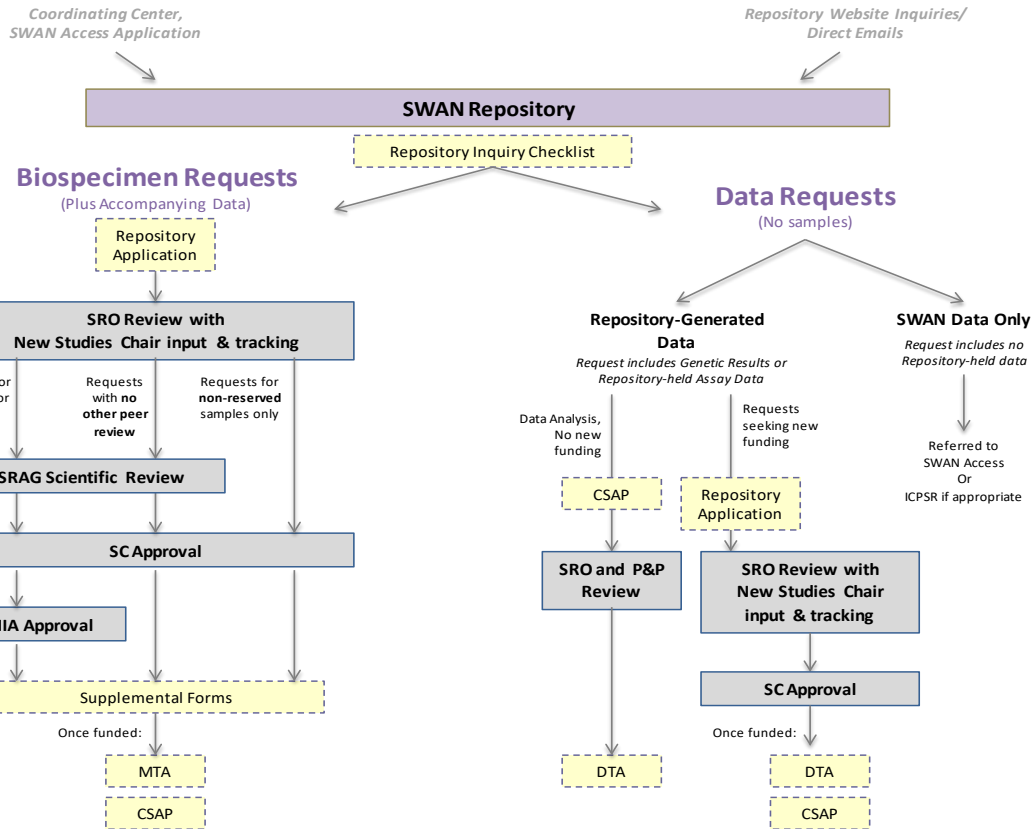
6.1.3.6. NIA Approval

A final approval from the Repository's NIA Representative is required for the release of any Reserved/Restricted or Genetic Samples. This is typically conducted by email from Repository PI to NIA Representative (Winnie Rossi).

6.1.3.7. Timing

Investigators are encouraged to have Repository Applications submitted, fully complete and with all questions answered, at least 6 weeks prior to a grant deadline. A letter will be provided to include with grant materials stating the approval to use Repository resources.

6.1.3.8. Schematic / Flow Chart of Repository Applications and Review



08.28.13

6.1.4. Application Revisions

Applications not approved through Repository review may be revised and resubmitted. In the Introduction of the revised application, these proposals should include replies to any issues or concerns expressed in the initial review. There should be substantial changes in the content of the application, and these changes should be clearly marked for re-review.

6.1.5. Changes to Approved Applications Requiring Re-Review

Recognizing that resubmissions of grant applications frequently require modifications to scientific aims or study design, which may or may not change the scope or specimen requirements, this policy specifies the two situations when the SWAN Repository requires re-review and approval of such modifications:

1. Additional samples are requested.

2. There is a *substantial* change in aims or scientific scope of the proposal.

In these situations, an Amendment Letter to the SRO is required. The Amendment Letter should clearly point out the changes in the request and reasons for them.

If no reserved samples are requested, the SRO will review and approve proposed changes in specimen request.

If reserved samples are requested or if there is a substantive change in scope or aims of the study, the usual approval process will be followed.

6.2 POST-APPROVAL ACTIVITIES

For applications approved through Repository review, several additional actions take place before samples are released.

6.2.1. Award Letter

Notice of the Repository review approval comes officially in an Award letter from the Repository PI to the approved applicants. This letters states specifically which samples are approved and the associated estimated fees, and marks the date from which progress reports are due annually.

6.2.2. Supplemental Form for use of Genetic Materials

A set of agreements between the Repository and investigators approved to receive genetic materials is compiled in a Supplemental Form, which requires the investigator's signature to proceed with the sample embargo. On this form, investigators must discuss the likelihood that the proposed investigation would lead to the need for genetic counseling and are advised that they must be prepared to provide individual results in the event a participant requests them. *The Supplemental Form can be found in the Section 6 Appendices.*

6.2.3. Embargo

The approved set of samples is embargoed for the investigator(s) while project funding is secured. The embargo is granted for up to two NIH review cycles. Prompt updates are expected from Applicants on the outcomes of their grant applications.

If after two grant cycles, funding is not secured for the Repository-approved application, the embargo is lifted from the samples and they are made available to other applications.

6.2.4. Material Transfer Agreements (MTA) and Data Use Agreements (DUA)

MTAs are agreements required to transfer materials (any specimens) from the Repository to another institution. The SWAN Repository begins the MTA process once the approved application is funded. The MTA sets forth the terms for accepting Repository specimens. This Agreement and the application's Award Letter are sent to the receiving institution from UM's Office of Technology Transfer (OTT). The Investigator and his or her institution's officials sign the MTA and return to the UM OTT. Finally, the Repository PI signs for the Repository and the MTA is then fully executed.

In the case of applications only requesting Repository data (and no specimens), a Data Use Agreement (DUA) is used instead of a MTA. The DUA works the same way, but lays out terms for the appropriate use of data and how it should be disposed of after a certain period of time. *The Repository's MTA and DUA forms can be found in the Section 6 Appendices.*

6.2.5. Sample and Data Destruction Verification Forms

Both the MTA and DUA are accompanied by Destruction Verification Forms, which investigators use to document and verify that the Repository resources they received were disposed of in an appropriate way – limiting their use to the approved investigators for the specified aims. *The Repository's Destruction Verification forms can be found in the Section 6 Appendices.*

6.2.6. Concept Proposals for Manuscripts - to SWAN

Concept Proposal forms (CSAPs) for new SWAN publications are distributed to Investigators as Repository materials are released. These forms are submitted by the Investigator, along with the SWAN sponsor, to the SWAN P&P committee when the applications are funded. The Concept Proposal form is also used as an application for requests for data only (no specimens) not seeking new funding.

6.3. RELEASE OF REPOSITORY RESOURCES

6.3.1. Release of Repository Samples

Once the above steps are taken, including fully executing the MTA, the final approved sample pull list is submitted to Precision by the Repository Manager, along with contact information of the receiving laboratory and personnel. The lists are ordered by vial ID or barcode. If any vials are found to be missing or damaged, substitutes may be provided. A timeframe for pulling and shipping the specimens is agreed upon.

Standard procedures are strictly followed by Precision personnel retrieving the specimens to assure the highest sample quality throughout the pulling, QC, and packaging processes. Samples are packed into shippers on dry ice, in accordance with federal and IATA shipping guidelines. Prior to shipment, email notifications are sent from Precision personnel to the contact person at the receiving lab and to the Repository manager with notice of the outgoing shipment and FedEx tracking numbers, and confirmation that the lab is able to receive the shipment.

The samples are shipped via FedEx overnight service. Precision follows up with the lab, confirming the receipt of shipments in good condition.

6.3.1.1. Relabeling Requirements for Release of Samples from 2 Sites

Following requirements set forth by the IRBs of 2 SWAN sites in 2014 (see Section 3.1.2.1 for details), all vials from non-consenting UC Davis participants and all Core vials from Chicago must be relabeled prior to release from the Repository. This involves removing the current label and replacing it with a new label displaying only the original barcode. Precision has developed appropriate procedures to include this extra step in processing outgoing SWAN batches. (This requirement does not affect DNA samples.)

6.3.2. ID Encryption of Released Samples

At the same time the samples are being shipped, the Repository manager sends an electronic manifest to the Investigator and receiving personnel at the laboratory. This manifest lists the samples in the shipment - by Barcode (Tube ID, matching the vial label's barcode) and Encrypted study ID (EID). The EID replaces the regular 7-digit SWAN ID, and is consistent for participants across all visit years. The EID is unique to each application, with the application number appended to the newly auto-generated alpha numeric code constituting the EID.

6.3.3. Release of Repository-coded data sets

SWAN phenotypic datasets without personal identifiers are built, including variables as specified by the end-user and encrypted IDs (EIDs) matching the electronic manifests. SWAN ID encryption adds another layer to assure confidentiality. Detailed codebooks are built for each data set including a data dictionary, methodological description and descriptive statistics.

6.4. DIFFERENT TYPES OF APPLICATIONS

The process above describes the steps taken for the application and review of new Repository applications requesting biospecimens. Below are the modifications in the process for different types of Repository applications.

6.4.1. New Applications, for Data Only

If a new Inquiry Checklist submission indicates interest in SWAN Core data only (no Repository specimens or data), Investigators are referred to the SWAN CC (SWAN Access) or to the publicly available Inter-university Consortium for Political and Social Research (ICPSR) data sets if appropriate.

If a new Inquiry Checklist submission indicates interest in Repository data (but no specimens), then these steps are followed:

6.4.1.1. Data-only Requests for Repository Data, Seeking New Funding

In these cases, applicants submit the full Repository Application to SRO, as usual, and must get SC approval. (SRAG and NIA approval is NOT required.) Once funded, these applications sign a DUA instead of an MTA.

6.4.1.2. Data-only Requests for Repository Data, Not Seeking New Funding

In this scenario, applicants need only to complete the CSAP (in place of the Repository application) and submit it to the SRO and P&P simultaneously, which is the only review necessary for approval. Once approved, a DUA is signed between the Repository and institution receiving Repository data.

6.4.2. Supplemental Applications

If an Investigator is interested in requesting additional data and/or specimens to expand the scope of a previously approved and completed project, *and is staying within the originally approved scientific aims*, a supplemental application can be submitted. A supplemental application includes a progress report and evidence that expanded access will substantially enhance the impact of the research findings. The original aims and sufficient information from the original application should also be submitted to allow evaluation of the proposed supplement in relation to the goals of the original proposal. Supplemental applications are reviewed by the same committees as a new application would warrant, depending upon what materials are being requested.

6.4.2.1. Extensions of the 2006 Genetics Supplement Papers

In the case of an investigator requesting to extend the protocols from the Papers of the 2006 Genetics Supplement of the Am J of Med (Repository Protocols P01-P19), the following application process should be followed: (2012 precedent, J Bromberger, extension of baseline study of depression (P16) to all of SWAN.)

Investigator should submit CSAP to Core SWAN and the Repository, which is reviewed by SWAN and SRO genetics members. The data will remain encrypted, with EIDs in place of SWAN IDs.

Note: a new or amended MTA is necessary if a) additional genetics data is requested (additional SNPs) or if b) the data will be received by an institution other than the original institution.

6.4.3. Hybrid Applications

In the situation where an application includes both a Repository request and a *new data collection* component, applications are submitted through the Repository, but must meet application requirements of both the SWAN Repository and New Studies. These applications will be reviewed by the SRO with an expanded application and review process to comply with New Studies Guidelines.

Most applications to the Repository require dialogue between the Repository and the PI submitting the hybrid application, by which the Repository requests clarification and additional information. The deadline for the submission of the initial draft of the proposal is 10 weeks before the grant proposal is due to the funding agency. The review of Hybrid Studies is anticipated to take 4-6 weeks, once a complete proposal is provided, having all questions from the Repository answered and resolved.

1. *Identification of the appropriate review process* will be addressed by including a question in the Repository Inquiry Checklist asking whether the Repository application also involves the collection of new data from SWAN participants.

2. *Additions to the Repository Application* if the application includes new data collection will include sections on:

    a. **Subject eligibility/recruitment**: Provide a detailed explanation of the study participants to be included in your proposal. Include which site(s) will be used in the proposal.
    b. **Data management issues**: If applicable, provide details on how the data will be collected, entered, processed and/or cleaned. Include information on quality control measures that will be implemented in your study.
    c. **Integration with and impact on core**: Explain how your proposal will be merged into the main SWAN study. State whether any SWAN Investigator can sign up for authorship during the publication phase or whether publication sign-up will be limited to ancillary studies.
    d. **SWAN participant burden**: Indicate whether participant time will be needed to accomplish your aims. If participant time will be involved, estimate the amount of time needed by each participant.

3. *The review process* for hybrid applications:
   a. The chair of New Studies will be appointed to be one of the SRO primary reviewers and review the application with respect to both Repository and New Studies requirements and guidelines.

   b. The New Studies chair will designate one additional reviewer from the New Studies Committee who will review the application with respect to the New Studies requirements and guidelines.

   c. A senior member of the CC will be asked to serve as the statistical reviewer for the Repository statistical review and will also review to assess the feasibility of the data collection and data management plan for the New Studies/new data collection component of the application.

7.0. SWAN REPOSITORY PRACTICES AND POLICIES

7.1. SAMPLE USE POLICIES

7.1.1. Furthering the SWAN Mission

A major factor influencing the decision to release SWAN Repository samples is whether the aims of the application align with the SWAN mission to help scientists, health care providers and women learn how mid-life experiences affect health and quality of life during aging.

7.1.2. Use of Validated Assays

Applications for Repository specimens should include evidence that the proposed assay has been fully validated. Repository specimens are not released to applications aiming to validate new assays, unless approved by the SRO. Tests for assay validation, such as “Test for Ligand Specificity” and “Test for Comparability of Results” are described in the “Assay Validation Criteria” found in the Section 7 Appendices and distributed to applicants when appropriate.

7.1.3. Reserved Samples

7.1.3.1. SWAN Definition of Reserved Samples

To help protect the overuse of the Repository’s most valuable samples, defined by both scientific importance and scarcity, the Repository has identified and flagged

certain specimens as "Reserved". The SRO and SWAN SC approved the following definitions for Reserved samples in the SWAN Repository:

a.) **Serum**: All of the baseline serum collection is considered reserved, due to both scarcity and increased scientific value. For all subsequent visit years, serum vials can be identified as Reserved based on the combination of two factors:

- "Clean Post" indicating whether or not this participant would go on to have a natural menopause transition (defined as "not reporting any Hormone Therapy use before or at the first visit she was classified as post-menopausal").
- "Scarcity" or Vial Count per Visit Year.

ORIGINAL, 2012

In SWAN studies participants with a clean menopausal transition have a higher scientific value, therefore vial counts of 10 or fewer per visit year are flagged as Reserved. Serum from women without a clean transition are Reserved when 5 or fewer vials remain. The last serum vial for any woman at a visit year is flagged as restricted.

The following table shows the cut-off points for reserving Repository serum samples based on these two values:

IF CLEAN POST=	And SCARCITY =	THEN 'RESERVE' FIELD =
Clean Post = No	2-5 vials left	Reserved
Clean Post = Yes	2-10 vials left	Reserved
(either)	1 vial left	Restricted

REVISED, 2015

In the Fall of 2015, the SWAN Repository examined the consistent voting patterns from reviewing committees. These patterns suggested it would be worthwhile to consider lowering the bar for Reserved serum to reduce time of application review, reduce committee effort and increase utilization.

Therefore, the recommendation was made and approved to move the Reserved cut-off line for the clean-post women down from 10 or fewer serum vials available to 5 or fewer for all women. With approval from SRO, SC and NIA, the current definition of Reserved Serum is:

IF SCARCITY =	THEN 'RESERVE' FIELD =
2-5 vials left	Reserved
1 vial left	Restricted

Note: Review would still give special consideration to requests for baseline and clean post samples.

b.) **Plasma**:

1. **EDTA Plasma** is all reserved due to scarcity. Across study visit years, only 1-3 vials of EDTA plasma remain.
2. **Citrated Plasma** is not reserved, as this resource has not been utilized.

c.) **Urine**: Urine samples are plentiful in the Repository and are therefore not considered Reserved.

d.) **DNA**: SWAN Repository DNA is renewable through expansion of the EBV-transformed cell line and extracting new DNA. It is therefore not considered Reserved.

7.1.3.2. Specific Guidelines for Use of Reserved Samples

The following guidelines were established to support decisions by the SRO and SRAG to release Reserved and Restricted samples:

- Reserved samples should not be used if the question can be answered using other available samples.
    - Other available samples include non-reserved SWAN samples from other comparable women, from different time points, and/or non-pristine samples.
- Reserved samples should not be used for exploratory investigations or very preliminary hypothesis testing.
    - Prior scientific evidence should exist to justify the hypothesis/proposed analyses.
- Reserved samples should be used to substantively advance scientific knowledge and for innovative science.
- The use of Reserved samples should be more strongly considered for studies that are consistent with the mission of SWAN.

7.2. THE COST RECOVERY PROGRAM

7.2.1. Initialization of the Repository's Cost Recovery Program

The SWAN Repository responded to the directive of NIA to establish a cost-recovery mechanism to help support the expenses involved in the biobanking of SWAN specimens. The fee schedule was developed based on actual collection, processing, storage and distribution costs, and is reviewed and approved by the University of Michigan's Office of Financial Analysis on an annual basis. Official quotes are provided to individual applicants when the final sample selection is defined. Fees are the same for both SWAN Investigators and non-SWAN Investigators.

7.2.2. Current Fee Schedule

SWAN Biospecimen Costs – per vial

	Serum, Plasma, Urine	Extracted DNA	Transformed Cell lines
Direct Costs	$18	$65	$148
Indirect Costs*	+30%	+30%	+30%

Rates are valid through 10/21/2015.

SWAN Application & Data set fees

	Application Processing Fee	Encrypted Data Set Construction Fee
Direct Costs	$400	$950
Indirect Costs*	+30%	+30%

Rates are valid through 10/21/2015.

*Indirect costs apply to non-University of Michigan applicants.

7.2.3. Exemption / Reduction of Repository Fees

All exemption/reduction requests require NIA approval. There have been instances when an exemption from or a reduction in recharge fees was granted to Repository applicants. Precedents include:

In 2013, Application #052 NELA (Lasley) requested a waiver of the Repository's recharge fee for his small intramural project using DHS urine specimens. Special consideration was given to this case because 1) the requested samples were urine (particularly, DHS urine) which is plentiful and being considered for reduction, and 2) it was for a pilot study being used to test an idea where limited, and often only internal, funds are available. NIA approved the exemption.

In late 2013 – early 2014, Application #054 Melatonin (Greendale) requested a reduction in Repository recharge fees to a feasible amount, in her request for over 50,000 DHS urine samples to measure melatonin in over 2,500 cycle in the DHS study. The recharge rates per vial, for that many samples, made the cost prohibitive (more than $1 million). Completely waiving the fee was not an option as the Repository did not have funding to cover the cost of pulling and shipping such a large volume of sample. Permission was requested from NIA to reduce total costs of the recharge (including UM required indirect on the recharge amount) to $220,000- the maximum amount Greendale was allowed to include in her grant budget. Special consideration was again given because the samples requested were urine (particularly, DHS urine), and the release of this large of volume of samples had the potential to reduce overall storage costs. NIA approved the reduction.

7.3. RETURN OF DATA TO THE REPOSITORY

New data created from the analysis of SWAN Repository specimens must be returned to the Repository within 3 years of end of grant funding, as specified in the MTA. It is the responsibility of the Study PI to return results in a timely manner. Once returned, these data become a part of the Repository's Data Warehouse and are available to the Scientific Community through future Repository applications.

7.3.1. Standard Data Return

Typically, approved and released Repository sample manifests are encrypted to match a limited set of SWAN data which specifically supports the study's approved aims. The encrypted IDS (EIDs) make the datasets unique and no longer linkable to the rest of SWAN data. (If additional variables are required during the course of analysis, they must also be encrypted to match.)

In these cases, the Standard Data Return process applies for returning newly generated data to the Repository. Investigators work with protected (genetic and non-genetic) assay results for a period of up to 3 years-post funding.

- Advantage: Additional protected time to publish from generated results.
- Disadvantage: Samples and SWAN data are encrypted (to match). Additional variables needed must also be encrypted.

7.3.2. Integration with SWAN Core

Non-genetic assay results returned to the Repository are eligible for integration with SWAN Core data. These data are then managed at SWAN's Coordinating Center (CC). EIDs are un-encrypted and these data contain the common SWAN ID. Approval for integration is granted after review and consultation with the Study PI and SRO. In general, only data that are obtained on all or a substantial proportion of SWAN participants is considered eligible for integration.

7.3.3. Expedited Data Return Option

SWAN investigators working with non-genetic Repository materials (serum, plasma, or urine) have an expedited data return option. They may opt out of the ID-encryption, allowing the sample results to be linkable to all SWAN data. However, in choosing this option, they must also agree to return the assay results immediately to the Repository and SWAN Coordinating Center, and thus the scientific community, without a protected window of time.

- Advantage: Repository samples and SWAN data sets do not get encrypted; SWAN data retains regular 7-digit SWAN IDs.
- Disadvantage: Generated results are open for use by other approved SWAN investigators and scientific community.

This option is available for any SWAN Investigator using non-genetic Repository materials, and is working under the auspices of the SWAN Laboratory (CLASS). The SWAN V work is one scenario where the expedited data return option was employed.

7.3.4. Steps to Return Data

1. Instructions to the requestor about materials to be returned should include:
    a. *A final dataset*, with EID and newly-generated values & variables (SAS or Excel format). This should include the name of the analyte(s), units of measure, EID and visit. If values are analyzed in duplicate or triplicate, individual values should be provided rather than a summary value.
    b. *A codebook*, with descriptions of all new variables along with relevant response codes, when applicable (Word or pdf format). This codebook should include documentation about all included variables and their coding.
    c. A 1-2 page *Assay Description and Protocol* (Word or pdf format)
    Identify if this is a proprietary protocol, and what kits or major reagents were used (identify with manufacturer's name and lot numbers of kits for future comparability, plus QA information about how well the assay has been performing). Include

boundaries of lower limits and upper limits of detection (if relevant). Also include the laboratory where the analyte was run, as well as the address and lab manager or director's contact information.

2. When these documents are assembled and complete, please contact the Repository for a secure transfer site (do not email data). A transfer site will be established and a link to the site will be emailed to you.
3. Data descriptions are then added to Repository Data Warehouse catalog. Data will be available to subsequent Repository applicants.
4. If a request to integrate with SWAN Core data is received:
    a. Review and consultation will be sought from the Study PI, and then from the SRO.
    b. Final dataset will be prepared for SWAN CC, decrypting EIDs back to normal 7-digit SWAN IDs.
    c. Data and documentation will be securely sent to CC.

NOTE: Data can be returned at any time prior to 3 years after grant funding ends (or as specified in the MTA). Investigators may continue to work on the papers relevant to the approved original aims of their study as long as CSAPs are submitted and approved.

7.4. MARKETING AND ADVERTISING CAMPAIGNS

An advertising campaign was launched in 2013 and again in 2014, to promote the utilization of specific types of specimens unique to the SWAN Repository. SWAN Repository resources were advertised in relevant scientific journals and at relevant scientific meetings.

Journals in which Repository ads were ran include: *Menopause, Fertility/Sterility, Climacteric, JCEM Endocrine News, J Gerontology, Epidemiology,* and *Am J Epidemiology*.

Annual scientific meetings at which Repository ads were included in program materials include: International Menopause Society (IMS), North American Menopause Society (NAMS), International Congress of Endrocrinology (ICE/Endo), and the American Society for Bone & Mineral Research (ASBMR).

These advertising campaigns were considered successful. After each publication or meeting, we saw an uptick in the number of hits to the data warehouse. Our web audience increased from an average of less than 50 new users per month to over 100 new users per month in 2013 and 2014 during advertising periods. We had planned to expand the scope of this advertising program to include scientific journals focused on genomics, metabolomics, and proteomics, and promoting utilization of SWAN resources to specific scientific groups and Institutes who have relevant research programs, however no funding for these activities was available in the no cost extension year.

7.5. PRECISION BIOSERVICES PROCEDURES (confidential – restricted access)

These procedures describe the processes for handling SWAN specimens at the Precision Bioservices facility. Because they are proprietary and confidential, requiring the expressed

approval from Precision Bioservices to copy or release them, they are only listed here by number and title, and are kept in locked file by the SWAN Repository manger.

Precision Bioservices is accredited by the College of American Pathologists (CAP) Biorepository Accreditation Program (BAP) and is ISO9001 and ISO 13485 certified. Precision performs tasks under Standard Operating Procedures, and follows ISBER and NCI Best Practices for Biorepositories and applicable IATA shipping procedures.

7.5.1. List of Precision Standard Operating Procedures (SOPs) on file:

SOP25330 (SWAN Study Specific Information) SWAN Core Procedures

SOP25220 (Receipt of Incoming Frozen Samples) Incoming shipments handling

SOP25205 (Inventory of Incoming Frozen Specimens) Sample inventory

SOP25104 (Forms data entry SOP)

SOP25204 (Outgoing requests)

SOP25347 (Aliquot Procedures)

SOP25308 (Inventory Investigations Protocols)

SOP25204 (Lab Processing, Outgoing Shipments)

SOP712.01 (Business Continuity, Disaster Response and Recovery Plan)

8.0 TOOLS: THE WEBSITE, THE WAREHOUSE, AND OTHER HELPFUL REPOSITORY TOOLS

8.1. TOOLS FOR INVESTIGATORS/REPOSITORY USERS

8.1.1. The Repository Website: SwanRepository.com

SWAN Repository provides for effective utilization of its specimens and data through online tools and resources. The SWAN Repository website (swanrepository.com) provides visibility for the Repository and is the primary point of contact for researchers who are interested in using SWAN Repository specimens. The website was designed to create a contemporary and user-friendly hub of information about the Repository and its resources. Several important tools for investigators are located there, including: the Data Warehouse, the automated online application submission system, summaries of available specimens, specimen-associated costs, forms and references for approved investigators, contact information, and answers to frequently asked questions.

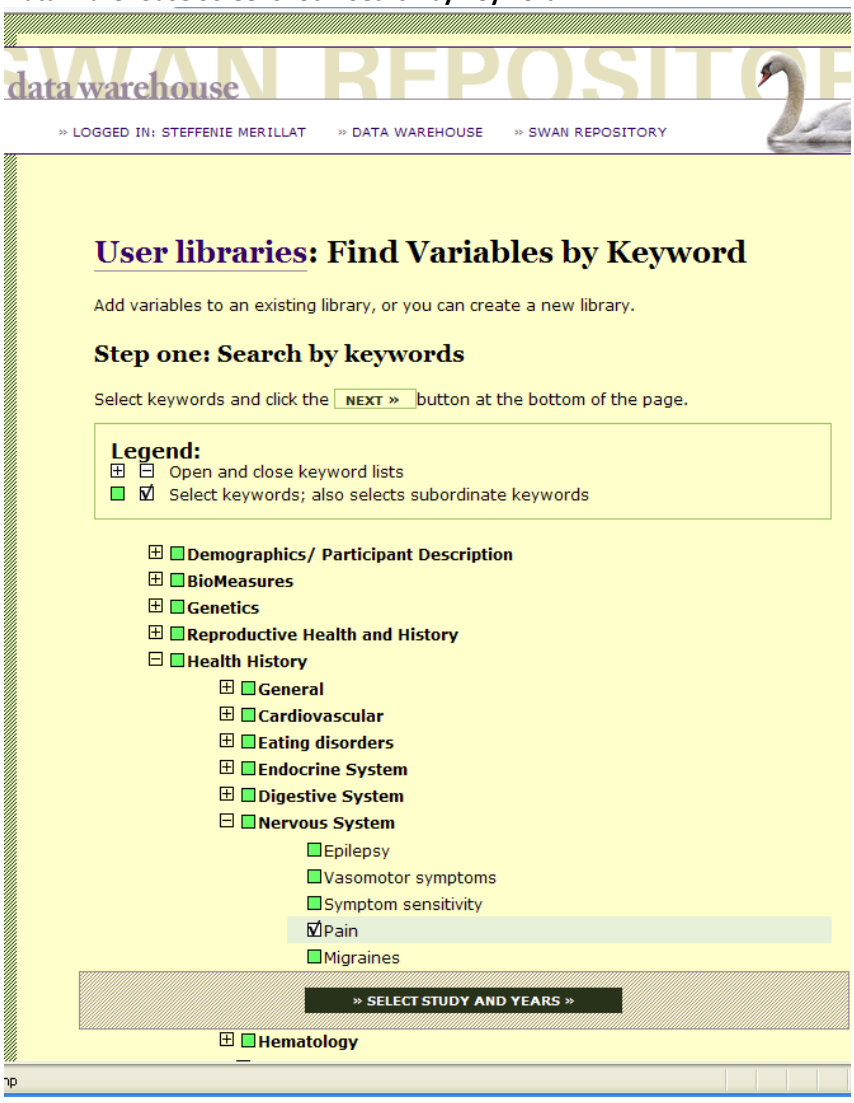
8.1.2. The Data Warehouse

8.1.2.1. Overview

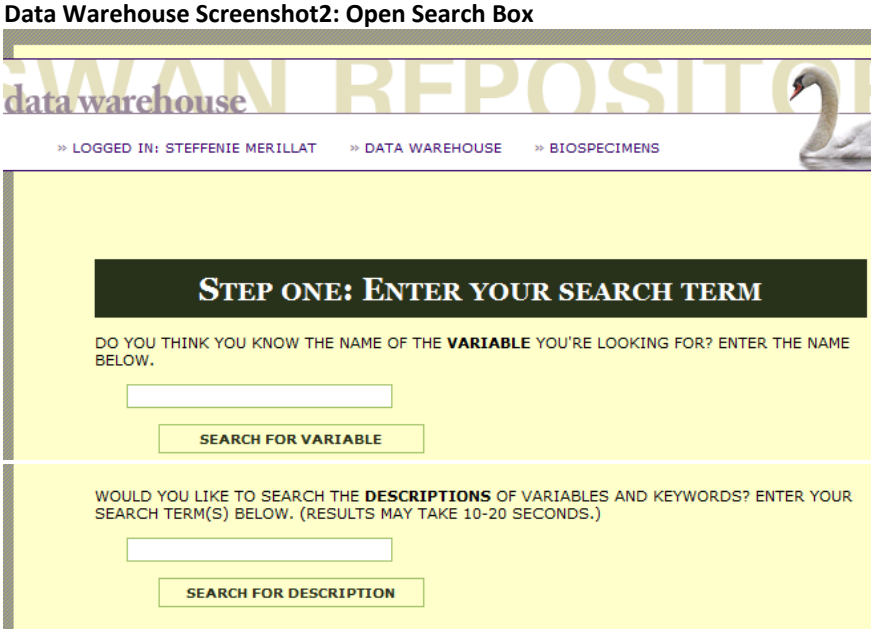
The Data Warehouse is a searchable directory or index of SWAN data, and has become a valuable tool in the management and utilization of SWAN resources. With nearly 20,000 variables collected thus far in the SWAN study, finding specific SWAN data can be daunting even for associated investigators. The Repository constructed the Data Warehouse to help make the data more easily navigated. Without allowing direct online access to the data (which is not allowable under current IRB approvals or SWAN study protocols), the Data Warehouse organizes the nearly 20,000 collected and created SWAN

variables into a searchable directory, based on a keyword search engine, and built using the internationally-recognized National Library of Medicine's MeSH (Medical Subject Headings) hierarchy of medical categories as a guide. Users can choose topics or keywords of interest, then drill down to more specific subtopics (see DWscreenshot1), or search broadly via an open search box (see DWscreenshot2). Users can view all associated variable names and descriptions, response codes, collection methods, assay descriptions, and study years collected, and then select variables, assemble and save them into personal libraries on the site. As studies use Repository specimens to generate more data, this data is returned and also made available through the Data Warehouse inventory and directory.

Data Warehouse Screenshot1: Search by Keyword



Data Warehouse Screenshot2: Open Search Box



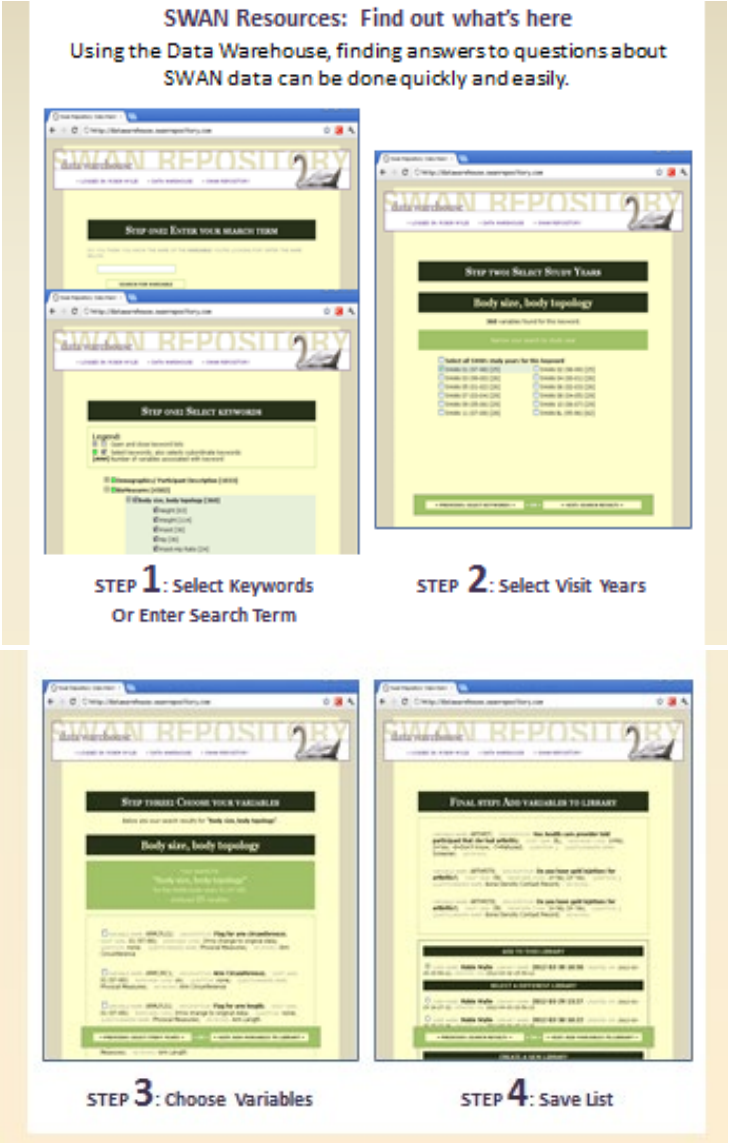
8.1.2.2. Data Warehouse Content and 4-Step Process

The Data Warehouse describes available data:

- **SWAN Data** By the end of SWAN's Visit 13 (2013), there were 154 different questionnaires or instruments used to collect or create the nearly 20,000 variables that make up the body of SWAN data. Each of these data variables has been individually linked to keywords in the Data Warehouse. Users can view descriptions of variables, wording of study questions, methodology behind created variables, then create and save lists of variables they are interested in (see 4-Step Screenshot below).

- **Repository-Generated Data** Data or results generated from SWAN Repository specimens are returned to the Repository within 3 years of close of funding. These new data are then incorporated back into the body of SWAN data, and are included in the Data Warehouse directory system. The origins and methodology behind these data are available as well.

Data Warehouse Screenshot3: 4-Step Process



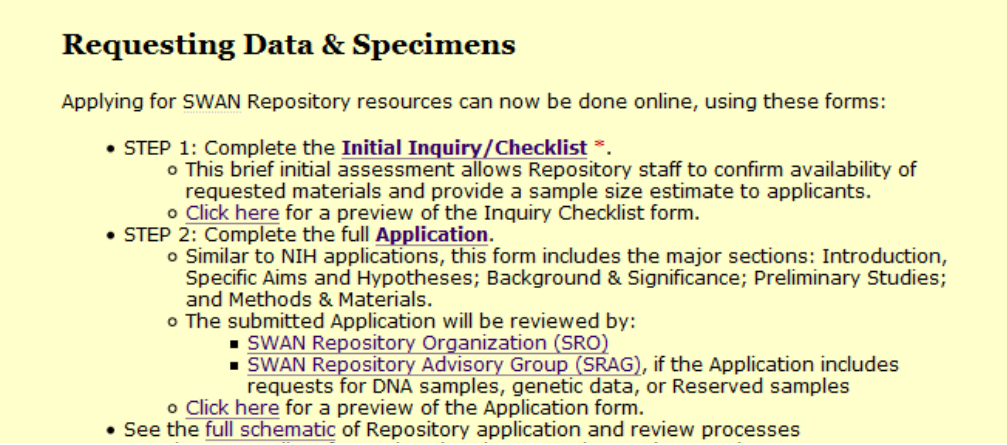
8.1.2.3. Updating the Data Warehouse

The Data Warehouse database is updated annually, as the SWAN Coordinating Center (CC) releases newly cleaned data from the prior visit. Newly released variables are linked to the keyword structure by Repository staff and added to the database, along with descriptions and other supporting documentation.

8.1.3. The Automated Online Application Submission System

Application forms are available to investigators via the Repository website. After analyzing the Data Warehouse, available biospecimen summaries and other online information, investigators can complete and submit online the 2 application forms necessary for requesting Repository resources – the Inquiry Checklist and the full Application (see screenshot below). PDF documents of both forms are available to preview on the website, prior to beginning the submission if desired. (See section 6 for complete application process.)

Website screenshot: Online Inquiry Checklist and Application Forms



Requesting Data & Specimens

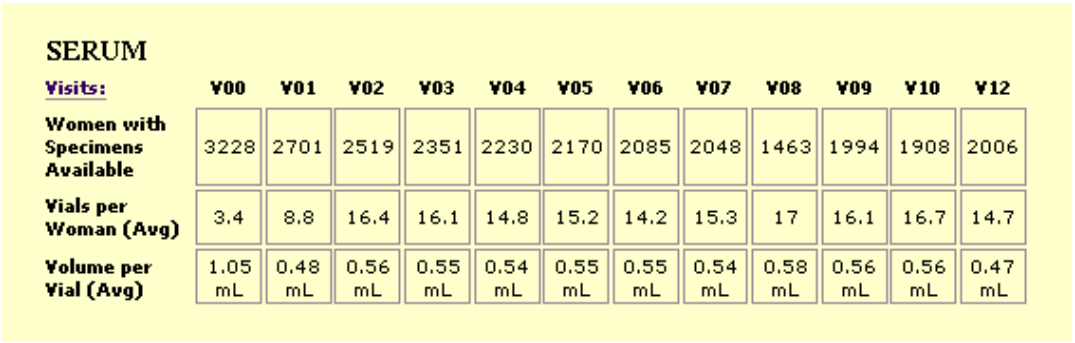
Applying for SWAN Repository resources can now be done online, using these forms:

- STEP 1: Complete the **Initial Inquiry/Checklist** *.
  - This brief initial assessment allows Repository staff to confirm availability of requested materials and provide a sample size estimate to applicants.
  - Click here for a preview of the Inquiry Checklist form.
- STEP 2: Complete the full **Application**.
  - Similar to NIH applications, this form includes the major sections: Introduction, Specific Aims and Hypotheses; Background & Significance; Preliminary Studies; and Methods & Materials.
  - The submitted Application will be reviewed by:
    - SWAN Repository Organization (SRO)
    - SWAN Repository Advisory Group (SRAG), if the Application includes requests for DNA samples, genetic data, or Reserved samples
  - Click here for a preview of the Application form.
- See the full schematic of Repository application and review processes

8.1.4. Biospecimen Tables

The Repository website also provides details about the SWAN specimens available for study. The *Biospecimens* page contains a summary table for each available specimen type (serum, plasma, urine and DNA) showing the number of women with samples available, average number of vials per woman, and average vial volumes. These tables are updated on an annual basis, or subsequent to a large sample pull.

Website screenshot: Available Biospecimen Tables



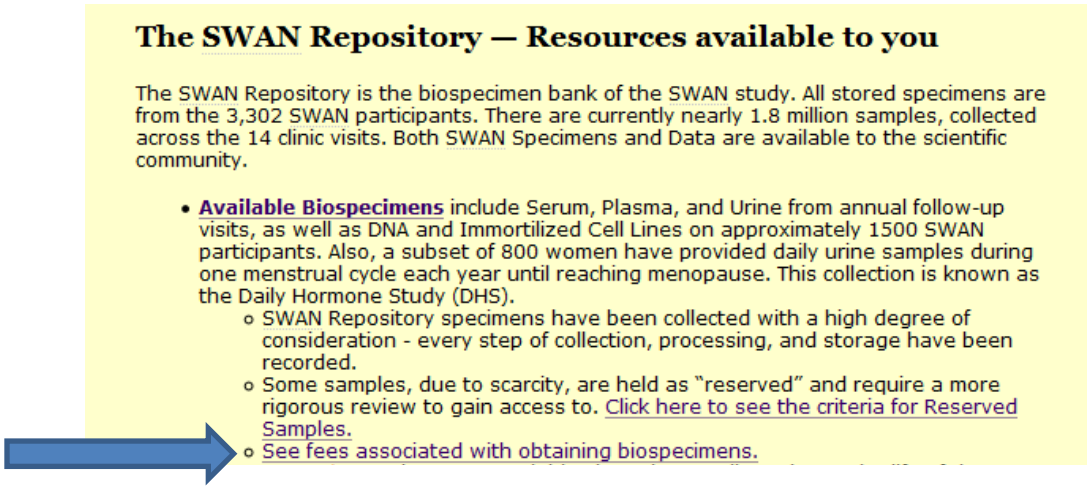
SERUM

Visits:	V00	V01	V02	V03	V04	V05	V06	V07	V08	V09	V10	V12
Women with Specimens Available	3228	2701	2519	2351	2230	2170	2085	2048	1463	1994	1908	2006
Vials per Woman (Avg)	3.4	8.8	16.4	16.1	14.8	15.2	14.2	15.3	17	16.1	16.7	14.7
Volume per Vial (Avg)	1.05 mL	0.48 mL	0.56 mL	0.55 mL	0.54 mL	0.55 mL	0.55 mL	0.54 mL	0.58 mL	0.56 mL	0.56 mL	0.47 mL

8.1.5. Fees

Specimen-related cost tables, which provide current cost-recovery rates for obtaining samples and creating individualized matching datasets, can be viewed on the Repository home page under *Available Biospecimens*.

Website screenshot: Link to Fees associated with Obtaining Biospecimens

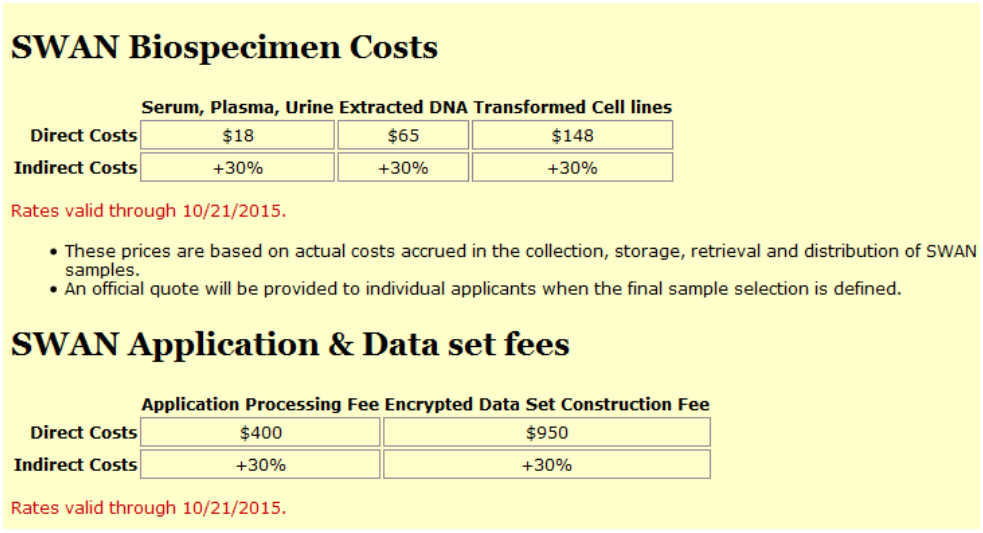


The SWAN Repository — Resources available to you

The SWAN Repository is the biospecimen bank of the SWAN study. All stored specimens are from the 3,302 SWAN participants. There are currently nearly 1.8 million samples, collected across the 14 clinic visits. Both SWAN Specimens and Data are available to the scientific community.

- **Available Biospecimens** include Serum, Plasma, and Urine from annual follow-up visits, as well as DNA and Immortilized Cell Lines on approximately 1500 SWAN participants. Also, a subset of 800 women have provided daily urine samples during one menstrual cycle each year until reaching menopause. This collection is known as the Daily Hormone Study (DHS).
  - SWAN Repository specimens have been collected with a high degree of consideration - every step of collection, processing, and storage have been recorded.
  - Some samples, due to scarcity, are held as "reserved" and require a more rigorous review to gain access to. Click here to see the criteria for Reserved Samples.
  - See fees associated with obtaining biospecimens.

Website screenshot: Current Biospecimen-Related Costs



SWAN Biospecimen Costs

	Serum, Plasma, Urine	Extracted DNA	Transformed Cell lines
Direct Costs	$18	$65	$148
Indirect Costs	+30%	+30%	+30%

Rates valid through 10/21/2015.

- These prices are based on actual costs accrued in the collection, storage, retrieval and distribution of SWAN samples.
- An official quote will be provided to individual applicants when the final sample selection is defined.

SWAN Application & Data set fees

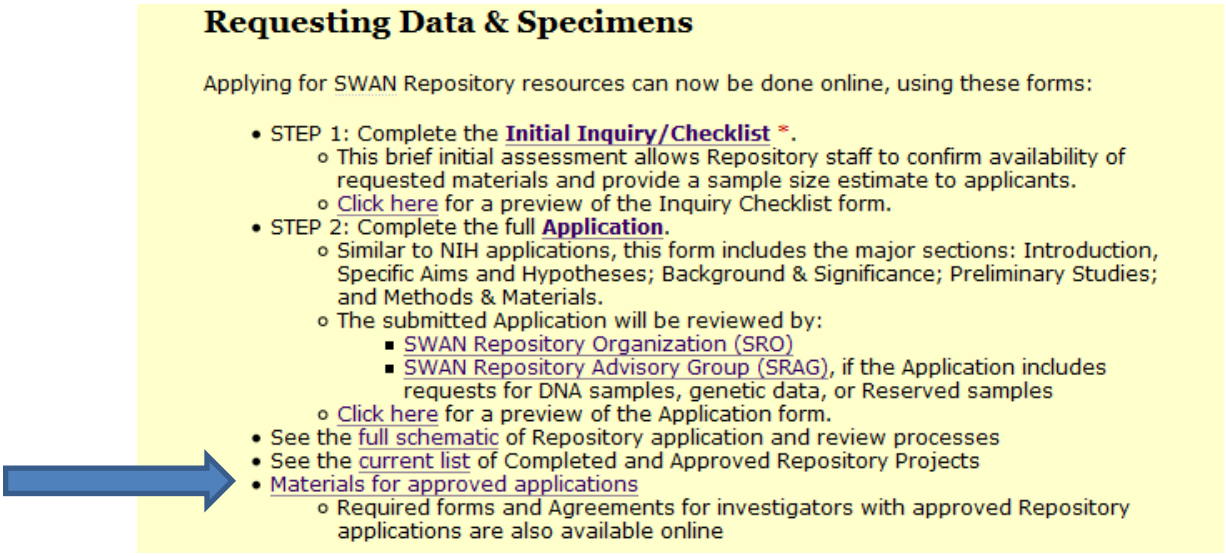
	Application Processing Fee	Encrypted Data Set Construction Fee
Direct Costs	$400	$950
Indirect Costs	+30%	+30%

Rates valid through 10/21/2015.

8.1.6. Materials for Approved Applications

From the website's home page, investigators approved to receive Repository resources can find a number of important forms and references.

Website screenshot: Link to Materials for Approved Applications



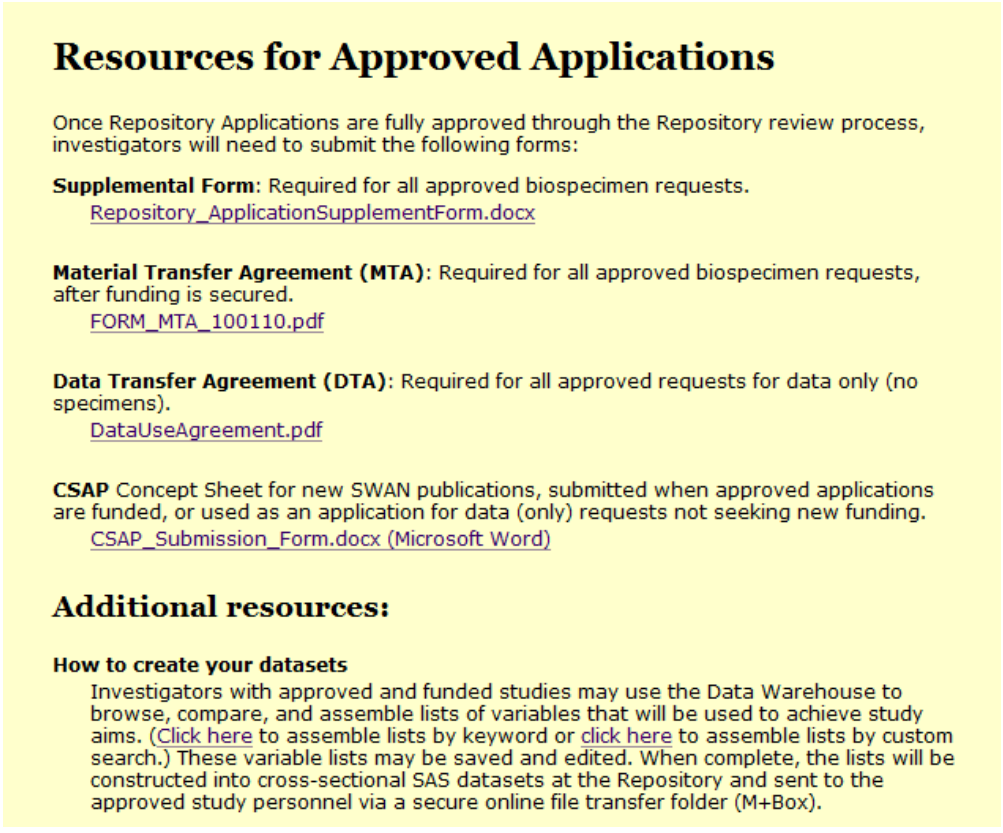
Requesting Data & Specimens

Applying for SWAN Repository resources can now be done online, using these forms:

- STEP 1: Complete the **Initial Inquiry/Checklist** *.
  - This brief initial assessment allows Repository staff to confirm availability of requested materials and provide a sample size estimate to applicants.
  - Click here for a preview of the Inquiry Checklist form.
- STEP 2: Complete the full **Application**.
  - Similar to NIH applications, this form includes the major sections: Introduction, Specific Aims and Hypotheses; Background & Significance; Preliminary Studies; and Methods & Materials.
  - The submitted Application will be reviewed by:
    - SWAN Repository Organization (SRO)
    - SWAN Repository Advisory Group (SRAG), if the Application includes requests for DNA samples, genetic data, or Reserved samples
  - Click here for a preview of the Application form.
- See the full schematic of Repository application and review processes
- See the current list of Completed and Approved Repository Projects
- Materials for approved applications
  - Required forms and Agreements for investigators with approved Repository applications are also available online

These include the Material Transfer Agreement (MTA) and Data Use Agreement (DUA) forms, tutorials for creating variable lists, and instructions for submitting annual progress reports, properly acknowledging SWAN, and returning new data to the Repository.

Website screenshot: Forms and References available to Approved Investigators



Resources for Approved Applications

Once Repository Applications are fully approved through the Repository review process, investigators will need to submit the following forms:

Supplemental Form: Required for all approved biospecimen requests.
Repository_ApplicationSupplementForm.docx

Material Transfer Agreement (MTA): Required for all approved biospecimen requests, after funding is secured.
FORM_MTA_100110.pdf

Data Transfer Agreement (DTA): Required for all approved requests for data only (no specimens).
DataUseAgreement.pdf

CSAP Concept Sheet for new SWAN publications, submitted when approved applications are funded, or used as an application for data (only) requests not seeking new funding.
CSAP_Submission_Form.docx (Microsoft Word)

Additional resources:

How to create your datasets

Investigators with approved and funded studies may use the Data Warehouse to browse, compare, and assemble lists of variables that will be used to achieve study aims. (Click here to assemble lists by keyword or click here to assemble lists by custom search.) These variable lists may be saved and edited. When complete, the lists will be constructed into cross-sectional SAS datasets at the Repository and sent to the approved study personnel via a secure online file transfer folder (M+Box).

Annual progress reports

1-2 page progress reports are due from all approved applicants annually from approval date (date of Approval Letter) until data has been returned to Repository.

Acknowledgements: For Manuscripts & for Abstracts, Posters, etc (short version)

ACKNOWLEDGMENTS_forReposWebsite.docx (Microsoft Word)

How to return data to the Repository; When data is considered for integration with Core SWAN data

Returning_Data_to_the_Repository.pdf (Adobe PDF)

8.1.2.7. Other Investigator Tools

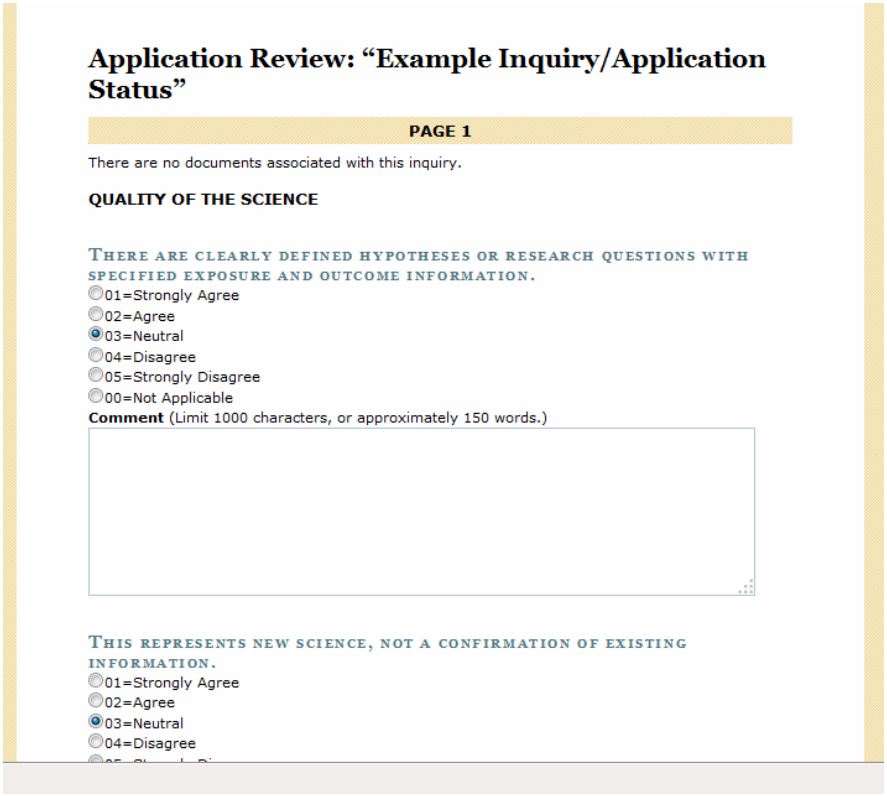
Other online tools for investigators include:

- description of the SWAN study design;
- the SWAN Study timeline;
- brief descriptions of completed and current Repository projects;
- schematic of the Repository application and review process; and
- FAQ page.

8.2. TOOLS FOR REVIEWERS

A second database, the Repository Admin Center, was built to automate and streamline the review of Repository applications. When a full application is submitted for SRO or SRAG review, individual reviewers can be selected online and a link to the application and review questions is automatically emailed to each reviewer. Reviewers can answer the objective review questions with a click of a button indicating his or her level of agreement, and provide comments on each point within the associated text boxes. Reviewers are asked to provide an overall recommendation and final comments before submitting online to Repository staff. When all reviews are submitted, the system automatically averages responses and compiles reviewers' comments and votes providing neat summaries for the applicants. (For more information on how this website and Application Reviews fit into the full Application and Review process, see section 6 of this manual.)

Admin Center screenshot: Reviewer Questions



Application Review: "Example Inquiry/Application Status"

PAGE 1

There are no documents associated with this inquiry.

QUALITY OF THE SCIENCE

THERE ARE CLEARLY DEFINED HYPOTHESES OR RESEARCH QUESTIONS WITH SPECIFIED EXPOSURE AND OUTCOME INFORMATION.

- 01=Strongly Agree
- 02=Agree
- 03=Neutral
- 04=Disagree
- 05=Strongly Disagree
- 00=Not Applicable

Comment (Limit 1000 characters, or approximately 150 words.)

THIS REPRESENTS NEW SCIENCE, NOT A CONFIRMATION OF EXISTING INFORMATION.

- 01=Strongly Agree
- 02=Agree
- 03=Neutral
- 04=Disagree

8.3. INTERNAL TOOLS FOR REPOSITORY STAFF

8.3.1. Application Management

On the same Admin Center system that handles the automated review process, the Application Management system is available to internal Repository staff. The *Application Management* site is a password-protected tracking system for all inquiries and requests to access SWAN Repository samples and data. It provides structure and space to store all communications and documents associated with each step of the life of an application – from the inquiry, application and review, resource transfer and data return, through the completion of the project. (http://applicationmanagement.swanrepository.com)

8.3.2. SAS Code Generation from the Data Warehouse

The Data Warehouse also contains useful tools for Repository staff. The DW is built using a MySQL database, and links to the SAS (Statistical Analysis System) platform. Because frozen SWAN datasets are stored on the server as SAS datasets, this link makes possible the automation of SAS code generation, thus forging individualized datasets and codebooks. Investigators select SWAN variables of interest on the DW and save them into personal libraries. These variable lists drive the production of SAS code which pulls the selected data into datasets. This automation saves all users a great deal of time and resources.

9.0 REPOSITORY SPECIMEN INVENTORY

The following tables summarize the specimens available through the SWAN Repository.

9.1. Annual Visit (Core) Samples

The table below shows the number of SWAN participants with data collected at each completed follow-up visit* and the number with Core serum/plasma/urine in the Repository.

	Base-line	V 01	V 02	V 03	V 04	V 05	V 06	V 07	V 08	V 09	V 10	V 12	V 13
In Core Study	3302	2867	2725	2694	2658	2604	2441	2320	2274	2246	2239	2399	2338
In Core Study with Repository specimens	3276	2773	2528	2357	2232	2174	2085	2048	1462	1994	1908	2006	1947
% in Core Study with Repository specimens	99%	97%	93%	87%	84%	83%	85%	88%	64%	89%	85%	84%	83%

*excludes refusals, persons active but not available to participate that visit, and the deceased. There are no visit 06-10 data from the New Jersey site due to a hiatus in data collection at that site. Specimens are not collected from participants participating by telephone interview, who at Visit 8 participated in the mailed protocol option. Visits 11 and 14 were interim years; therefore no Repository specimens were collected.

9.1.1. Available Serum from SWAN Core, as of **MARCH 1, 2016**

Visit	Material type	Vial status	Vial Count	Women with Serum	Avg Vials Per Woman	Avg Vial Volume (mL)
00	Serum	In	13,903	3269	4	1.1
01	Serum	In	24,490	2723	9	0.59
02	Serum	In	40,662	2518	13	0.58
03	Serum	In	34,313	2346	14	0.58
04	Serum	In	31,534	2278	12	0.60
05	Serum	In	30,843	2188	12	0.62
06	Serum	In	30,099	2096	13	0.63
07	Serum	In	31,026	2064	14	0.61
08	Serum	In	24,161	1471	15	0.64
09	Serum	In	29,884	2046	14	0.65
10	Serum	In	32,364	1916	14	0.61
12	Serum	In	24,619	1995	12	0.52
13	Serum	In	30,370	1900	16	0.59
15	Serum	In	4,841	725	7	0.49
	Total serum vials		383,109			

9.1.2. Available Plasma (EDTA and Citrated) from SWAN Core, as of **MARCH 1, 2016**

Visit	Material type	Vial status	Vial Count	Women with Plasma	Avg Vials Per Woman	Avg Vial Volume (mL)
00	EDTA PLASMA	In	3179	3151	1	1.3
01*	EDTA PLASMA	In	2796	2701	1	1.0
02*	EDTA PLASMA	In	6130	2229	3	1.0
03*	EDTA PLASMA	In	2242	2231	1	1.1
04*	EDTA PLASMA	In	4378	2262	2	1.0
05*	EDTA PLASMA	In	2242	2126	1	1.0
06*	EDTA PLASMA	In	4001	2066	2	1.0
07*	EDTA PLASMA	In	2770	1878	1	1.0
08*	EDTA PLASMA	In	4059	1370	3	1.0
09*	EDTA PLASMA	In	3638	1833	3	1.0
10*	EDTA PLASMA	In	3899	1858	3	1.0
12	EDTA PLASMA	In	2992	1522	2	1.0
13	EDTA PLASMA	In	3568	1812	2	1.0
15	EDTA PLASMA	In	5084	739	7	0.5
	Total EDTA plasma vials		50,978			

*All Repository EDTA plasma from Visit 01-10 are samples recovered from MRL.

Visit	Material type	Vial status	Vial Count	Women with Plasma	Avg Vials Per Woman	Avg Vial Volumes (mL)
00	Citrate PLASMA	In	3183	3167	1	1.2
01	Citrate PLASMA	In	9534	2697	4	0.9
02	Citrate PLASMA	In	9275	2492	4	0.6
03	Citrate PLASMA	In	6765	2326	3	0.5
04	Citrate PLASMA	In	6344	2158	3	0.5
05	Citrate PLASMA	In	6218	2089	3	0.5
06	Citrate PLASMA	In	5930	2021	3	0.5
07	Citrate PLASMA	In	5827	1983	3	0.5
08	Citrate PLASMA	In	5438	1449	4	0.6
09	Citrate PLASMA	In	7453	1970	4	0.6
10	Citrate PLASMA	In	7389	1890	4	0.6
12	Citrate PLASMA	In	5963	1979	3	0.5
13	Citrate PLASMA	In	5398	1847	3	0.5
15	Citrate PLASMA	In	2195	744	3	0.5

Total Citrate plasma vials 86,912

9.1.3. Available Urine from SWAN Core, as of **MARCH 1, 2016**

Visit	Material type	Vial status	Vial Count	Women with Urine	Avg Vials Per Woman	Avg Volume/ Vial (mL)
00	Urine	In	5345	2375	2	0.95
01	Urine	In	8199	2063	4	1.00
02	Urine	In	12551	1967	6	0.51
03	Urine	In	14555	1864	8	0.63
04	Urine	In	13946	1814	8	0.68
05	Urine	In	12323	1770	7	0.70
06	Urine	In	13911	1790	8	0.69
07	Urine	In	13439	1751	8	0.68
08	Urine	In	9556	1251	8	0.69
09	Urine	In	14654	1655	9	0.61
10	Urine	In	14969	1698	9	0.59
12	Urine	In	13089	1639	8	0.50
13	Urine	In	12563	1573	8	0.50
15	Urine	In	1843	463	4	2.00

Total urine vials 160,943

9.2. Daily Hormone Study (DHS) Samples, collected 1997-2008

9.2.1. DHS Urine

Visit	Material Type	Vials In	Women with DHS Urine	Avg large vials per woman (5mL)	Avg aliquoted tubes per woman (0.5mL)
H1	Urine	280,276	884	29	291 (10/day)
H2	Urine	240,882	727	31	306 (10/day)
H3	Urine	202,897	645	32	320 (10/day)
H4	Urine	128,094	539	44	317 (7/day)
H5	Urine	88,988	403	35	326 (9/day)
H6	Urine	60,754	271	38	417 (11/day)
H7	Urine	8,274	208	40	*not aliquoted*
H8	Urine	6,624	171	39	*not aliquoted*
H9	Urine	4,534	124	37	*not aliquoted*

1,021,323

9.2.2. DHS Serum

Visit	Material Type	Vials In	Women with DHS Serum	Avg Vial Volume (mL)
H1	Serum	4,652	888	1.8
H2	Serum	3,771	725	2.0
H3	Serum	3,419	644	1.8
H4	Serum	2,417	522	1.6
H5	Serum	2,022	401	0.5
H6	Serum	843	263	0.5
H7	Serum	1,163	207	0.5
H8	Serum	876	166	0.5
H9	Serum	592	119	0.5
H10	Serum	342	84	0.5

1,021,323

9.3. Genetic Materials

9.3.1. DNA

The SWAN Repository offers diluted extracted DNA to the scientific community. The DNA comes from EBV-transformed cells, see processing details in Section 4. The total volume of DNA per stock vial is 5µg. The vials are stored on VP-LN2 (liquid nitrogen vapor).

Because the DNA resource comes from renewable cells, and can be replenished, DNA is not considered Reserved. The number of SWAN participants who have DNA available is 1538.

9.3.2. EBV-Transformed Cells

The Epstein-Barr Virus-transformed, or immortalized, cell lines are also available to the scientific community. (See processing details in Section 4.)

9.3.3. Buccal Cells

The buccal cell samples collected during the special one-time collection are another source of DNA from the SWAN participants.

9.3.4. The SWAN Population with Genetic Materials

1538 women from the SWAN Study population have consented and contributed to the Repository DNA specimen bank. The race/ethnicity breakdown of the contributors is shown below. Note that no New Jersey site samples are included in any of the genetic materials (see comment in section 3.3.1).

DNA Ethnic Breakdown	
BLACK	412
CAUCA	807
CHINE	151
JAPAN	168
TOTAL	**1538**

9.4. Costs associated with obtaining biospecimens

Cost recovery efforts have been put into place, following directives from NIA (section 7). The following recharge rates apply to specimens released from the SWAN Repository:

SWAN Biospecimen costs

	Serum, Plasma, and Urine	Extracted DNA	Transformed Cell lines
Direct Costs	$18	$65	$148
Indirect Costs	+30%	+30%	+30%

Rates valid through 10/21/2015.

SWAN Application & Data set fees

	Application Processing Fee	Encrypted Data Set Construction Fee
Direct Costs	$400	$950
Indirect Costs	+30%	+30%

Rates valid through 10/21/2015.

These prices are based on actual costs accrued in the collection, storage, retrieval and distribution of SWAN samples. An official quote will be provided to individual applicants when the final sample selection is defined.