

DNA Banking for Epidemiologic Studies: A Review of Current Practices

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Abstract: To study genetic risk factors for common diseases, researchers have begun collecting DNA specimens in large epidemiologic studies and surveys. However, little information is available to guide researchers in selecting the most appropriate specimens. In an effort to gather the best information for the selection of specimens for these studies, we convened a meeting of scientists engaged in DNA banking for large epidemiologic studies. In this discussion, we review the information presented at that meeting in the context of recent published information. Factors to be considered in choosing the appropriate specimens for epidemiologic studies include quality

and quantity of DNA, convenience of collection and storage, cost, and ability to accommodate future needs for genotyping. We focus on four types of specimens that are stored in these banks: (1) whole blood preserved as dried blood spots; (2) whole blood from which genomic DNA is isolated, (3) immortalized lymphocytes from whole blood or separated lymphocytes, prepared immediately or subsequent to cryopreservation; and (4) buccal epithelial cells. Each of the specimens discussed is useful for epidemiologic studies according to specific needs, which we enumerate in our conclusions.

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Key words: biological specimen bank, cryopreservation, DNA, lymphocyte transformation, blood spots.

As the Human Genome Project provides the foundation for understanding the genetic basis of common disease,¹ population-based genetic studies will provide the information needed for the practical application of genetic risk factors to public health practice. To this end, researchers have begun collecting specimens for molecular analysis in epidemiologic studies and surveys (Table 1).^{2–16} Here we address factors to

be considered in choosing appropriate specimens for epidemiologic studies, including convenience of collection and storage, quantity and quality of DNA, and ability to accommodate future needs for genotyping. We focus on four types of specimens that are stored in these banks: (1) whole blood preserved as dried blood spots, (2) whole blood from which genomic DNA is isolated, (3) whole blood from which lymphocytes are isolated and immediately transformed or cryopreserved for later Epstein-Barr virus (EBV) transformation, and (4) buccal epithelial cells.

Blood Spots

During the past 10–15 years, dried blood spots have been used in state newborn screening programs to identify an increasing number of disorders.^{17–20} As a result, dried blood spots from these programs make up at least two population-based repositories.^{21,22} Further, blood spots from military personnel are stored to serve as biologic “dog tags” for identification purposes.^{22a}

Blood spots are a stable, inexpensive source of DNA, useful for genotyping polymorphisms for association studies.²⁴ Those collected in newborn screening programs can serve as samples from which to determine

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TABLE 1. Selected Studies with Specimen Repositories in United States

Study	No. of Participants with Specimens for DNA Studies ^{§§}	Type of specimen used to obtain DNA
Atherosclerosis Risk in Communities (ARIC)*	15,792	Buffy coats (EDTA and ACD)
Physicians Health Study [†]	≈13,000	Buffy coats (EDTA and ACD)
Women's Health Study [†]	≈30,000	Buffy coats (EDTA and ACD)
Women's Antioxidant Cardiovascular Study (WACS) [‡]	≈6,000	Buffy coats (EDTA and ACD) vapor phase of liquid nitrogen
Nurses' Health Study [§]	32,826	Buffy coats (vapor phase of liquid nitrogen)
Women's Health Initiative	161,000	Buffy coats (EDTA) -70°C
Prostate, Lung, Colon, Ovarian Cancer Study (PLCO) [¶]	≈65,000	Whole blood; buffy coat; transformed lymphocytes
CPS-II Lifeline [¶]	100,000	40,000 buffy coats (EDTA) 60,000 buccal cells
Genetics of Non-Insulin-Dependent Diabetes Mellitus (GENNID) [#]	≈6,000	Immortalized lymphocytes
Cooperative Family Registry for Breast Cancer Studies (CFRBCS) [#]	14,000	Cell lines established from cryopreserved lymphocytes; whole blood; buccal cells collected with mouthwash; blood spots
Carotene and Retinol Efficacy Trial (CARET)**	12,426	Whole blood
NHANES 99+ ^{††}	12,606	Blood spots
NHANES III ^{††}	1,206	Extracted DNA
Multi-Ethnic Cohort Study ^{‡‡}	6,462	Extracted DNA -20°C
	≈8,300	EBV-transformed lymphocytes (vapor phase, liquid nitrogen)
	12,041	Buffy coats in vapor-phase liquid nitrogen

EBV = Epstein-Barr virus, NHANES = National Health and Nutrition Examination Survey, EDTA = ethylenediaminetetraacetic acid, ACD = acid citrate dextrose.

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 ‡‡ Brian Henderson, personal communication, 2001.
 §§ As of November 2001.

population gene frequencies. However, use of these specimens in any way other than anonymously is problematic because the specimens may not have been collected with adequate informed consent to perform genetic studies.^{21,23} Blood spots can be collected without a phlebotomist and safely transported by regular mail. Blood spots yield enough DNA to genotype multiple gene variants^{25,26} (Table 2²⁷⁻³⁵).

The New York State Department of Health has developed a method for simultaneously genotyping polymorphisms related to hereditary hemochromatosis, sickle cell disease, methylene tetrahydrofolate reductase deficiency, and medium-chain acyl CoA dehydrogenase deficiency. Genotyping is performed by allele-specific oligonucleotide hybridization in a 96-well format using 1-mm punches that yield 0.8 μL of whole blood. Up to 40 1-mm punches have been obtained from 50- μL blood spots. By combining 4 markers per punch, a minimum of 800 markers could be genotyped from one 250- μL aliquot of blood, that is, five 50- μL blood spots obtained in one specimen.²⁵

To ensure the quality of blood spots for newborn screening, the Centers for Disease Control and Prevention (CDC) evaluates the chromatographic properties of filter paper, the variation of blood volume among different lots of filter paper, and the effects of differences in hematocrit on blood volume. Two brands of filter paper are approved by the Food and Drug Administration (FDA): Schleicher and Schuell (Keene, NH) Grade 903 and Whatman (Clifton, NJ) BFC 180. The NCCLS, formerly the National Committee for Clinical Laboratory Standards, has published guidelines about how blood should be collected on filter paper for newborn screening programs.³⁶

Whole Blood from Which Genomic DNA Is Purified

Many large epidemiologic studies with a genetic component include whole-blood specimens for obtaining genomic DNA. This approach provides high-quality DNA in amounts sufficient for current applications, including genome scans using single-nucleotide polymorphisms, microsatellite repeat polymorphisms, and polymorphism identification (using methods such as single-strand conformational polymorphisms and restriction fragment-length polymorphisms), as well as for genotyping loci (using methods such as allele-specific oligonucleotides or sequencing).

The National Heart, Lung, and Blood Institute (NHLBI) has published guidelines for obtaining specimens,³⁷ and we have discussed some of these issues in a previous review.²⁴ Blood is most often collected using ethylenediaminetetraacetic acid (EDTA), although anticoagulants including heparin and acid citrate dextrose (ACD) have also been used. Cells can be stored in

whole blood, either anticoagulated or as a clot, or in buffy coats.^{4,5,11-13,38-42}

Buffy coats have been obtained in large epidemiologic studies for isolation of DNA and establishment of transformed cell lines. However, obtaining consistent DNA yields from buffy coats requires careful technique and is time-consuming. Because of ease of specimen handling and storage, DNA is often isolated from fresh whole blood or blood stored at -80°C in large epidemiologic studies.¹¹⁻¹³ DNA can also be extracted from blood clots, and improved commercial methods now allow yields that are similar to those from anticoagulated whole blood (Puregene, Minneapolis, MN). The use of clotted blood allows the investigator to obtain serum for other analyses including environmental toxicants and, at the same time, to obtain DNA from clots.

Polypropylene rather than glass containers should be used to store frozen blood, and blood should be divided into aliquots to prevent freeze-thaw cycles. Purification methods include use of (1) enzymes (including proteinase K and RNase)⁴³; (2) organic solvents or organic solvents with enzymes⁴⁴; (3) salt precipitation⁴⁵⁻⁴⁷; and (4) resins or affinity gels, which are also the basis for many commercial kits.^{48,49}

Several large epidemiologic studies collect anticoagulated whole blood from which to extract DNA or for storage and later extraction (Table 1). The National Health and Nutrition Examination Survey (NHANES) that began in April 1999 is a continuous survey that collects specimens from approximately 5,000 people in 15 U.S. locations each year.¹³ Because of limited resources, lymphocytes are not being transformed as was done for NHANES III,²⁴ but instead approximately 250-700 μg of DNA per participant is isolated from two 10-mL tubes with EDTA following the Puregene (D-50K) kit protocol (Gentra Systems, Inc., Minneapolis, MN).

Transformed Fresh or Cryopreserved Lymphocytes

EBV-transformed lymphocytes provide an unlimited source of high-quality genomic DNA for genotyping large numbers of polymorphisms requiring microgram quantities of DNA as well as cells that may prove to be useful for functional studies. Traditionally, blood specimens have been sent to a central laboratory within 48 hours of collection for separation and EBV transformation of lymphocytes. However, an appealing alternative would be to cryopreserve initially and transform lymphocytes later. This option would allow the investigator to transform selected subsets of specimens for nested case-control studies, but it presupposes that lymphocytes can be cryopreserved for long durations and then transformed.

Although two published articles have reported viability of B-lymphocytes after cryopreservation and long-

TABLE 2. Comparison of Specimens for DNA Banking for Epidemiologic Studies

Specimen Type	DNA Yield	Advantages	Disadvantages
Blood spots	12–42 ng/ μ l (adults) ¹⁷ 43–78 ng/ μ l (neonates) ¹⁷ 1/4-inch punch from 75- μ l volume yields about 12 μ l of blood ²⁷	Small sample size Ease of sample collection Ease of shipping (regular mail) Stability and low cost storage Offers a source for study of exogenous or endogenous compounds other than DNA Genotyping generally requires 10 ng/genotype, and with current technology as little as 2.5 ng per SNP so that scores to hundreds of genotypes could be obtained from one blood spot	Low DNA yield: may not be suitable for whole-genome amplification Nonrenewable Smaller amplicons
Blood cells Whole blood - anticoagulated or blood clots Buffy coat	100–400 μ g/10 ml ^{28–31*} \approx 200 μ g/ml ^{28–31*}	Relatively low-cost storage (-80°C) Yields large quantities of high-quality, genomic DNA Offers a source for study of exogenous or endogenous compounds other than DNA	Invasive sample collection Shipping (special requirements) Nonrenewable
Transformed lymphocytes	10^6 cells = 6 μ g ^{28–31*} 1–2 $\times 10^6$ cells = 5–10 μ g	Renewable source of DNA Yields large quantities of high-quality, genomic DNA	Labor-intensive preparation High-cost storage (liquid nitrogen and periodic reculture) Does not offer a source for study of exogenous or endogenous compounds other than DNA or RNA
Buccal cells	49.7 μ g mean; 0.2–134 μ g range (mouthwash - total DNA) ³² 12–60 μ g range (mouthwash - total DNA) ³³ \approx 16–30 μ g median; 1–290 μ g range (mouthwash - hDNA) ³⁴ 32 μ g median; 4–196 μ g range (mouthwash - hDNA) ³⁵ 1–1.6 μ g/2 cytobrushes median; 6 ng–13 μ g range (hDNA) ³³ 1–2 μ g/ cytobrush (total DNA) ³¹ 1–2 μ g/ swab (total DNA) ³¹	Noninvasive collection Ease of sample collection (allows participant to collect and mail specimen) Genotyping generally requires 10 ng/genotype, and with current technology as little as 2.5 ng per SNP so that many thousands of genotypes could theoretically be obtained from a buccal cell specimen	Low DNA yield; not in general use for whole-genome amplification Highly variable yield Does not offer a source for study of exogenous or endogenous compounds other than DNA or RNA Bacterial contamination must be addressed

SNP = single-nucleotide polymorphism; hDNA = human DNA.
* Whole-blood, buffy-coat, and cell values are a range derived from expected yields given by manufacturers.³¹

term storage,^{50,51} we found only one published report of a systematic study of the effects of long-term cryopreservation on transformation success rates.⁵² In most cases, cells are cryopreserved in RPMI with 30% fetal bovine serum, dimethylsulfoxide (DMSO) is added to a final concentration of 6–10%, and cells are frozen in a programmable freezer and stored in liquid nitrogen (vapor or liquid phase). Some scientists have suggested that cryopreserved cells may have a higher rate of transformation than fresh cells because of treatment with DMSO before EBV exposure.⁵¹

Investigators conducting the Multi-Center AIDS Cohort Study published data on the viability and ability to EBV-transform specimens stored for up to 12 years with a 90% transformation success rate.⁵⁰ The authors emphasized the importance of cell separation and storage within 6 hours of collection and use of a controlled-rate freezer when cryopreserving cells—procedures that may not be possible in many epidemiologic studies.

The Cell Biology Program of the American Type Culture Collection (ATCC, Manassas, VA) reported their experiences with a project of the National Cancer Institute (NCI) during the past 10 years in which specimens were obtained for study from people who either have cancer or are at high risk for cancer.⁵² A typical specimen comprised 20–30 mL of whole blood from which DNA was immediately extracted and lymphocytes separated on a density gradient for EBV transformation (for which ATCC reports a 97.5% success rate). Lymphocytes have been successfully transformed from 250–500 μ L of fresh or cryopreserved whole blood, and all attempts to EBV-transform lymphocytes from 245 cryopreserved whole-blood specimens that had 10% DMSO and were frozen in a programmable freezer were successful. Whether the whole blood was fresh or cryopreserved, culture conditions included irradiated MRC-5 cells as a feeder layer with exposure to EBV. On the basis of this information, ATCC now cryopreserves 2 mL of whole blood for later EBV transformation.

NCI's Cooperative Family Registry for Breast Cancer Studies (CFRBCS)¹¹ includes cell lines and a DNA repository, some of which is maintained by the Coriell Institute for Medical Research (Camden, NJ). Repository specimens include blood and buccal cells. For each blood specimen, a tube of blood is stored at -80°C for direct DNA isolation, another is used for the isolation and cryopreservation of lymphocytes for future transformation or nucleic acid preparation, and a third tube is used for preparation of plasma and blood spots for identity checks. When venipuncture is not possible, DNA is prepared from buccal cells obtained using mouthwash. Cryopreserved cells are held in the liquid phase of liquid nitrogen⁵⁴ as four aliquots of about 10 million cells each.

Attempts to transform freshly isolated lymphocytes from CFRBCS participants yields a success rate of 93%

and for cryopreserved lymphocytes a 90% success rate.⁵² In addition, the data suggest that the length of time lymphocytes are held cryopreserved has little or no effect on the time to transformation, an observation noted also by Penno *et al.*⁵⁵ Coriell receives specimens 1–6 days after collection with no significant difference in time required to transformation over the 6 days whether the lymphocytes are fresh or cryopreserved. Successful lymphocyte transformations are obtained up to 10 days after collection in ACD.

The National Marrow Donor Program (Minneapolis, MN) was established in 1986 to provide donors for bone marrow transplantation.⁵⁶ This program has established a repository, managed by Blood Centers of the Pacific (San Francisco, CA), comprising serum, lymphocytes, and EBV-transformed lymphocytes. Blood is collected in ACD and is usually received less than 48 hours after collection, although international specimens can take up to 5 days to arrive. Blood Centers of the Pacific has transformed 95% of donor specimens on the first attempt, which can be augmented with a second try.

The Biotech Research Laboratories of Boston Biomedical, Inc. (BBI, Rockville, MD), works in the area of infectious disease, particularly human immunodeficiency virus and other retroviruses. BBI processes specimens for NCI, NHLBI, and FDA repositories in three ways: Ficoll separation, nucleic acid extraction, and EBV transformation of lymphocytes. BBI is involved in studies to determine optimal specimen handling for these repositories.

As an index of cell viability, BBI measures lymphocyte apoptosis using Hoechst 33342 dye.⁵⁷ To determine whether cryopreserved lymphocytes should be shipped in a dry shipper or on dry ice, particularly for situations in which samples are delayed for several days using this system, lymphocytes were isolated using Ficoll-density gradient centrifugation, frozen in a controlled-rate freezer with 7.5% DMSO, or placed into liquid nitrogen for 2 weeks. Aliquots were then transferred to -70°C or left in liquid nitrogen for 4 days. All aliquots were then placed into liquid nitrogen. The rate of apoptosis for cells that had remained in the vapor phase of liquid nitrogen was 6.8% and that for cells moved from liquid nitrogen to -70°C and back to liquid nitrogen was 54%.

Transformation rates were virtually identical in cells that were stored at -70°C and those stored in liquid nitrogen for up to 2 months, although lymphocytes stored at -70°C took longer to transform. For the present, BBI recommends that specimens be shipped in dry shippers to maintain viability.

NCI reported on a cohort study within a program that evaluates strategies for early detection of cancer. Blood specimens collected at each of six annual examinations are used to search for early markers and etiologic factors for cancer. Specimens include plasma, buffy coats, and

whole blood (in ACD) for cryopreservation. Approximately 95% of the specimens arrive within 24 hours, and virtually all within 48 hours. After receipt by NCI, the whole blood is divided into 10–12 aliquots in 1.8-mL vials with 10% DMSO, frozen in a rate-controlled freezer, and placed into the vapor phase of liquid nitrogen.

In collaboration with ATCC, NCI is evaluating EBV transformation after long-term storage of lymphocytes. NCI finds that lymphocytes from blood collected with ACD maintain viability longer at room temperature than blood collected with heparin. However, in all cases, viability drops by 3 days after collection, an experience similar to that of CDC.²⁴

Despite the virtually unlimited supply of DNA furnished by transformed cell lines, this type of specimen has drawbacks, not the least of which is the high cost. Another difficulty is the absence of serum or whole blood that could be used to measure compounds that may be important study variables.

Buccal Cells

Buccal cells can be obtained for DNA isolation using cytobrushes, swabs, or oral lavage.^{23,53,57,58} Buccal cells are being collected in several large epidemiologic studies as the primary specimen or as a supplement to whole-blood specimens (Table 1). Although there are few systematic studies that compare the various methods of collecting buccal cells in terms of their yield of human DNA (hDNA), a growing consensus suggests that the use of mouthwash gives a greater yield and higher-quality hDNA (excluding bacterial contamination) than other methods of collection.^{32–35} The use of alcohol-containing mouthwash has been proposed as the optimal collection medium to prevent bacterial growth on swabs, given the bacteriostatic properties of the alcohol. Alternatively, some have proposed the use of cytobrushes to exfoliate buccal cells, followed by expectoration of fluid that is spotted onto Guthrie cards treated with bactericidal and nuclease-inhibiting compounds.⁵⁹

One published study reported the quantity, quality, and stability of hDNA collected with mouthwash.³⁵ Specimens yielded a median quantity of at least 32 μg DNA (2–194) if specimens were held for up to 5 days, with yields declining to 21 μg (5–56) at 30 days. Polymerase chain reaction (PCR) success rates were greater than 94%, and high-molecular weight DNA (>23 kb) was found in all but 1 of the 24 specimens. Yields were greater when specimens were collected before brushing teeth.

Another recent publication reported hDNA yields in a comparison of cytobrush and mouthwash collection methods as well as DNA extraction methods.³⁴ Median yields for cytobrushes were between 1 and 2 μg com-

pared with yields between 16 and 27 μg for mouthwash. Although PCR success rates were similar between the methods, mouthwash specimens were superior to cytobrushes for obtaining high-molecular weight DNA.

ATCC is developing methods for use of buccal cells as a cost-efficient, noninvasive source of DNA and is shifting its emphasis from DNA derived from whole blood and EBV-transformed lymphocytes to greater use of buccal cells. Oral rinses are collected on filter-paper cards⁵⁹ and mailed to a central laboratory. DNA is then extracted and its quality evaluated by electrophoresis. However, quantities of DNA collected on these cards have not been sufficient for spectrophotometric detection and fragment sizes from only 536 base pairs (bp) to 989 bp have been amplified.

Methods such as the mouthwash method that require participants to expectorate are not an option for infants and small children. Because many studies report lower yields using cytobrushes, particularly in children and infants, methods for optimizing yield from buccal specimens obtained with cytobrushes are needed. Primer extension preamplification (PEP) is one method for whole-genome amplification (WGA).^{60,61} Zheng *et al.*⁶² reported improved DNA yields using PEP with WGA, which allowed about 900 PCR reactions per cytobrush with results verified with blood or marrow specimens.

To determine the quality of buccal-cell DNA as well as the feasibility of whole-genome amplification, Coriell developed a protocol for obtaining buccal cells from registry participants who declined to have blood drawn. After collection with mouthwash, DNA is prepared with kits from Qiagen (Valencia, CA), quantified with SYBR Green (Applied Biosystems, Foster City, CA), and characterized by pulse field electrophoresis. DNA 20 to 40 kb in length was obtained and was suitable for PCR-based assays. The DNA supported long PCR using a 5.6-kb segment of the calmodulin gene.

Because of the limited amount of DNA obtained from buccal specimens (0.2–6.0 μg), Coriell used the PEP method of Zhang *et al.*⁶¹ to determine whether the DNA could serve as a template for WGA. Using mouthwash DNA as a template, DNA can be increased 500- to 1,000-fold using PEP with sizes ranging from 500 to 4,000 bp. However, a portion of the amplification is attributed to bacterial DNA. PEP appears to be suitable for PCR-based assays such as the amelogenin allelic discrimination assay, genotyping with di-, tri- and tetranucleotide markers, and detection of the trinucleotide repeats in the androgen receptor and polymorphisms in mitochondrial DNA. Although PEP is a method for whole-genome amplification yielding sufficient DNA for extensive genetic analysis, it is not routinely done or routinely successful. First, the fidelity of repeat sequences is problematic because telomere repeats appear to shorten, and amplification of trinucleotide repeats in

Huntington's disease is difficult. Others have also noted the need for validation of nucleotide repeats.⁶² Second, the product size is limited, which may preclude its use for long PCR.

Sources of variation in yield from buccal cells include the method of collection, the DNA extraction method (which may vary with the type of column used), and the unsupervised collection of specimens in the homes of participants and subsequent transport to central laboratories.

Conclusions

The basic sequence of the human genome has been completed and in the coming years a majority of human genes will be identified. The next step is to elucidate the differences among people in sequences, genes, and gene expression patterns, to explain what role these differences play in disease, and in some cases to develop genetic tests for these variants. Specimens such as those described here will be used in studies to identify genetic risk factors for disease. The type of specimen collected in epidemiologic studies will depend on the study needs, as follows.

1. In most cases, genomic DNA extracted from whole blood for immediate use or storage will be the safest assurance that sufficient material will be available for most current and future molecular applications at a cost for storage of specimens that is sustainable.

2. Blood spots should be considered as alternative to whole blood when protocols call for easier collection and cheap room-temperature storage. Buccal cells should be considered when noninvasive, self-administered, or mailed collection protocols are required. However, these alternative collection protocols will yield only limited amounts of DNA with wide interindividual variation when buccal cells are collected. Also, the strategy for shipment of DNA-containing samples may need to be modified if sterilization procedures such as E-beam radiation are put in place by postal services and other carriers. In any case, scores to thousands of genotypes can be theoretically obtained from blood-spot or buccal-swab specimens.

3. If a virtually unlimited source of DNA is needed for repeated or collaborative studies, or if studies of gene expression using RNA or protein are needed, and if sufficient long-term funding is available, then lymphocytes should be transformed. Although cryopreservation and later transformation of selected specimens could reduce the number of specimens to be transformed, the high costs of maintaining the cell lines created later is still a factor, and data are insufficient to confirm that this strategy would ensure viable cell cultures upon transformation.

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