Large scale, complex biobanking of biofluids for immunology research and testing

Rohit Gupta[1], Vanitha Sampath[2], Kari Nadeau[3], and Holden Maecker[3]

[1]University of California San Francisco
[2]Stanford University School of Medicine
[3]Stanford University

July 23, 2020

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Rohit K. Gupta[1], Vanitha Sampath[2], Kari C. Nadeau[2], Holden T. Maecker[3],

[1] Biospecimen Resource Program, Office of Research, University of California, San Francisco, CA, USA.

[2] Sean N. Parker Center for Allergy and Asthma Research at Stanford University and Division of Pulmonary and Critical Care Medicine, Department of Medicine, Stanford University, Stanford, CA, USA.

[3] Institute for Immunity, Transplantation, and Infection, Stanford University, Stanford, CA, USA.

Corresponding Author: Prof. Holden Maecker, Department of Microbiology and Immunology, Fairchild Science Building, room D039, 299 Campus Drive, Stanford, CA 94305-5124, USA, Email: maecker@stanford.edu, Phone: (650) 723-1671

Funding: Sean N. Parker Center for Allergy and Asthma Research at Stanford University and Sunshine Foundation.

Conflict of Interest: Dr. Nadeau reports grants from National Institute of Allergy and Infectious Diseases (NIAID), Food Allergy Research & Education (FARE), End Allergies Together (EAT), Allergenis, and Ukko Pharma; Grant awardee at NIAID, National Institute of Environmental Health Sciences (NIEHS), National Heart, Lung, and Blood Institute (NHLBI), and the Environmental Protection Agency (EPA); is involved in Clinical trials with Regeneron, Genentech, AImmune Therapeutics, DBV Technologies, AnaptysBio, Adare Pharmaceuticals, and Stallergenes-Greer; Research Sponsorship by Novartis, Sanofi, Astellas, Nestle; Data and Safety Monitoring Board member at Novartis and NHLBI; Cofounded Before Brands, Alladapt, ForTra, and Iggenix; Chief Intellectual Office at FARE, Director of the World Allergy Organization (WAO) Center of Excellence at Stanford, Personal fees from Regeneron, Astrazeneca, ImmuneWorks, and Cour Pharmaceuticals; Consultant and Advisory Board Member at European Academy of Allergy and Clinical Immunology (EAACI) Research and Outreach Committee, Ukko, Before Brands, Alladapt, IgGenix, Probio, Vedanta, Centecor, Seed, Novartis, NHBLI, EPA, National Scientific Committee of Immune Tolerance Network (ITN) and NIH Programs; US patents for basophil testing, multifood immunotherapy and prevention, monoclonal antibody from plasmoblasts, and device for diagnostics. HM, RG, and VS indicate no conflict of interest.

Author Contributions : All authors wrote and edited the final manuscript.

Word Count: 978

To the Editor:

Biobanks have evolved from simple localized storage of samples in individual labs and clinics to large industrialized repositories with sophisticated sample life cycle infrastructure. By enabling collaborations between researchers working on different aspects of a disease, biobanks can bridge the gap between clinical care and research, accelerating medical care towards precision medicine. The concomitant advances in trans-omic technologies, big data analytics, and biorepositories make possible a coordinated, robust systems biology approach. Biobanks can be envisioned as a central hub responsible for compliant custodianship of specimens and associated clinical and biological data. Operationally, biobanks should strive to provide universal consent, standardized processing, cold-chain management, and quality control checks. Here, we discuss biobanks with respect to optimal utilization of biofluid derivatives, such as cells, supernatants, and genomic material, for immunology research and testing.

A number of parameters need to be considered for specimen optimization and standardization based on sample type and downstream assays to be performed. Choices begin with the blood collection tubes to be used. For DNA and RNA analysis, EDTA anticoagulated blood is most common, as heparin can inhibit downstream polymerase reactions. However, at least one source suggests that citrate may provide higher quality RNA and DNA than other stabilizers.[1]

For immunoassays, either serum or plasma can be effectively used, but there are subtle differences for some cytokine analytes.[2] As such, a minimal requirement should be to use the same matrix (serum or plasma) and same anticoagulant if using plasma, for all samples to be compared in a study. For metabolome and lipidome studies, a report by Yin et al suggests EDTA plasma as the preferred matrix, since clotting in serum tubes activates additional processes, including the release of metabolites and enzymes from activated platelets.[3]

For cellular assays such as flow cytometry, CyTOF, or single-cell RNAseq, viably cryopreserved peripheral blood mononuclear cells (PBMCs) or other cells of interest are key. Protocols for cryopreservation are readily available, but careful attention to both freezing and thawing protocols is particularly important to maintain viability and recovery. Traditionally, heparinized blood is used for Ficoll isolation of PBMCs, but other anticoagulants are generally equivalent for functionality of cryopreserved PBMC. Concomitant use of the whole blood for other purposes (e.g., stimulation or DNA isolation) may dictate the optimal anticoagulant—for example, EDTA would inhibit T cell receptor-based stimulation, but would be compatible with molecular assays). There are also variations to traditional Ficoll protocols, using Cell Preparation Tubes (CPTs) or SepMate tubes.[4] These should be considered, as they can save time and labor and overcome some hurdles for standardization of the Ficoll procedure. The main drawbacks are a slight reduction in yield or increase in erythrocyte contamination. Importantly, training and protocol adherence are still important to prevent, for example, breakage of CPT from improper centrifuge holders, inadequate PBMC separation from improper spin speed, or loss of separation if CPT are shipped in very cold temperatures. Another variable to be considered is time to processing[5], which is of course highly related to whether samples are shipped prior to processing (see Figure 1). This is particularly relevant to functional cellular assays. An alternative to overnight shipping and PBMC cryopreservation for functional assays is to perform on-site stimulation and stabilization of whole blood (e.g., Smart Tube Inc., http://smarttubeinc.com); however, proper monitoring of cold-chain storage is critical to ensure frozen specimens are not compromised. For example, when using the Smart Tube system, biobanks must maintain the frozen samples at -80°C, as micro-fluctuations in temperature can cause the specimens to coagulate, rendering them unusable. In any case, there are a number of potential variables that can be detrimental to downstream analysis and even reproducibility; biobanks should strive to harmonize collection, processing, and storage of samples related to biofluids.

Research institutes often have multiple laboratories, each of which may be supporting various collections of human specimens. Unfortunately, most labs have employed their own data solutions to track and search for specimens, which has led to fragmented processes and inconsistent ontologies. Utilization of biospecimens that have been collected for scientific purposes continues to be problematic and may be more effective when paired with informatics tools that enable researchers to track, annotate, and interrogate.[6] Biobanks should have a sample management system (SMS) which permits labs to accurately register, label (Figure 2), and track biospecimen inventory related to study participants[7]; in addition, the software should be configurable

to align with lab workflows, while maintaining best practices for biobanking and ensuring governance can be maintained by the individual laboratory or institute. Further, for bioinventory tracking, it is critical to connect de-identified clinical attributes from electronic health records to biological assays following analysis of specimens in a central ecosystem; this enables researchers to rapidly search and request specimens for further analysis.[8] To date, although many solutions have been developed to support virtual sample catalogs, most require extensive software engineering support in order to be deployed and require data to be migrated to a central database; robust and innovative solutions for identifying unused biospecimens in the life sciences are still desired.

Long thought of as freezer farms, a biobank's primary role has always been to provide proper cold-chain storage and logistics related to biospecimens. While much literature exists on optimal storage conditions and management,[9] biobanks have evolved to now facilitate research in the life sciences that extend from the physical management of the sample life cycle to supporting standardized processing, assay optimization, and modernized data infrastructure. As compliant use of biospecimens continues to be a major component being addressed through community engagement, biobanks are poised to play an important role in medical research with increasing demand for high quality biospecimens. However, a number of questions and challenges exist regarding standardization, classification, management, sustainability, as well as ethical considerations including ownership and informed consent. Ultimately, improving how biospecimens are utilized for downstream analysis can accelerate our understanding of biological mechanisms and fuel a better tomorrow.

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Holden T. Maecker[3]

1 Biospecimen Resource Program, Office of Research, University of California, San Francisco, CA, USA.

2 Sean N. Parker Center for Allergy and Asthma Research at Stanford University and Division of Pulmonary and Critical Care Medicine, Department of Medicine, Stanford University, Stanford, CA, USA.

3 Institute for Immunity, Transplantation, and Infection, Stanford University, Stanford, CA, USA.

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FIGURES

Figure 1: Options for sample processing workflows. In general, sample quality is compromised as one moves from the top to bottom scenario. However, batching is desirable for efficiency and comparability of assay results, such that freezing specimens is typical for all but the most sensitive analytes. It also allows for biobanking for unspecified future purposes.

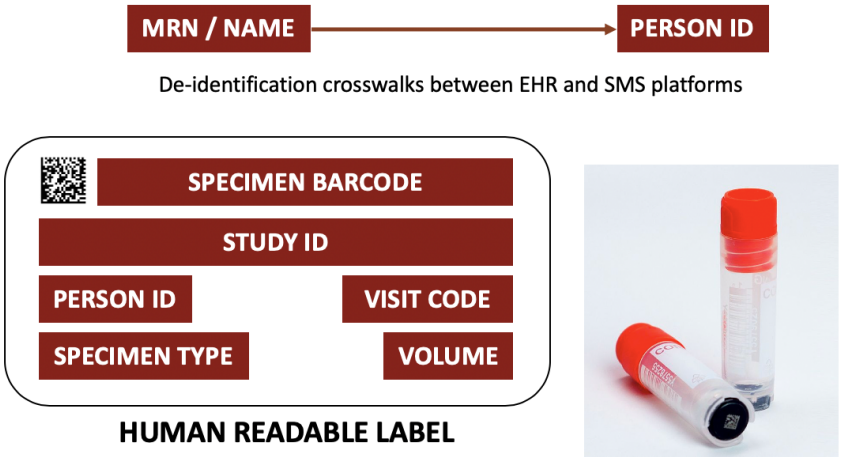

Figure 2: Sample labeling needs to be de-identified and should ideally include both a specimen barcode and human-readable information pertaining to the study, person, visit, and specimen.