Available online at www.sciencedirect.com

SCIENCE DIRECT®

Toxicology and Applied Pharmacology 206 (2005) 261–268

Toxicology and Applied Pharmacology

www.elsevier.com/locate/ytaap

Molecular epidemiology biomarkers—Sample collection and processing considerations

Nina T. Holland[a,*], Laura Pfleger[a], Eileen Berger[b], Alan Ho[a], Maria Bastaki[a]

[a]*Environmental Health Sciences, School of Public Health, University of California, 317 Warren Hall, Berkeley 94720-7360, USA*
[b]*Input Automation Inc., Sonoma Mountain Road, Glen Ellen, CA 95442, USA*

Received 17 March 2004; accepted 26 October 2004
Available online 30 March 2005

Abstract

Biomarker studies require processing and storage of numerous biological samples with the goals of obtaining a large amount of information and minimizing future research costs. An efficient study design includes provisions for processing of the original samples, such as cryopreservation, DNA isolation, and preparation of specimens for exposure assessment. Use of standard, two-dimensional and nanobarcodes and customized electronic databases assure efficient management of large sample collections and tracking results of data analyses. Standard operating procedures and quality control plans help to protect sample quality and to assure validity of the biomarker data. Specific state, federal and international regulations are in place regarding research with human samples, governing areas including custody, safety of handling, and transport of human samples. Appropriate informed consent must be obtained from the study subjects prior to sample collection and confidentiality of results maintained. Finally, examples of three biorepositories of different scale (European Cancer Study, National Cancer Institute and School of Public Health Biorepository, University of California, Berkeley) are used to illustrate challenges faced by investigators and the ways to overcome them. New software and biorepository technologies are being developed by many companies that will help to bring biological banking to a new level required by molecular epidemiology of the 21st century.

Keywords: Molecular epidemiology; Biorepository; Biomarkers; Quality assurance; Quality control; Databases; Barcoding; Cryopreservation

Introduction

Advances in molecular biology and cytogenetics, ultra-sensitive methods of exposure assessment and susceptibility analysis of genetic polymorphisms by real time PCR and microarrays are providing new means of measuring biomarkers in epidemiological studies and understanding mechanisms of adverse health outcomes in response to environmental exposures (Fig. 1). With these advances, the number and variety of samples collected and stored for each epidemiological or clinical study increases significantly. The new procedures of collection, processing and banking are much more expensive and complicated, leading to a need to maximize the amount of information gained from each sample. This paper addresses issue in collection, processing and banking samples with a focus on examples of biorepositories today and future directions in molecular epidemiology.

Sample collection and processing issues

The main goals of a biological bank or biorepository are to store and process samples collected from ongoing studies in a way that maximizes the amount of information obtained from each sample, makes possible future analysis for currently unknown biomarkers and minimizes research costs for future studies. Fig. 2 shows the logistic parts of a biomarker study. Samples obtained in some of the studies are available on a one time-only basis making it critical to

* Corresponding author. Fax: +1 510 642 5764.
E-mail address: ninah@berkeley.edu (N.T. Holland).

0041-008X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2004.10.024

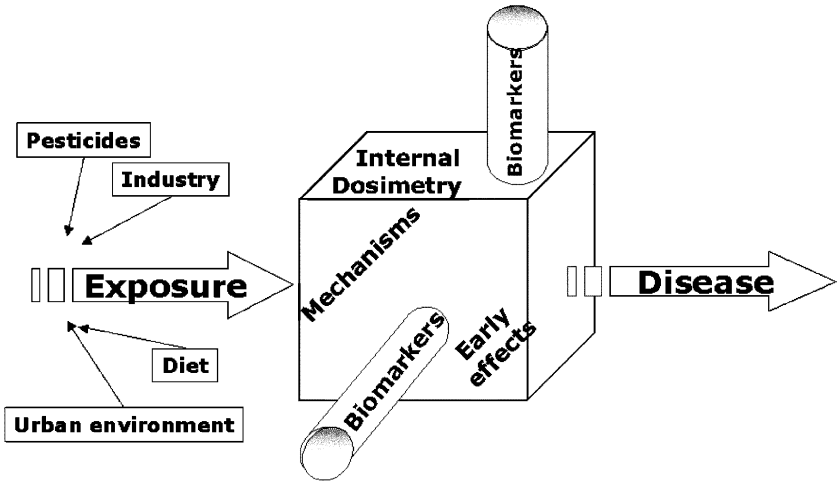

Fig. 1. Schematic representation of the role of biomarkers in molecular epidemiology and toxicology.

handle and store these precious samples carefully. Here, we will briefly discuss main issues and challenges for all steps of a molecular epidemiological study. For more detailed review, see (Holland et al., 2003; Schulte and Perera, 1993).

Study design

With the emergence of new molecular tools such as real time PCR and DNA/RNA microarrays, the variety of types of samples collected for epidemiological studies has expanded. Samples need to be collected more carefully and processed more extensively, frequently following more complicated protocols (Albertini et al., 2000). Identifying the relevant biomarkers and choosing the target tissue are very important in the planning phase of a study. This is achieved through pilot studies. Pilot tests need to be done to ascertain the optimum collection and handling of the samples since many factors can affect the levels of a biomarker. For example, many biomarkers such as chromosome aberrations, micronuclei, comet assay, etc. can reveal genetic damage. However, they must be narrowed down to find the most informative marker/s for the particular study goal with consideration of availability of specimens, locations of the collection site and processing laboratory and timing required to assure the quality of results. Furthermore, the study must be designed so that different study groups are collected at the same time so that the effects of time and storage are equal in control and case/exposure groups.

Informed consent

Sample banking for future use gives rise to new concerns regarding a patient's privacy and consent. New regulations are making informed consent an increasingly complicated issue. Researchers need to obtain consent for not only the current use of their samples but also for future studies which may yet be unknown. Many resources addressing the issues of bioethics (http://nih.gov/sigs/bioethics), informed consent and human subject research (http://ohsr.od.nih.gov) are available on the Web and through International Society of Biological and Environmental Biorepositories (http://www.isber.org).

The Health Insurance Portability and Accountability Act (HIPAA) was passed to protect a patient's personal and health information in April 2003. Without specific consent, a researcher cannot obtain or release a patient's name, addresses, social security number, birthdate, photographs or a number of other identifying pieces of information. In other words, each subject must have a unique number in no way related to his/her identity and a very limited number of researchers/personnel should have access to the identifying information. More information and training about HIPAA can be found at http://www.hhs.gov/ocr/hipaa/.

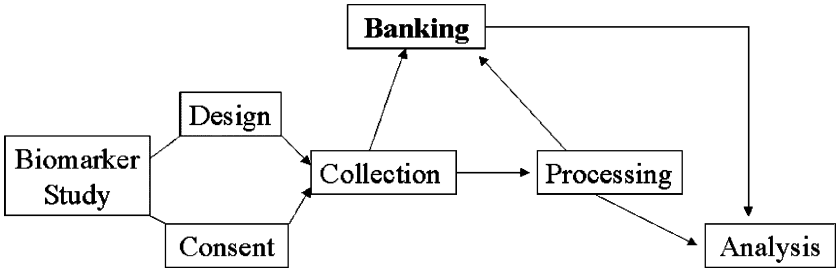

Fig. 2. Biological and environmental sample handling for molecular epidemiological studies.

Sample collection

Clear communication among study subjects, field personal and researchers is vital for consistent sample collection. Researchers must establish written protocols (SOPs) for collection staff with unambiguous instructions regarding collection time, fasting instructions, volumes required, container type for sample and types of equipment to use for the blood draw or other procedure. Clear written or verbal instructions should also be provided to the study subjects. Communication between field personal is especially important when the samples need to be shipped or transported from the field to the laboratory (Eskenazi et al., 2003). A system of e-mail and/or phone messages and confirmations should be established with back-up contacts to avoid any missed shipments or improper handling on arrival.

Non-invasive as opposed to invasive methods should be used whenever possible (Table 1). Using non-invasive samples such as exfoliated cells from the mouth (buccal) or urine (urothelial) tend to increase participation in a study since more participants are willing subject themselves to a buccal swipe than a blood draw. Non-invasive sample collection also eliminates the need for highly trained personnel needed for a blood draw or biopsy. Some specimens, such as buccal cells, can even be obtained by the participant at home and mailed to the researcher making studies in the remote areas more feasible.

Safety issues are a consistent area of concern when working with biological samples. All researchers and staff involved in a study must be trained in the proper handling of biological samples in order to protect themselves and others who may come in contact with the samples. Risk is obviously higher when working on infectious disease studies or conducting international studies in countries with high rates of infectious diseases. However, unless samples are specifically screened, even samples from healthy volunteers and children should be considered infectious. Extra care must be taken when sample collection involves needles or other "sharps." More information on proper human sample handling can be found at the Occupational Safety and Health Administration (OSHA) website (http://www.osha.gov).

Due to the instability of some biomarkers, factors like timing of processing, temperature, addition of stabilizing chemicals and buffers and sterility are important considerations. Adherence to established protocols decreases variance in stability between samples and increases stability of biomarker assessment within each sample. Anticoagulants and stabilizing agents should be considered carefully and tested in pilot studies before using in large-scale study. Timing between collection and processing of a sample is a vital concern. Some processes such as cell culture can be done 24–48 h after collection, whereas others such as cytokine analysis must be started immediately after collection (Duramad et al., 2004). Samples that must be shipped to a processing laboratory cannot be used for the most unstable biomarkers or processing should begin in the field to ensure stability during transportation. Temperature control between collection and processing as well as during storage is necessary for sample stability. The fact that different components of blood must be stored at different temperatures should be considered in the study design. Repeat freeze–thaw cycles may be detrimental to some of the samples (e.g., RNA) and should be avoided by creating multiple aliquots upfront rather than delayed aliquoting when specific assay is conducted. Frequently, this means the researcher must choose the most important biomarker/s for his particular research project, and store the sample according to those biomarkers requirements. Sterility is important for RNA isolation and cell culture since

Table 1
Examples of specimens useful for measuring biomarkers

Non-invasive		Invasive	
Type of tissue	Mode of collection	Type of tissue	Mode of collection
Buccal epithelia	Swab of inner lining of cheek with tongue depressor or cytobrush	Blood	Venipuncture or finger prick
Saliva	Sterile plastic pipette or specially prepared cotton swab	Bronchial, esophageal, GI tract epithelia	Biopsy material
Urine and urothelial cells	Separated by centrifugation	Bone marrow	Spinal tap
Nasal epithelia	Swab of inner lining of the nose with cytobrush or cotton swab	Amniotic fluid	Amniocentesis (Mother)
Cord blood	Drained into sterile container from the cord after delivery	Adipose tissue	Biopsy
Expired air	Spirometer attachment		
Hair	In container after cut or fallen out		
Fingernails	Clippings in sterile container		
Extracted teeth	Collected in sterile container after loss		
Cervical epithelia	Scraping with small spatula		
Breast duct epithelia	Nipple Aspirate		

contamination can grossly affect biomarkers measured. Samples intended for protein or RNA analysis are vulnerable to degradation to naturally occurring enzymes. Commercially available protease inhibitors and RNAse-free materials can protect these samples.

In addition to the preservation of biological components in the collected samples, it is important to ensure that measurement of environmental pollutants present in the biological sample is not confounded from contaminants in the containers or tools used for sample collection. Prescreening of these materials maybe necessary depending on the toxicant of interest.

Many strict regulations are in place for the shipping of biological samples, including International [International Civic Aviation Organization (ICAO) (http://www.icao.int) and International Air Transport Association (IATA) (http://www.iata.org/whatwedo/dangerous_goods)] laws and Federal regulations [Department of Transportation (DOT) (http://hazmat.dot.gov/)]. If a package is not properly labeled or packed, shipping companies, airlines and customs will refuse to ship it causing delays and most likely affecting the sample's and the study's integrity. It is in the best interest for a researcher to thoroughly know and adhere to these regulations. Training and certification is available via classes or on CD through Saf-T-Pak (http://www.saftpak.com).

Quality assurance

Good laboratory practices (GLP) and quality assurance/quality control (QA/QC) programs address everything from properly calibrated equipment to raw data maintenance. Every laboratory should have a program to assure GLPs and QA/QC procedures are followed. Table 2 lists many of the components of GLPs and QA/QC. Documentation for QA/QC programs is provided by the EPA at http://www.epa.gov/quality/qatools.html and http://www.epa.gov/quality/exmural/html.

In addition, testing of human samples performed in non-research laboratories in the US is subject to regulations by Clinical Laboratory Improvement Amendments (CLIA) (http://www.cms.hhs.gov/clia/).

A paper trail for each sample is necessary to ensure the integrity of the sample. A complete paper trail includes collection details (date, sample number, type and volume), shipping information (receipts and tracking numbers) and chain of custody forms. In accordance with HIPAA, personal information of the participant must be encrypted. Electronic databases are quickly replacing hard copies and barcodes allow for quick and accurate encoding and processing of the samples. In addition to sample collection and processing information, electronic logs should have all protocols stored in a secure location.

Table 2
Examples of QA/QC procedures

Record keeping (archives[a] and documentation[a])	Storage	Sample integrity	Equipment
Sample labeling	Back-up system	Standard operating procedures[a]	Maintenance records
Chain of custody forms	Temperature monitoring	Replicates	Calibration
Laboratory log (master schedule[a])	Retrieval	Internal standards	System tests[a]
Database data entry (audit trail[a])		Pilot analysis	
Problem resolution			

[a] Included in good laboratory practices (GLP).

Sample processing

Processing of blood, buccal cells, urine and other samples produces a number of types of samples to be analyzed or banked for future use. A variety of processing protocols exist to prepare samples for biomarker studies. Many of the processes are discussed at length in Holland et al. (2003). Full protocols are available on the website http://ehs.sph.berkeley.edu/holland/ProtocolLibrary.html. Frequently, one sample will be divided and undergo several processes to obtain the maximum amount of information in one sample (Fig. 3). In addition to flow-charts as shown in this figure, we recommend using pre-made time schedules and tables for recording volumes, times and calculations of cell densities, concentrations, etc.

Several considerations are important when planning sample processing. As mentioned in previous sections, biomarkers may decay soon after collection. It is essential to process the sample as soon as possible after collection. If the collection site is not close to the laboratory (Eskenazi et al., 2003) simple processes like aliquoting, serum and clot separation, freezing at −80 °C can be done on site. However, more complicated processes often need to be done in a laboratory with sterile facilities and sophisticated equipment.

Blood components are separated via centrifugation with a density gradient such as Ficoll or Lymphoprep (http://www.axis-shield-poc.com/optiprep/C04.pdf; http://www.progen.de). It is common to set aside aliquots of the unaltered sample, for example, whole blood before beginning processing. A lymphocyte/granulocyte layer (known as buffy coat) can be isolated using a simple centrifuge; however, more advanced Ficoll isolation will be able to separate lymphocytes, granulocytes, plasma and red blood cells from initial vacutainer with either heparin, EDTA or other anticoagulant. Finally, it is important to divide the samples that result from processing into multiple aliquots. It is best to store duplicate aliquots in different locations to protect against equipment failure and natural disasters. Also storing small aliquots (the size needed for an assay) reduces cell damage caused by multiples freezes and thaws to access the sample.

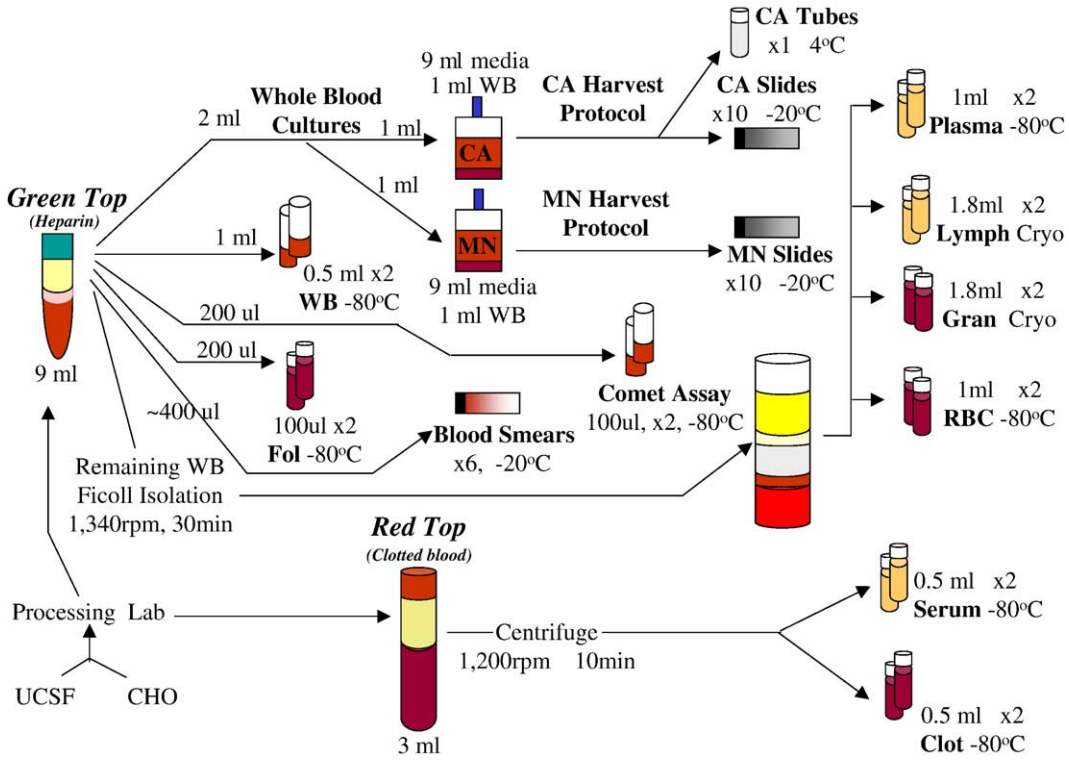

Fig. 3. Schematic representation of clotted and heparinized blood sample processing. The original sample is divided into multiple aliquots according to the final purpose of the analysis. Part of the original blood is separated into plasma, lymphocyte, granulocyte and red blood cell fractions through a Ficoll gradient. Another part is used to set up whole blood cultures for different cytogenetic assays. Storage conditions are noted on the flow chart, including storage at 4 °C, and in −20 and −80 °C freezers, and cryopreservation in liquid N_2. More information can be found at our website: http://www.ehs.sph.berkeley.edu/holland/ProtocolLibrary.htm l. (CA: chromatid aberrations; Fol: folate; Gran: granulocytes; Lymph: lymphocytes; MN: micronucleus; RBC: red blood cells; WB: whole blood).

Cell culture is necessary for cytogenetic analysis such as chromosomal aberrations, sister chromatid exchanges, and micronucleus analysis. Cells for culture must be processed under sterile conditions within 48 h of collection or cryopreserved. Factors important in cryopreservation include addition of DMSO to prevent cell damage during the freezing process, storage in liquid nitrogen to maintain temperatures below −132 °C to prevent cell death and tests during pilot studies to measure cell viability upon thawing. Recently, new approach to cell cryopreservation is being explored to enhance viability and functional integrity of various sample types (Mathew et al., 2003).

Today's biorepositories

A biorepository is "a system which will store one or many types of biologic specimens for later analysis from single or multiple studies under conditions which permit efficient retrieval and optimum stability of the sample" (Winn et al., 1990). Epidemiology studies generate any-

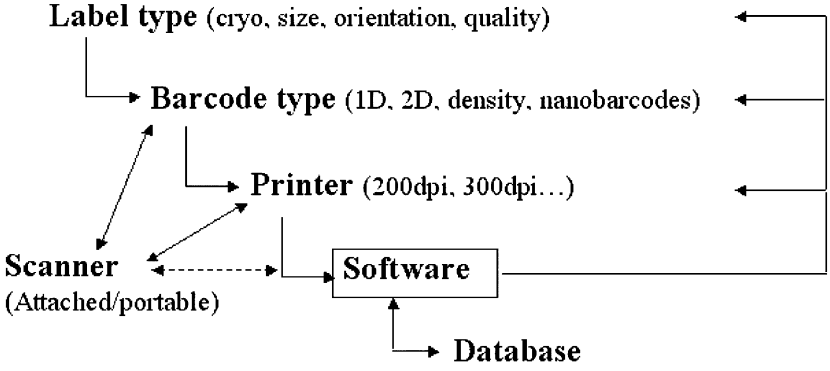

Fig. 4. Decision tree for the selection of the appropriate hardware and software to support barcoding of biological samples and database development. The selection process varies based on individual needs.

where from a couple hundred samples to several million samples. It is important to have the physical space for these samples as well as an effective labeling and tracking system. Storing these samples in a biorepository has several advantages. Sample utility is maximized in that they can be used in the future as new biomarker tests develop. Student projects become more feasible and costs of future studies are decreased with the elimination of the sample collection stage. A specimen bank is available for future intra-center collaboration and for development of new methods. Also in today's tight budget environment, biorepositories are providing additional incentives for extramural funding. Challenges of physical space, labeling and data management arise with a large bank of samples (Fig. 4). Here, we discuss examples of three biorepositories of different size and main focus, and compare their approach to handling numerous challenges they are facing.

European cancer bank

The European Cancer Study houses one the largest biorepositories of human samples in the world. Over 380,000 blood samples have been collected and divided into 28 half-milliliter aliquots, making the total number of storage units more than 10 million. Samples are stored in −80 °C freezers or in liquid nitrogen vapor (−120 °C) in cryo-sleeves. These straw-like containers are color-coded for sample type and stamped directly with identification numbers to eliminate label loss. For the security of the samples half of each subjects aliquots are stored in regional collaborating centers and half at the International Agency for Research on Cancer (IARC) (Bingham and Riboli, 2004).

National cancer institute biobank

The National Cancer Institute Division of Cancer Epidemiology and Genetics (NCI DCEG) biorepository has over 7 million samples from 500 different studies (http://www.cnio.es/es/meetings/descargas/tumour-banks/12-11-2002/11_Vaught.pdf).

Most of the samples are whole bloods or separated blood components in 2 ml cryovials stored at −80 °C or in liquid nitrogen. The NCI contracts with commercial biorepositories who also offer support with collection, processing and shipping. The samples are labeled with a 2-D bar code system rather than a standard barcode. The 2-D barcode or data matrix code has more individual codes and can store more pieces of information than a tradition bar code system. The data management system to keep track of the samples is a real time web-based database with provisions for multiple user and group passwords. Currently six inventories ranging from 500,000 to 7 million specimens are on the database. Quality control is the current primary concern of the NCI DCEG biorepository. QA/QC practices include standard operating procedures, cGMP, alarms, remote equipment monitoring, back-up generators (with weekly tests) and a disaster plan. NCI uses an automated DNA extraction system (Gentra AutoPure; http://www.gentra.com/products.asp?product_family_ID=7), which processes up to 96 samples in 8 h with yields similar to manual processing.

School of Public Health biorepository, University of California, Berkeley

The biorepository at the School of Public Health, UC, Berkeley, has a large variety of sample types including blood samples and their components, breast milk and dust samples. The samples number more than 100,000 (Fig. 5) and are stored in a large variety of container types from 0.5 ml tubes to 15 ml falcon tubes and cytogenetic slides. All specimens are labeled with standard barcodes on cryolabels.

The database system was designed with the assistance of a local software programming company Input Automation (http://www.inputautomation.com). The goals in design were to centralize and streamline information, optimize the use of storage space, minimize errors and inconsistencies, facilitate sample tracking and allow for flexibility and expansion. Some of the decisions involved in designing a database are shown in Fig. 4. They include the choice of development platform, application development and automatic generation of barcoded labels. SQL Server was chosen as the database platform for its performance as well as business convenience reasons. Those include the number of users and the size of the database it can accommodate, speed, scalability, cost and popularity with the users. Microsoft Access was used as the front end user interface because of its tight integration with SQL Server and because of its ease of use by non-programmers. Power users can create their own custom reports and have them integrated within the application. When deciding to undertake a custom software approach, there are two facts to consider. First, a solution designed to meet user exact specifications will be delivered, and, second, it will require a significant investment of time and money. The end user will need to work closely with the developer and understand that the software will be a work in progress for some time.

A custom application database was developed for SPH Biorepository, UC Berkeley covering more than a dozen projects. Each project requires slightly different information to be collected and recorded for each sample. To accommodate this requirement, a unique input form was designed for each project. The basic database structure, however, remains the same. For each project, the system can maintain completely separate databases running on separate SQL servers, separate databases within the same SQL Server, or a shared database. Each solution has advantages and disadvantages. The flexibility is there to administer the system in a way addressing unique requirements of each project while keeping it centralized and cost effective.

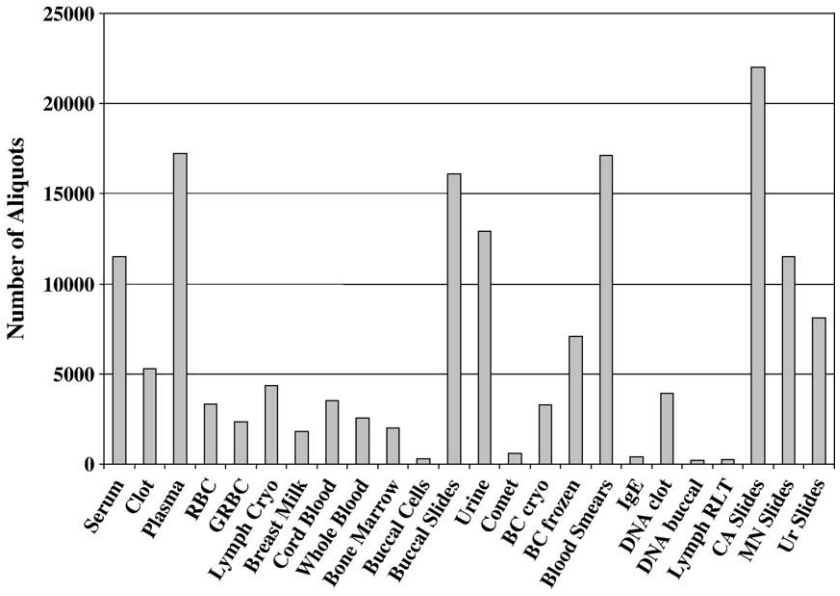

Fig. 5. Representative chart of the sample types collected and stored in a biorepository. The total number of banked specimens depends on the size of the original human sample (e.g., volume of blood), the diversity of sample types derived from processing the original human sample, and the number of aliquots one wishes to store for multiple analyses.

Though information collected for each project may vary, the basic workflow is the same. The main input to the database occurs on the Sample Master form. This form contains coded information about the study subject, the original sample ID, and a listing of all the aliquots generated from the original sample. Prior to inputting data into the Sample Master, several support tables must be populated. These tables contain the static information such as Employees who conducted processing, Sample Types, and Receiving Facilities. Data in these tables is input once and then rarely changes. The information in these tables is used to make data entry easier, faster, more accurate and consistent on the Sample Master form through the use of limited pull down lists.

An important support table for the system is the process templates table. Here, the user defines what sample types and quantity of aliquots will be generated from the original sample. Once the user defines these templates, the processing of samples is accomplished with very little data entry. For example, when a blood without anticoagulant is received, the system will process that sample into five aliquots of serum and two aliquots of clot without having to input this information each time they process this sample types for a specific project that has this requirement. A barcode label is automatically printed and assigned to each generated aliquot.

Once a barcode has been attached, physically to the sample and electronically within the database, tracking the location and disposition of the sample can be automated with the use of a barcode scanner. The place available for attaching barcodes on vials is usually very limited. The use of 2D barcodes can address this problem. Data Matrix, for example, can store 800 characters per square inch.

The future of banking and biorepositories

As biorepositories grow the number of samples they must store, trace and process grows exponentially. A small biorepository associated with the University or clinical research laboratory may have 100,000 samples or less; larger biorepositories such as the CDC or NCI have many millions of samples. With such vast quantities of samples, many issues arise. Sample size and storage space are very important. Samples need to be stored in the smallest form possible to maximize the number of samples that can be stored in any space. However, the samples also need to be labeled clearly and accurately for easy retrieval. Tracking samples at these large quantities also becomes a problem. Each sample needs to have secure chain of custody, processing, location and temperature stability records compiled in an easy to access location. Nanobarcoding and partial to complete automation of a biorepository both help address these arising issues.

Nanobarcoding

To minimize the coding information that can be incorporated into the samples rather than attached on the label, nanobarcoding is a very promising approach. Reif et al. have developed a method of using two kinds of DNA strands to form a bar code like lattice. These lattices can be sensed with an atomic force microscope and read similarly to bar codes. The barcodes can contain a large amount of information in a very small area making small sample containers feasible to label. Prototypes contain five individual codes in one 75-nanometer lattice. This technology should develop for use in the next 5–8 years. For more

information see http://www.trnmag.com/Stories/2003/070703/DNA_makes_nano_barcode_070203.html.

Another approach to solving nanobarcoding challenge has been developed by SurroMed, Inc. (http://www.surromed.com/news/press_archive.asp?query=10052001). Their nanobarcodes are composed of cylindrically shaped "striped" metal nanoparticles. The stripes are comprised of different metals and are varied in width and composition to make a large number of unique codes. The particles can be used as a barcode with an optical microscope or to do many biological assays in a small volume of sample.

Cryobanks

The European Cellbank and Center for Cryo-Biotechnology was founded to address the growing "need for cell preservation and revitalization." The focus of the research is to freeze and thaw cells in a way that maximizes cell viability. The technology use at this center is referred to as "intelligent" sample storage. The samples stored at this facility are miniaturized and modularized, stored in small plates that hold many samples and are attached to a memory chip containing the information for each sample. The system has been developed to access samples and the data attached individually without any thawing to the rest of the plate. The center consists of over 150 large cryotanks. Many areas of cryopreservation are being developed at this center including full automation of cryofacilities through research in low-temperature electronics and robotics and improved sample life through reduction of toxic agents like DMSO. The website http://www.ibmt.fraunhofer.de/Produktblaetter/cryo-at-ibmt.pdf contains more information about the centers research and technologies.

Automation

With the large number of samples to be handled by many biorepositories, the issue of automation of some or most of the procedures becomes increasingly important. Biophile, Inc (http://www.biophileinc.com) offers −20 °C, −40 °C and −80 °C freezers with automated storage and retrieval systems. Internal robotics improve temperature stability of samples by decreasing retrieval time and eliminating open freezer time. Small volumes (as low as 1 μl) of samples can be retrieved at a rate of 20 s per sample. These freezers can store up to 90,000 samples in 96-well plates or 22,572 samples in cryovials each identified with its own barcode. Each freezer has a touch screen interface. It can be set up with individual and group passwords and be accessed remotely. The interface can provide customized reports such as chain of custody, sample history and temperature stability.

The next step to handling samples without a touch by the hand of technician is being developed to completely automate DNA isolation and banking (Medical Automation Research Center, http://www.marc.med.virginia.edu/pdfs/library/biological_repository.pdf). This system is made up of a number of robotic components that would carry out all steps of DNA banking. The system starts with purified DNA in 50 ml tubes. From there, it can prepare dilutions in 96-well plates (and make replicate daughter plates at a later time), analyze these plates with a microplate reader and/or transports all items to freezer or other storage unit. This system would need to be linked to a data facility to manage all information and provide it to all interested investigators.

In summary, biorepositories are becoming an essential part of the large-scale molecular and epidemiological studies. Many new technologies are being developed to increase an efficiency of samples preservation and banking for already available and novel emerging biomarkers.

References

Albertini, R.J., Anderson, D., Douglas, G.R., Hagmar, L., Hemminki, K., Merlo, F., Natarajan, A.T., Norppa, H., Shuker, D.E., Tice, R., Waters, M.D., Aitio, A., 2000. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. International programme on chemical safety. Mutat. Res. 463 (2), 111–172.

Bingham, S., Riboli, E., 2004. Diet and cancer—The European prospective investigation into cancer and nutrition. Nat. Rev., Cancer 4 (3), 206–215.

Duramad, P., McMahon, C.W., Hubbard, A., Holland, N., 2004. Flow cytometric detection of intracellular Th1/Th2 cytokines using whole blood: validation of immunological marker for use in epidemiological studies. Cancer Epidemiol., Biomarkers Prev. 13 (9), 1452–1458.

Eskenazi, B., Bradman, A., Gladstone, E., Jaramillo, S., Birch, K., Holland, N., 2003. CHAMACOS, a longitudinal birth cohort study: lessons from the fields. J. Child. Health 1 (1), 3–27.

Holland, N.T., Smith, M.T., Eskenazi, B., Bastaki, M., 2003. Biological sample collection and processing for molecular epidemiological studies. Mutat. Res. 543 (3), 217–234.

Mathew, A., Van Buskirk, R., Baust, J., 2003. Improved hypothermic preservation of human renal cells through suppression of both apoptosis and necrosis. Cell Preserv. Technol. 1 (4), 239–253.

Schulte, P.A., Perera, F.P., 1993. Molecular Epidemiology Principles and Practices. Academic Press, San Diego.

Winn, D.M., Reichman, M.E., Gunter, E., 1990. Epidemiologic issues in the design and use of biologic specimen banks. Epidemiol. Rev. 12, 56–70.