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Ambient Biobanking Solutions for Whole Blood Sampling, Transportation, and Extraction

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Abstract

Biobanking increases the rate at which precision medicine can be used to successfully refine currently existing medical treatment methodologies. The purpose of precision medicine is to increase a patient's likelihood of defeating a chronic disease, by creating a unique and personal treatment method. However, the research necessary to develop precision medicine requires thousands of biospecimens, which is why biobanking is necessary to move precision medicine forward. Traditional biobanks are a library of preserved biological specimens, such as tissue and whole blood, that can be later accessed for further testing and analysis. Maintaining these types of biobanks is cumbersome and expensive, due to freezer care. Biobank samples are used to support therapeutic drug monitoring in clinical trials, epidemiology, public health screening, and biomarker discovery. Collecting samples for large translational studies requires making regular trips to the phlebotomist or a clinic, which is an inconvenience that is exacerbated when collecting samples in remote and/or resource-limited locations. Inconsistencies in sample collection can affect downstream clinical studies. Remedies for these procedural issues include the development of a medium that effectively preserves the samples at ambient temperature and developing a virtual biobanking system that allows for long-distance access to bioinformatic data of previously analyzed biospecimens.

Keywords: biobanking, precision medicine, dried blood spots (DBS), nucleic acid, ambient temperature storage, translational studies

1. Introduction

Biobank acts as a library for genotypic and phenotypic data for a variety of biological samples. It is the process of acquiring, storing, processing, and distributing biological materials for the purpose of clinical use, including for the development of precision medicine. The term biobanking covers a broad range of samples, including those of animal, plant, and microbial origins. For instance, animal samples can be organ tissue, marrow, and synovial fluid, while plants can be roots, leaves, bark, flowers, and lastly microbial samples. The biobanking arena has seen significant advances from collecting and cataloging samples to having detailed archives of genotypic and phenotypic information. The storing of this information is part of the newest wave in biobanking, virtual biobanking. Virtual biobanks contain full

genomes of previously collected specimen that may be accessed through specialized software or portals. Virtual biobanking assists investigators in searching multiple sites for specimen worldwide, essentially, allowing for the mining of data remotely [1]. Integration of genomics, proteomics, and metabolomics, as well as introduction of highly sensitive analysis methods, has translated into a demand for high-quality specimens and the need for accurate, reliable, and standardized clinical data. However, current methods in collecting samples are strenuous, expensive, and unreliable. Samples collected in the field have to be chilled or frozen until analysis, but the shipping of large chilled containers and powering freezers are cost limitations affecting research projects. Furthermore, there is a relatively small window of time between sample collection, storage, and analysis to preserve sample integrity; reducing the reliability of data. As a result, there has been a growing demand in the market for developing ambient temperature storage methods. In the following sections, the relevance of biobanking to precision medicine will be discussed as well as advances in sample collection and ambient temperature storage methods to reduce the cost of acquiring and storing precious biospecimen.

2. Precision medicine

In the last decade, there has been a push to understand factors that affect an individual's health on the molecular level. These factors include an individual's unique lifestyle and environment because it is now understood that epigenetics plays a large role in a person's health as well as their development of chronic diseases, such as cancer [2]. Epigenetics and its effects on multiple "omics" (e.g., proteomics) require more than a snapshot of a single person's life. Instead, large data sets ranging from local population (i.e., a neighborhood or city block) to a statewide population, or larger, are required to truly understand the connection between lifestyle and health; but this is not the only advantage of having a large sample size. Determining treatment for a disease requires the largest possible sample size, in order to account for all the possible variables that lead to developing the illness. The marriage of genetic sequencing and external factors that affect health (i.e., lifestyle and environmental) is the foundation of precision medicine. Precision medicine is the use of multiple facets of an individual's health to develop a unique treatment plan [3] (**Figure 1**). With the cost of sequencing decreasing, it has become possible to query the whole genome in search of variants that are known to cause certain disease, and, thus, develop targeted therapies and reduce the overall strain placed on the body [4, 5]. Analysis of the human genome in the context of diagnostic medicine is one of the main facets of precision medicine. The current methodology for designing treatment plans is based on general information obtained from clinical trials; however, every person is unique and there are numerous instances where these "umbrella" treatment methods prove to be unsuccessful [3]. Thus, by being able to determine the root cause of the disease, be it lifestyle affecting gene expression, genetic inheritance of a mutation, or a random mutation itself, precision medicine provides the opportunity to have a focused approach in diagnostic medicine.

Precision medicine, and the initiative to push it forward, was strongly endorsed by the Obama administration after a young woman was able to determine the cause of her extremely unusual form of liver cancer through virtual searches of sequenced genomes of donors with the same disease [6]. The synergy between genetic markers and new therapies for cancer treatment is one powerful example of precision medicine. Typically, biopsied tissue samples or whole blood samples are used as the material for sequencing [6, 7]. For example, liquid biopsies are now routinely used

Figure 1.
Precision medicine is a holistic approach to treatment where for the first time, the phenotypic, proteomic, metabolomic, and the genomic composition of the individual as well as their links to other factors such as the microbiome and even the environment will be taken into consideration. This means treating the patient as a “whole” in contrast with the current isolated symptom-treatment approach. The goal of precision medicine is to develop a personalized treatment regimen, a one-of-a-kind approach that would have a better clinical outcome for the patient.

for the analysis of cell-free DNA to look for progression/regression of cancer as well as additional genomic mutations [8]. As technology advances, instant interdisciplinary integration has become a reality and bioinformatic biobanking makes this integration possible [4]. However, there are many instances when hospitals or research facilities will not release the sequenced data of the samples that were collected or preserved specimens, and literature sources have inadequate information of the biospecimen used [6, 8]. This increases the necessity to have both a national and global data base available so that invaluable information could be accessed seamlessly. One of the first steps in creating such a large data bank is the development of the Million Donor cohort.

Precision medicines’ Million Donor cohort comes with a great responsibility for those institutions preserving these samples to answer future research questions [8]. The purpose of the cohort is to begin collecting data from one million individuals across the nation with diverse backgrounds [3, 9]. Building such a large cohort proves to be a daunting task; however, Terry [6] has shown that, when able to, patients will take the initiative to be active members in their health maintenance. Although questionnaires will be used to develop the cohort data, the success of precision medicine depends on the number of available electronic medical records (EMRs) to gain valuable insight into quantitative medical data [3]. Developing such a sample pool that can be easily accessed requires storing the data in virtual biobanks, a topic that will be discussed in detail later in the chapter [9, 10]. Biobanking and the accessibility of data with a user-friendly network for the purpose of data mining are crucial not only to both short-term and long-term goals of the Precision Medicine Initiative but also to the future. Access to samples is necessary to fulfill the vision of combining established clinicopathological parameters with emerging molecular profiling approaches to create diagnostic, prognostic, and therapeutic solutions that are precisely tailored to an individual patient’s unique requirements. Sample availability and sample preservation via biobanking are key to the future of the Precision Medicine Initiative and beyond.

3. Biobanking

In this section, the growth of biobanking for the purpose of current research interests will be discussed. Biobanking has been implemented in the scientific community for over 100 years by various institutions worldwide [11, 12]. Biobanks are large repertoires of biospecimen, ranging from animal samples to plant and microbes, that are used for research purposes [12]. Biobanking itself is a relatively simple concept (**Figure 2**). All types of biospecimen are stored in a biobank for long, yet finite amounts of time. These repositories of specimen in traditional biobanks remain in large freezers and other storage facilities until needed [12, 13]. Thus, biobanks are extremely valuable for translational research studies since generations of specimen may be stored and received.

Standardization of samples is key to successful biobanking. Reliability of samples collected in an ethical and legal manner with the oversight of the Institutional Review Board (IRB) or equivalent ethics committee for the biobanking institutions in their respective countries is crucial to ensuring reproducibility of results. International standards are being established by both the European Union [14] and International Society of Biological and Environmental Repositories (ISBER) in the United States [15] to establish standardization metrics for biobanked samples. ISBER coordinated the launch of the International Repository Locator (IRL) website in early 2015. This centralized locator, analogous to a “repository directory,” was created to increase the profile of individual repositories including ISBER, researchers, funding bodies, governments, and private industry. However,

Figure 2.

Infrastructure needed to biobank samples in a laboratory (left to right). Blood, buccal swab, or tissue biopsy/aspirate samples collected from donors are transported to the research facility if the collection site is remote to the sample processing laboratory. Maintaining traceability and transparency should be mandatory to all human samples, the samples are cataloged electronically when received and connected with the donor's electronic health record (EHR) if available, along with other relevant information before either biobanking or processing the sample for nucleic acid or other biomolecule (DNA, RNA, buffy coat, proteins) extraction. Depending on the size of the donor pool, the most efficient means of processing the large cohort of samples is by automation. The biomolecule extracted is analyzed for quantity and quality before storage in a biobank at -80°C , or -196°C (liquid nitrogen). Consequently, for an economical means of storage of a large cohort of samples, the extracted biomolecules can be stored in a chemistry matrix for dry state or “glassy state” such as RNA/DNA stable (Biomatrica), RNasecure (ThermoFisher) or GenTegra RNA/DNA (GenTegra LLC) or on treated paper such as Whatman FTA or GenSaver or untreated paper such as Whatman 903 or GenCollect. The choice of media for storage for biobanking is institute dependent.

not all facilities with biobanks are willing or able to share these invaluable biospecimens [3, 12, 13]. With such limitations on the use of biobanks, the importance to develop new methods for biobanking continues to grow as technology and research methods have advanced and become more refined to solve previously, seemingly impossible medical mysteries. The Million Donor cohort acts as one solution to the problem of free-data sharing since the project's purpose is to create a comprehensive virtual biobank for the purpose of precision medicine and bettering healthcare [3, 16]. Making biobanking a realistic tool and more accessible requires the blending of specimen collection and analysis.

To streamline biobanking and research analysis, a new concept in biobanking is bioinformatic biobanking. This refers to the querying of sequenced genomes that have been stored in virtual biobanks. Bioinformatic biobanks are large databases of information pertaining to the sequenced and analyzed specimen [12, 17]. In this concept of a biobank, an individual's immutable genetic markers form a library to be queried over a lifetime for continuing patient management. Once a sample is analyzed and the data stored, it is then a simple matter of querying the data when and as required for specific gene regions, biomarkers, variations, etc. This approach is currently being implemented by Helix personal genomics with whole genome sequencing as the first step. Using this method negates the need for long-term sample storage because the whole genome can be virtually analyzed for specific biomarkers that may correlate with a disease. Once the genomic analysis is completed, any future test queries involve only a bioinformatic search, as opposed to additional sample collection and repeated analysis. This reduces the cost of biomedical research significantly; however, the collection, transportation, and storage of samples until analysis occurs still pose a significant cost and slow efforts in developing a globally available virtual biobank. In the next section, the sample types used for biobanking will be discussed, and advances that will eliminate the cost of storing and shipping such liquid samples.

3.1 Sample types used for biobanking

The most common sample types collected for precision medicine and biobanking of human specimen are tissue samples and whole blood. Tissue samples can be further subdivided into liquid biopsy samples for circulating tumor cells (CTC), tissue biopsy samples such as formalin-fixed paraffin-embedded (FFPE) tissue, and fresh frozen tissue samples or wet mount tissue slides. Whole blood samples can be: peripheral blood mononuclear cells (PBMCs), serum, or plasma. Additional, albeit less common, sample types collected are cerebrospinal fluid (CSF), urine, and fecal material. When collecting these samples, it will be imperative to have "True Control" samples from surrounding disease-free tissue and corresponding known disease-state samples for comparisons; but, it is not always practical for tissue biopsy samples or CSF. In such instances "external" matched controls must serve as acceptable substitutes for "True Control" samples.

3.1.1 Tissue

Tissue samples such as formalin-fixed paraffin-embedded (FFPE) blocks have been stored since the early twentieth century. FFPE tissue samples are a common sample type collected from biopsies. Although core biopsy samples yield a healthy amount of tissue, tissue biopsy procedure is a painful process for the patient and can potentially cause considerable trauma to the surrounding tissue. Fine needle aspirate (FNA) biopsy with a 21-gauge needle to remove tissue samples for pathology is less traumatic to the patient and to the surrounding tissue. Compared to core biopsy

samples that are typically about 17 mg or more, the FNA samples are just 2–10 mg and the amount of sample that is donated to research is often less than 1 mm as priority for testing of the biopsy sample is to perform cytopathology. The best outcome for nucleic acid-based testing from tissue samples is to isolate nucleic acid from fresh or flash-frozen at -196°C tissue samples. There is no ambient temperature method available to preserve tissue samples for extracting good-quality nucleic acid.

FFPE is the most common method of preserving tissue samples at ambient temperature. FFPE tissue storage has been used for three decades [18] as a means of keeping tissue samples at ambient temperature for future research [19, 20]. This has created a large resource of pathologically interesting human and animal samples. Fixing tissue samples with formalin and embedding in paraffin preserves the pathology of the tissue. But formalin fixation can cause both inter and intra protein cross-linking [21–23] as well as cross-linking of histones to DNA [24]. Other factors affecting the quality of nucleic acid from FFPE samples include buffering formalin, time and temperature of fixation and penetration of formalin into the tissue by stasis, or by ultrasound, or microwave irradiation. The nucleic acid and protein quality are additionally dependent on the time of collection of tissue following postmortem interval and cold ischemia. Acceptable time for collection of tissue samples is between 4 h postmortem and 12 h after cold ischemia has set in. Acceptable time for formalin fixation of tissue postmortem is <48 h for RNA [25, 26], <24 h for proteins [27–32], and <72 h for DNA [33–36]. It would be best to isolate the nucleic acids from FFPE samples within the acceptable time to ensure the best outcome for the quality of the nucleic acid isolated. The isolated nucleic acid can be further stored at ambient temperature by removing the aqueous media from the nucleic acid sample or by adding some commercially available stabilizers for ambient temperature storage of nucleic acid. Although cross-linking of nucleic acid is of concern with aged FFPE samples [18, 33], nucleic acid extracted from FFPE samples have been successfully used for amplification, single cell analysis, and methylation studies. Decalcification of the FFPE sample using EDTA allows for longer PCR product [37], stronger fluorescence in situ hybridization (FISH) signals, lower background staining [38], and superior comparative genomic hybridization [1] results as compared to other methods.

3.1.2 Blood and blood components

Perhaps the most economical samples are blood samples collected in EDTA tubes [35]. A host of specialized blood collection tubes are commercially available for stabilization of transcripts such as Tempus Blood RNA tubes and PAXgene Blood RNA tubes [39]. The strategy for storing the samples for short-term usage and long-term biobanking needs will determine the quality of the sample. Acceptable short-term storage of weeks to months of blood and blood components such as serum, plasma, peripheral mononuclear cells (PBMCs) etc. is at 4 to -20°C and long-term storage is at -80 to -196°C . Liquid samples such as whole blood, saliva, plasma, and serum samples as dried spots can be stored for decades at ambient temperature if sampled on chemically treated substrate such as FTA or GenSaver paper cards [40, 41].

Serum and plasma samples can be stored at ambient temperature for extended periods of time on chemically treated bead matrix such as GenTegra LLC's Matrix Chaperone (MC) (**Figure 3**). Up to 250 μL of serum or plasma sample can be applied to the MC for storage and for biobanking. Downstream analysis of the MC can be performed simply by adding back equivalent volume of water to the MC. The full complement of analytes, proteins, enzymes, and nucleic acid in serum and clotting factors in plasma samples (data not shown) have been successfully stored for 25 days at ambient temperature on MC consisting of a randomly packed chemically

Figure 3.
Ambient storage of serum samples. It is possible to store the entire complement of biological molecules in serum (or any other biological fluids) on a simply made storage device consisting of 2-µm polystyrene beads coated with stabilization chemistry collectively called matrix chaperone (MC) for ambient storage and transportation. The collection device was a simple three-component device (i), containing a cap (A) with the resuspended stabilization chemistry in a matrix of polystyrene beads and the holding chamber (B) with silica gel (C) to facilitate drying. A volume of 250 µL of serum sample was added to the cap containing the chemical matrix of polystyrene beads (iib) and capped. The assembly was placed in an upside-down position for at least 12 h to facilitate drying. To initiate analysis, the sample was reconstituted with 250 µL of water (iic). The sample is recovered by transferring the sample to a 1.5-mL tube (iid) and centrifuging the sample at maximum speed for a minute (iie). A complete metabolic panel and a lipid panel test were performed on this reconstituted serum sample (Table 1).

treated microsphere wafer when compared to pristine always frozen at -20°C serum samples (Table 1).

3.1.3 Dried blood spots microsample biobanking

Dried blood spots (DBSs) can be used for both real-time microsampling and subsequent ambient temperature biobanking for epidemiology and biomarker discovery (Figure 4). DBS samples can be particularly effective as a means of sample collection from participants in clinical trials. A survey by Tasso Inc. determined that a trial candidate may be more compliant to sample collection when given a less painful option for sample collection such as the OnDemand automated blood collection device for DBS collection and when done in the comfort of their own home (data unpublished) (Figure 5). Blood stabilized on the DBS can then be mailed by local postal services at the patient's own convenience. Although storage of whole blood as DBS is an old technology, historically poor stability outside the lab environment, as well as low recovery levels and generally low quality of extracted nucleic acids and numerous blood proteins, has hindered its acceptance. In recent years, there has been development for a completely new, "smart health care," paper-based sampling technology, which overcomes many of these known drawbacks. Deployed as a simple, painless skin prick onto a chemically treated collection card, the dried blood may then be recovered by ordinary magnetic bead or column-based DNA purification. With the resurgence of interest in the use of DBS for sample collection, research is being done to develop novel chemistries to yield RNA, DNA, and

Table 1.a: Enzyme Panel				
	Normalized to Frozen	Always Frozen	Initial Raw Values	Normal Range
Enzymes	Ambient	Values	Ambient	(IU/L)
ALT	75%	25	19	7 – 40
AST	70%	34	24	Oct-36
CK	74%	187	138	30 - 336
ALP	43%	68	29	44 - 147
Amylase	84%	61	51	26 - 102
GGT	77%	42	32	0 - 51

Table 1.b: Lipid Panel				
	Normalized to Frozen	Always Frozen	Initial Raw Values	Normal Range
Lipids	Ambient		Ambient	
Cholesterol	76%	254	192	≤200
Triglycerides	78%	227	177	≤150
HDLc	97%	53	51	≥60

Table 1.c: Protein Panel				
	Normalized to Frozen	Always Frozen	Initial Raw Values	Normal Range
Proteins	Ambient	Values	Ambient	
Total Protein	83%	7	6	6 – 8.3 g/dL
Albumin	78%	5	4	3.1-5.4 g/dL
Ferritin	75%	255	190	24-330mcg/dL
TSH	79%	11	9	0.4-4.0mIU/L
T4	95%	5.9	5.6	4.5-11.2mcg/dL
SHBG	77%	40	31	10-157nmol/L
IgA	79%	195	153	80-350mg/dL
IgG	77%	927	716	620-1400mg/dL
IgM	68%	95	65	45-250mg/dL

Table 1.d: Metabolite Panel				
	Normalized to Frozen	Always Frozen	Initial Raw Values	Normal Range
Metabolites	Ambient		Ambient	
Creatine	54%	1	0.5	0.6-1.3mg/dL
Cortisol	76%	17	13	7-25mcg/dL
Testosterone	121%	165	199	250-827 ng/dL

Table 1. Stability of serum enzymes, proteins, lipids, and metabolites at ambient when stored on the polystyrene bead matrix, MC, containing ambient stabilization chemistry for all biomolecules. A volume of 250 µL of CAP-certified serum samples spotted on the polystyrene matrix (MC) tubes and dried before storing for 25 days at ambient. Corresponding control serum samples were stored at –20°C. After 25 days of storage at ambient, the experimental and control samples were hydrated with 250 µL of water. All the analytes from the rehydrated MC serum samples and the fresh always frozen –20°C control samples were quantified for the complete metabolic panel and the lipid panel with the cobas® 6000 analyzer. The percent recovery of the analytes was calculated for the ambient stored samples compared to the control serum samples from the initial raw values of the test biomolecules. International units per liter (IU/L) of the enzyme panel for alanine amino transferase (ALT), aspartate aminotransferase (AST), enzyme marker creatine phosphokinase (CK), amylase, and gamma glutamyl transferase (GGT) are within the normal range for the ambient stored serum samples when compared to –20°C control samples. The level of the alkaline phosphatase (ALP) was low compared to the control serum samples indicating that the stabilizer is not able to protect the labile ALP enzyme. All the molecules tested in the protein panel and the lipid panel were in the normal range and maintained at between 68% and 83% of control indicating stability at ambient of all biomolecules in this panel on the MC. Of the three metabolites tested in the metabolic panel, the stabilizing matrix of the serum sample stored at ambient could not stabilize the metabolite creatine but cortisol and testosterone were stabilized. Normal ranges for panel values were taken from <http://www.mayoclinic.org/> (April 3, 2013) except for the testosterone normal range, which was taken from <https://www.questdiagnostics.com/home/> (January 31, 2020).

proteins with quality and quantity enough to support advanced analytical methods such as next generation sequencing and multiplex proteomics.

DBS is also associated with a 100-fold lower carbon footprint, being 100 times more compact (in terms of sample size) and it is readily suited for automated recovery from such solid-state blood specimens [42]. Long-term storage for multiple decades requires storage at –196°C under liquid nitrogen or on treated paper such as Whatman® FTA, Ahlstrom-Munksjö GenSaver™ cards. For short-term storage (weeks to a month), untreated paper such as Whatman 903 paper, Ahlstrom-Munksjö GenCollect™ paper, etc. may be used. The paper products work by drawing the water out of the sample causing localized dehydration of the sample. Specifically treated papers such as Whatman FTA and Ahlstrom-Munksjö GenSaver cards further stabilize the sample by either lysis of the cells and/or by prevention of various oxidative damage to the sample. Ribosomal RNA (18S and 28S rRNA) is more labile when stored in DBS, as demonstrated with a less than ideal RNA integrity number (RIN) below 6.5 (RIN values will be explained later on). Storage of blood on treated paper is superior to untreated paper for decades-long storage of DNA but to date there is no product available for storage of total RNA in whole blood for decades other than storing at –196°C.

Advances in non-invasive diagnostics for cancer where routine blood sample collection can be used for tracking progression of the disease is much more affordable and less painful than a solid tissue biopsy alternative. DBS microsample is a good alternative to collecting liquid whole blood in EDTA tubes by phlebotomy for individuals where tracking of progression of disease is crucial for prescribing treatment options. Advances in the quality and availability of highly sensitive

Figure 4. Commercially available DBS collection devices for ambient storage and transportation. Biological samples collected remotely or stored at designated biobanks can utilize any one of the various products available for ambient storage of liquid samples. A number of formats of high-quality fiber-based media are available from Ahlstrom-Munksjö, Whatman-Qiagen, and others. The colorless format of cards is ideal for storage of colored biological samples such as fecal matter, plants, and whole blood. The colored cards are for storage of colorless biological samples such as serum, saliva, and other organics, at ambient. Biosample TFN card, AutoCollect card from Ahlstrom-Munksjö and Guthrie card, protein saver card from Whatman-Qiagen are ideal for collection of DBS needed for protein and small molecule analysis. The VAMS storage device from Neoteryx™ is convenient for patient-centric remote collection of microvolume samples. Some of the collection cards such as FTA, GenSaver, GenSaver Color cards and GenPlates are chemically treated for long-term preservation of DNA at ambient. These cards are ideal for biobanking and forensics application. AutoCollect card with perforated DBS circles are designed for automated sample preparation. GenPlates allow for high-throughput automated spotting of biological samples. The Tasso OnDemand collection device with integrated VAMS or paper cards is a painless alternative for volumetric collection of DBS. GenSaver, GenPlates, and GenReleaz cards allow for the convenience of direct downstream analysis (PCR, NGS, STR, etc.), from a 1-mm punch of DBS without any need for sample extraction. The 96-well format is ideal for storage of biobank samples and for screening and health monitoring applications. GenSaver 96 color, Indicating CloneSaver and GenPlates are designed for high-throughput biobanking needs.

Figure 5. Patient survey for acceptance of blood collection method. Convenience, lack of pain, and simplicity of sample collection from donor will ensure compliance. As determined from a survey of 146 subjects, by Tasso Inc., on a pain scale of 1–10, the surveyed subjects graded the OnDemand device at 1.25, venipuncture at 2.25, and the lancet method of blood collection at >3.0. Of the 146 subjects, 83.4% preferred the least painful OnDemand method of blood collection compared to 15.9% by the lancet method (data courtesy of Tasso Inc.).

instruments coupled with the development of software and methodological platforms for improved qualitative and quantitative analyses have made adoption of microsampling mainstream [43]. At home, blood collection of finger stick blood redundancy on microsampling devices such as GenSaver or FTA paper or on polymer compound, for example, Volumetric analytical MicroSampling (VAMS), is a convenient and a less painful alternative to phlebotomy. Advances in automation of almost pain-free microsampling of blood with the OnDemand by Tasso Inc. or Tap by Seventh Sense Biosystems are great alternatives for finger prick collection. Although opponents of precision medicine [34, 44] argue that matching the genotypic and phenotypic makeup of the individual to the treatment will not work, thus far, an individual-refined approach to selecting treatments has yielded demonstrable if still limited success.

3.1.4 Postmortem samples biobanking

Living donors contribute tissue samples only if it is a medical necessity. A possible viable source of large quantities of tissue samples is through postmortem collection of whole organs and tissues from consenting families. This avenue incurs a whole host of new challenges such as the donation consent process, recovery of organs and tissues in a limited time frame, postmortem, impact of donation on the donor families and steps necessary for creating a postmortem biobank such as IRB and registries. Carithers et al. [45] describe development of eligibility requirements aligned with scientific needs of the project and implementation of a successful infrastructure for biospecimen procurement to support the prospective collection, annotation, and distribution of blood, tissue, and cell lines and associated clinical data from postmortem samples. The development of donor eligibility criteria is crucial since limited donor history is available within the time frame needed for the collection of potential donor samples as degradation of biomolecules starts immediately with death. This proposition incurs a whole host of new challenges such as the donation consent process, recovery of organs and tissues in a limited time frame, postmortem, impact of donation on the donor families, and steps necessary for creating a postmortem biobank such as IRB and registries.

Sample collection is an intricate process that involves having many facets of the process coming together such as participant willingness, maintenance of anonymity of the sample source, and collecting samples in an ethically appropriate manner. Primarily, sample collection depends on the individual's willingness to participate in the clinical study and their trust in the collecting institution. Higher participation rates may be anticipated if the need for the study results is focused on the greater good of the community [46]. Often the most problematic aspect of sample collection when collecting large number of donor samples is maintaining donor anonymity. The Kaiser foundation admits that donor personal information is vulnerable and has placed controls to mitigate the issue such as educating the donor as well as assigning an alternate identification to donor samples that are for the Precision Medicine Initiative [47].

Both private and federally funded institutions have set up repositories to collect and archive biological samples to be then made available to researchers globally through for-profit, paid services or for free through not-for-profit organizations. One such globally recognized institute, the Kaiser Foundation, launched their initiative in October of 2015 and has thus far accumulated over 220,000 samples through volunteers with an end goal of collecting a total of 500,000 samples [47, 48]. The Kaiser Foundation has the added advantage of possession of the patient's lifestyle and EMRs that can be integrated along with the sample.

3.1.5 Nucleic acid extraction and storage

The quality and quantity of nucleic acid (DNA and RNA) depends on the quality of the nucleic acid in the starting material and the extraction method. There are many commercially available kits for extracting nucleic acids from varied sample types such as blood, PBMCs, serum, DBS, and fresh or frozen tissue samples. Decalcified FFPE samples are treated in the same manner as tissue samples. The common mechanism by which most nucleic acid extraction kits work is through lysis of the cells to release the nucleic acid followed by capture of the nucleic acid in chaotropic agents such as guanidinium salt on paramagnetic silica beads or on glass fiber filters. The silica beads or glass fiber filters are then washed to remove the proteins and cellular debris leaving relatively clean nucleic acid on the beads/filter. The nucleic acid is then released with a buffered elution solution most commonly Tris-EDTA at pH 8.3.

Extraction methods or kits must be chosen so they are well suited to the sample type [49–52]. Nonetheless, there is varying opinion in the literature regarding the choice of nucleic acid extraction kit to use for different sample types. Molteni et al. [50] determined that the efficiency of extracting DNA from DBS on plain paper and Whatman FTA paper was better with Masterpure kit than with Qiagen's QIAamp Blood mini kit and GenSolve DNA extraction kit (GenTegra LLC) being next best. In contrast, Daniels et al. of Broad Institute [52] determined that the efficiency of extracting DNA from DBS on Whatman FTA paper was superior with GenSolve DNA extraction kit than Qiagen's QIAamp Blood mini kit. McClure et al. [53] report comparable quality of DNA extracted from DBS on Whatman FTA paper with the GenSolve DNA extraction kit to that extracted from whole blood samples. These DNA samples were compared on the Illumina BovineSNP50 iSelect BeadChip, which requires unbound, relatively intact (fragment sizes ≥ 2 kb), and high-quality DNA. Superior-quality total RNA can be extracted with the time-tested phenol extraction using the commercially available Tri Reagent, although good-quality total RNA can also be obtained by using commercially available RNA extraction kits (Zymo Research, Qiagen, and ThermoFisher). Agitation of the DBS sample in lysis solution at 40°C ensures more efficient extraction of RNA.

Clearly, the choice of method of nucleic acid extraction is dependent on prior sample expertise and analysis methods to be used for the study. A distinction between the quantity of nucleic acid extracted vs. the quality of nucleic acid is crucial, since having a large quantity of compromised nucleic acid will still result in an unsatisfactory outcome (**Figure 6**). A good check for the quality of DNA and RNA is by calculating the DNA Integrity Number (DIN) [54] or the RNA integrity number (RIN) [15, 55] of the sample by electrophoresis in the Agilent TapeStation or similar devices. Alternatively, the quality of DNA can also be assessed by amplification of a 3-kb to 7-kb fragment of a low copy housekeeping gene such as glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [56] and for RNA fragment >0.9 kb of a low copy gene such as RNase P [51].

Nucleic acid (both DNA and RNA) extracted from samples can be stored either at very low temperatures of -20 , -80 , or -196°C or in a dry state by spray drying; lyophilization; air drying in the presence of commercially available protective chemistries such as RNastable, DNastable, (Biomatrica Inc., San Diego, CA), GenTegra-DNA [57] or GenTegra-RNA (GenTegra LLC, Pleasanton, CA); or by spotting on paper. The ribose-phosphate backbone in RNA molecules makes them susceptible to degradation. RNA consequently needs to be stored short term at -80°C or long term at ultra-low temperature of -196°C or in a precipitated form under ethanol. It is also possible to store RNA vitrified in the dry state at ambient temperature in the presence of protectants (GenTegra-RNA) that form the “glassy

Figure 6.

Quality and quantity of DNA from DBS. A volume of 125 μ L of whole blood spotted on GenSaver, GenCollect, and Paper F (FTA) paper cards, these were stored for up to 10 years at ambient temperature (A). DNA extraction yields obtained from three 6-mm DBS punches of GenSaver and FTA paper cards, the amount of DNA obtained from the sample reduces with aging of the DBS. The quality of the DNA is influenced by the chemical protective agents added to the card (B). A 7.5-kb fragment of a single copy gene (GAPDH) was amplified from 5 ng of DNA from DBS on GenSaver, GenCollect, and FTA cards. DNA in DBS on GenSaver cards is 18 $\times$ more stable than FTA and 4 $\times$ more stable than GenCollect cards when a 7.5-kb fragment of a single copy gene (GAPDH) was amplified by polymerase chain reaction (PCR). A volume of 20 μ L of the PCR product was subjected to electrophoresis on a 1.2% agarose gel (C). Gel electrophoresis of GAPDH 7.5-kb PCR product. Bands showing the PCR product from DBS in lanes 1–3 (GenCollect) and lanes 7–9 (FTA) are less intense than those of lanes 4–7 (GenSaver) for the 7.5-kb GAPDH product (D). Gel electrophoresis of GAPDH 3.8-kb PCR product. Product on lanes 1–3 comes from GenCollect DBS, product on lanes 4–6 comes from GenSaver DBS, products on lanes 7–9 comes from FTA DBS, lane 10 is the negative control and 11 is the positive amplification control, the intensities of the PCR product is not distinguishable among paper types. The differences in the intensities of the 7.5-kb and the 3.8-kb product indicate that the DNA is better protected from environmental effects in GenSaver cards. Samples were run on an E-gel.

state” to prevent oxidative, hydrolytic, or RNase damage to the ribonucleic acid. The protective chemistries also allow the dry nucleic acid to re-dissolve easily because the chemistries prevent the formation of the gel-state that pure nucleic acids often form at high concentration. The gel-state makes solubilization very difficult without using mechanical forces that will also break the nucleic acid strands.

Oxygen and water are essential components in the generation of reactive molecules with the degradation process accelerating with increased temperature, reduced ionic strength of storage solution, increased concentration of divalent cations (greater than 5 ppb) or nucleolytic enzymes. In aqueous solutions (a convenient format for storage), nucleic acids are sensitive to depurination, depyrimidination, deamination, and hydrolytic cleavage. To inhibit this acid-catalyzed degradation of DNA, sample storage solutions for DNA need to be slightly alkaline buffered solutions such as tri-buffered to pH of 8.3. Nucleic acid extracted from clinical samples likely contain up to 30–40 ppb of iron (from heme or haem). Presence of even trace amounts of divalent cations (greater than 5 ppb) increases the oxidative degradation of nucleic acid due to the formation of highly reactive free radicals via Fenton reaction [58]. Adding chelating agents such as EDTA and EGTA to a concentration of 500 mM to the nucleic acid storage solution would ensure that the intrinsic divalent cations present in the clinical samples are chelated.

Dry storage of nucleic acids in the presence of protective chemistries causes the molecules to lose the ability to diffuse as the sample undergoes a non-crystalline

4 Years at Ambient (26°C)									
	HeLa RNA			WBC RNA				Rat Liver RNA	
Buffer	Recovery	Bioanalyzer	Strand Breaks	Recovery	Bioanalyzer	Strand Breaks	Recovery	Bioanalyzer	Strand Breaks
	%	RIN	Average per kb	%	RIN	Average per kb	%	RIN	Average per kb
Water	115	8.2	0.08	116	5.3	0.37	110	4.9	0.50
	121	8.2		118	5.4		109	4.8	
	116	7.7		115	4.8		113	5.0	
Citrate	115	8.4	0.08	112	5.0	0.38	109	4.3	0.67
	116	7.7		111	4.1		110	3.8	
	112	8.5		111	4.8		111	4.3	
EDTA	115	8.2	0.08	115	4.7	0.38	115	4.8	0.46
	118	7.9		112	3.6		114	4.3	
	115	8.3		121	3.8		114	4.7	

Table 2.
HeLa RNA, WBC RNA, and rat liver RNA in water, citrate, or EDTA buffer were stored in GenTegra-RNA for 4 years at ambient temperature (25°C). All of the samples were hydrated with water at the end of 4 years. RIN scores were analyzed by Bioanalyzer and average strand breaks calculated per kilobase (as determined by the negative natural log of ratio of peak heights of 28S-18S) at time equals 4 months to time zero (R_n) for the sample groups. The source of RNA and degree of RNase carryover was the key factor in determining the maintenance of a stable RIN score and development of number of strand breaks per kb and is independent of the type of buffer used for storage of the RNA.

amorphous phase or a “glassy state.” In this dry “glassy state,” the movement of protons is expected to be approximately one atomic diameter in 200 years, thus preventing both oxidative and nucleolytic degradation of the nucleic acid. Storage at ultra-low temperatures of −196°C also vitrified as the water becomes solid ice and the molecules lose their ability to move. If moisture is added to the dry sample or the temperature is raised in ultra-cooled samples above the glass transition temperature of water, DNA/RNA damage can occur as the proton movement and reactivity resume [59]. Trace amounts of RNase would also become active upon rehydration causing RNA damage.

Successful storage of biomolecules including nucleic acid is ultimately dictated by the purity of the extracted material. Highly pure total RNA samples from HeLa cells with a RIN of 10 can be stored dry for up to 6 years and 2 months at room temperature in GenTegra-RNA (**Table 2**) without appreciable loss of RNA integrity or strand breakage, but rat liver RNA that carries along cellular impurities in the extracted total RNA shows degradation of up to 0.2 strand breaks per kilobase, deterioration in RIN from 9.0 to 4.0 and a short storage life of 1 year and 8 months. Human blood lymphocyte RNA, like rat liver RNA (at an intermediate level of residual purity), displays more damage after 4 years as assessed by RIN analysis, suggestive of 0.4–0.5 RNA breaks per kilobase after 4 years of ambient temperature dry-state storage in GenTegra-RNA. WBC RNA, like rat liver RNA samples stored with additional 1 mm of EDTA, incurred much less damage upon 7 months of storage at 56°C (only about 0.1break/kb) (data not shown). RNA strand breakage (X) is determined from the calculated RIN value of the aged RNA to the RIN value of the unaged RNA stored at −20°C [60].

4. Conclusion

Located in hospitals, universities, non-profit organizations, and pharmaceutical companies, biobanks are key infrastructures for research and development; however, these vaults for biospecimen are expensive to maintain and are precious samples that

are not willingly shared. Nonetheless, biobanking provides invaluable insight into biomedical mysteries. The long-term translational studies allowed by maintaining archives of samples could provide valuable insight for future generations to treat chronic diseases. Currently, the research community hopes to use biobanking to push forward precision medicine, an initiative set forth by the Obama administration to form unique, targeted treatments for each individual [3, 6]. There is a two-fold challenge for sample collection, ensuring privacy and getting volunteers. Transparency and traceability of samples are key to governance of all human biospecimens. Living donors contribute tissue samples only if it does not directly affect the quality of their life. The development of donor eligibility criteria is crucial since limited donor history is available within the time frame needed for the collection of potential donor samples as degradation of biomolecules starts immediately with death. Thus, it is incredibly important to minimize the amount of time samples spend out of storage, in biobanks.

Biobanking biospecimens is an expensive endeavor both in terms of manpower and natural resources used. For example, a single -80°C freezer consumes as much energy as a small studio apartment. Most biobanks install a bank of -80°C freezers to store the biospecimen samples as each sample needs to be stored for 10 years as per CLIA and CAP guidelines. Many institutions store samples for longer than a decade for research, test development, and validation purposes. FFPE blocks are cataloged at ambient temperature room temperature making them the most efficient way of storing biospecimen samples. Most other sample types are presently stored in -80°C freezers for short-term storage or -196°C under liquid nitrogen for longer term storage. Although not yet mainstream, advances in microsampling analytical technologies has popularized ambient temperature biobanking of whole blood, whole blood components, fecal, urine, plasma, and serum biospecimens on paper such as treated GenSaver or FTA papers or on VAMS tips or Matrix chip-one. Nucleic acid can be stored at ambient temperature in the presence of protective stabilizers in a dry state with a choice of commercially available time-tested products such as GenTegra-DNA, GenTegra-RNA, DNASTable, RNASTable, RNAlater, etc. Ambient temperature storage is the most economical, environmentally friendly, low-carbon footprint, and practical way of storage when long-term storage for decades is needed. In addition to reducing molecular mobility, drying the samples removes water that can participate in hydrolytic reactions. Furthermore, storing samples in a dry state at ambient temperature is independent of environmental factors such as electrical supply, temperature, and humidity.

The Precision Medicine Initiative aimed at precisely and rapidly analyzing many more cancer genomes will bring about a deeper understanding of cancers fueled by discoveries of molecular diagnostic methods. The first fruits of precision medicine are already apparent as a wide range of nucleic acid and antibody/protein-based drugs have been optimized for individuals with favorable genetic makeup. With a goal of collecting a million samples for the Precision Medicine Initiative, storing the samples such as blood at -80 or -196°C for prolonged period (decades) is going to become impractical at some time. Consideration needs to be given to space and energy requirements for such an undertaking. A more practical approach is to consider dry ambient temperature storage of biomolecules that have a commercially available solution for storage. Although dry storage of nucleic acid and DBS at ambient temperature is an economical alternative, adoption of this concept by the research community would be a paradigm shift from the time-tested method of preservation by cryogenics. This could be due simply to availability of freezers for storing other sample types that yet do not have an ambient temperature storage method. A new technology introduced to the marketplace has a 30-year adoption cycle and dry storage is a couple decades into that cycle with increasing number of research facilities converting to ambient temperature storage where applicable. Biobanking of human samples has many ramifications that

go beyond the science and technology of their storage. There are national, state, and even local regulations that must be met to ensure the protection of individual rights and individual privacy. Educating donors on the purpose of biospecimen collection and assurance of maintaining the privacy of the donor has favorable outcome. Perhaps it is reasonable to consider in a future review these legal and privacy issues.

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