

Using human medical waste to build biobanks

Jugnu Jain, Sreevatsa Natarajan, Soma Chatterjee

Sapien Biosciences Pvt. Ltd., Apollo Health City, Hyderabad, Telangana - 500 096, India

Correspondence to: Jugnu Jain, E-mail: Jugnu@sapienbio.com

ABSTRACT

India has a high disease burden and a large number of patients. There is a tremendous need for Indian biobanks to preserve Indian samples, to capture the great diversity of diseases to spur research into earlier, more precise diagnosis and better treatments for diseases plaguing India. This review article summarizes the key components of building a systematic comprehensive biobank, type and formats of sample and data, and the ethical and regulatory guidelines in India. An example of a growing Indian biobank, Sapien Biosciences, is shared along with its business model to reach sustainability. This is done by developing clinical diagnostics internally using the annotated samples but also sharing samples and outcomes data with external investigators. Our goal is to highlight the immense untapped value of Indian biospecimens and data, to catalyze the formation of collaborative networks of biobanks both within and outside India.

Key words: Personalized medicine, Precision Diagnostics, Biorepository, Tissue bank, EMR, Outcomes Research

What is Biobanking

India has a high disease burden and a large number of patients. Yet, there are very few collections of human disease specimens and data, appropriately termed biobanks, in India. Globally, there are 350-plus biobanks that spur research into diseases to identify risk factors for the development of a disease, for diagnosing diseases earlier and, more accurately, for screening family members at risk and for customizing a patient's treatment to improve outcomes.^[1-4]

Leftover tissues from surgery or diagnostic procedures, such as cancer tissue, blood, or urine, are precious resource, and highly sought after worldwide by diagnostics, biotech, and pharma companies to validate their drug candidates in target patient population samples, before launching clinical trials. Results from such studies can augment, sometimes even replace the need to test new drugs, biologicals, nutraceuticals, or cosmetics in animal models.

Need for Biobanking

There is a tremendous need for biobanking in India to preserve the Indian germplasm, to capture the great diversity of diseases, spur R&D into new treatments for diseases plaguing India, using Indian samples.^[2-5] When our preferences for tea, with or without spices, with or without sugar, with milk or almonds, boiled or not, specific flavor of tea leaves, and frequency of service can be customized, why not healthcare, an issue that has a much longer-term and profound impact on us?

A biobank needs to have a diverse sample collection as well as well-curated matched patient medical data, comprising demographics, disease diagnosis, and preferably treatment outcome.^[2] Patient-identifiable information is removed from the sample to protect patient privacy, and the sample is coded further by a unique identity by the biobank. A biobank-specific database is used to track acquisition and distribution of samples, and data including longitudinal visit data for the same patient.

The samples stored in research biobanks are typically not used for therapy purposes such as transplantation or cord blood banking but are used rather for research toward the development of more sensitive, accurate or point-of-care diagnostics, or for tailoring the use of existing medicines using the patient's genes/proteins, and monitoring response to specific drugs. Living cells derived from tissue samples can also be used to repurpose existing drugs for new diseases, determine the mechanism of action of novel drugs, or identify new targets to develop drugs.

There are guidelines issued by the Indian Council of Medical Research (ICMR) that specify the use of human biomaterial for research into diseases.^[6] The ethical framework allows for biobanking activities while also safeguarding the interest of the patients and protecting their confidentiality. Institutional Ethics Committees (ECs), as well as biobank ECs, provide oversight of biobanking activities; scientific projects utilizing samples are reviewed and approved by the hospital and/or biobank-associated ECs chaired typically by legally trained advocates to

ensure compliance with ICMR's ethical as well as regulatory norms.

Type and Utility of Samples in a Biobank

There are different types of samples that are used for different kinds of research. Most common sample type is that of tissue preserved in formalin then encased in wax molds (also called formalin fixed paraffin embedded [FFPE] blocks) that are used by pathology labs to diagnose disease and its stage. Another type of sample is fresh surgical material that is snap frozen in liquid nitrogen at -190°C to arrest metabolism, prevent degradation, and preserve the tissues. Such frozen samples are ideal for conducting genetic and proteomic studies. The third type of sample is where part of the sample is maintained live to study cells that comprise the disease (see below) and compare them to similar cells from healthy people. A biobank may have any or all of these formats of tissues depending on their focus of research, infrastructure, and technical expertise.

Most large Indian hospitals store a vast number of FFPE blocks every year. Since the blocks are stable at room temperature for decades, they are simply stored away for years; these represent a huge untapped resource to perform clinically meaningful research. The current standard for storage of blocks and associated slides is 5 years as per NABL guidelines;^[7] hence, most accredited hospitals have at least that many years' worth of samples. Some hospitals such as Apollo Chennai have 20-plus years of valuable FFPE samples that represent a virtual goldmine of knowledge if used properly. Good organization of blocks in well-lit, moisture-proof containers is important to maintain their quality for future research use. Otherwise, poorly ventilated, damp basement storage conditions attract fungus and silverfish that not only destroy samples but also pose health hazard to people accessing the storage areas.

One of the common uses of FFPE blocks is creation of tissue microarray (TMA). A large number of sample "cores" can be placed on one slide that can be used to evaluate a protein of interest across many patients or normal samples readily and economically. Biobanks often have core facilities to imprint high-density TMAs that can cover many diseases, or normal tissues, or be disease focused. An example of a disease-focused TMA would be 5–10 cores each of stage 1–4 of breast cancer, different types of breast cancers (estrogen receptor [ER], progesterone receptor [PR], and Her2 subtypes) along with 5–10 samples of normal breast tissue.

In addition to FFPE blocks, surgical samples may also be used fresh, and dissected in a sterile laboratory facility under conditions that enable live cells to grow from the tissues, e.g., cancer cells. These patient cancer cells better represent the complexity of the tumor and can be used to screen new drug candidates for their efficacy compared to existing drugs. Several tumor types - breast cancer, prostate cancer, and gliomas (brain cancers) - have been cultured successfully

by us and can be used as models to investigate mutations or other changes that may have caused cancer, or its spread, i.e., metastasis, or its resistance to therapy. The cultured cells can also be used to derive immortalized cell lines of the Indian genotype as a renewable resource of human cells for life science researchers. This has been done successfully by highly regarded organizations, including American Type Culture Collection (ATCC), Coriell Repository abroad, and the cells are in demand world over by academia and industry alike to understand disease biology and validate novel drugs.

There are many other applications of fresh surgical leftover samples. Animal models that more closely mimic human disease, e.g., cancer, are increasingly being built with fresh tissue fragments implanted into small fish or immunocompromised mice, in which the human tumor cells recreate the tumor. Different stages and grades of cancers may be used to create a panel of models that between them capture the diversity and complexity of actual human cancer. Use of these translational models, called patient-derived xenografts (PDx) for PDx, better predicts the efficacy and tolerability of new drugs as would be reflected in actual human clinical trials, hence such humanized models are highly prized. Some Chinese companies have built thousands of cancer PDx models that are used by international pharmaceutical companies to screen drug molecules; same can be done in India readily by a biobank in partnership with a biotech/pharma group.

In addition to disease samples, there is a need for normal or non-diseased tissues to serve as controls in experiments. Controls are needed to distinguish biology of a disease from normal biology. A biobank can be of enormous help here since it also has access to samples from a small number of people that turn out to be unaffected with the disease they were screened for. Those samples, as well as samples to be discarded from cosmetic surgeries, may be used for controls with the patient's consent.

There is great scope for biobanking to create a focused collection of leftover blood, urine, or feces from healthy individuals undergoing preventive health checks along with the complete blood profile, family history, risk factors such as diabetes and smoking, current medications, etc. Repeat sampling could be done every 3–5 years for 10 years along with accumulation of longitudinal data. Such an effort can generate an unbiased database of Indian samples and information that can be analyzed to identify non-invasive markers for screening populations, for identifying better treatment paradigms for Indians, e.g., assessing effectiveness of statins in Indians known to have higher triglycerides and body mass index, predict risk of recurrence of cancer as exemplified by the partnership between Sapien and OncoStem where validation of a breast cancer recurrence predicting test was made possible by Sapien's breast cancer samples and 5 years outcomes data.^[8] Population-based biobanks in the UK and the USA have helped shed light on environmental risk factors for developing diseases and innovative diagnostics to detect

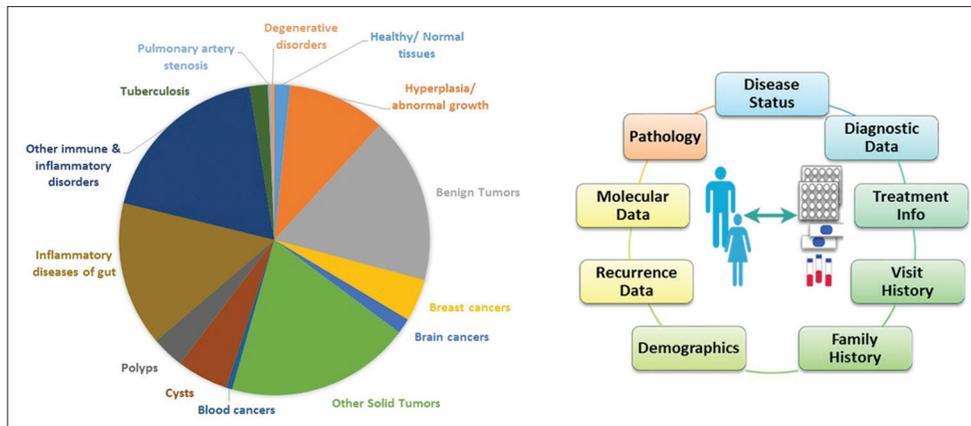


Figure 1: Summary of disease samples and associated data biobanked at Sapien

diseases earlier; same could be the case in India catalyzed by Indian biobanks. Another application of an unbiased database of samples and associated data could be the creation of an Indian reference genome, pooled from many healthy individuals, to serve as the baseline for genetic changes that are functionally significant in Indians.

Challenges and Way Forward

There are many challenges in establishing and sustaining a systematic biobank. It is time and money consuming. The initial infrastructure and effort needed are high to retrieve samples at hospitals, collate and manually curate scattered/incomplete medical records across multiple hospital departments. Specialized biobank databases and servers are required to track acquisition and distribution of tissues, code samples to protect patient privacy, store data, etc.

A biobank requires consistent support and alignment between many hospital stakeholders including hospital administrators, pathologists, nurses and physicians, IT, medical records department, EC members to ensure smooth operation, and adherence to protocols.

Public-private partnerships are the best model for creating a nation-wide biobank resource in India as has been the case globally since neither hospitals nor the government can do it alone.^[2] Most government or charitable hospitals have a high burden of patients, and therefore, unable to spare resources for storing samples and medical records. Private hospitals have better sample storage and patient records but are not oriented toward research into diseases and cures. Catalyzing a deeper partnership between public and private hospitals to preserve samples and data in an organized biobank to digitize records and match them to samples would create a goldmine of health-care information that can be turned into actionable knowledge benefiting Indian patients and researchers. The vision of Digital India if expanded to include digitization of at least 10% Indian population's health records can help us understand what can keep us healthy.

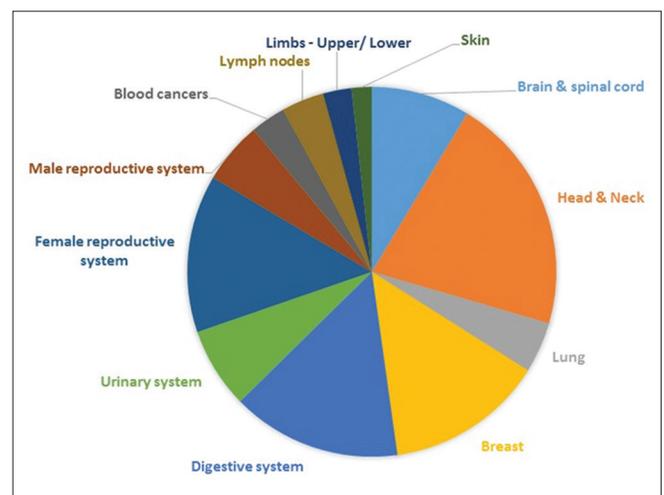


Figure 2: Samples of many different cancer types are available at biobank for evaluating biomarkers and drug molecules

An Indian Biobank: Sapien

Sapien Biosciences were born out of a partnership between Apollo Hospitals and Saarum Innovations with a shared vision of creating a high-quality biobank comprising ethically approved collection of samples and associated curated data. Over the last 3 years, Sapien has obtained approval from six of the largest Apollo Hospitals ECs to retrieve retrospective FFPE blocks and associated diagnostic data for applied research purposes. The samples have been de-identified and coded to protect patient privacy. A specialized open source database, developed specifically by National Cancer Institute to track acquisition and use of samples along with patient and diagnostic data over multiple visits, has been implemented [Figure 1].

Approximately 50,000 patients' blocks, leftover after the completion of pathological diagnosis, have been retrieved. There are an estimated 600,000 patients' blocks that could be retrieved from just the top six Apollo Hospitals, a virtual treasure trove waiting to be tapped. The sample collection is

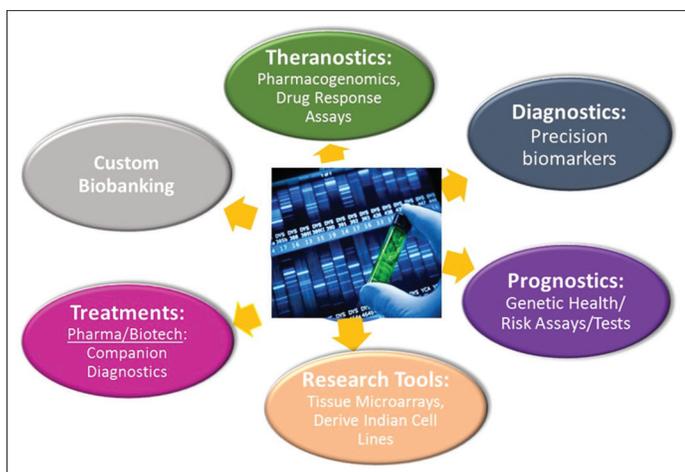


Figure 3: Services and products enabled by the biobank

drawn from hospitals across India without any geographic or ethnic bias. Sapien is well on its way to becoming India's largest, most comprehensive, and diverse biobank [Figure 2].

Fresh tumor tissues have been successfully cultured at Sapien to obtain cancer cells from breast, prostate, blood, brain, and oral cancers. Where available, a matched set of fresh tumor sample and blood from the same patient has been banked to allow study into hereditary causes of cancer.

Panels of related cells, e.g. combination of different subtypes of breast cancer (ER±, PR±, and Her2±) or gliomas representing a range of grade and stage of disease, have been derived knowing their value in target validation and testing efficacy of drugs. These primary cells can be immortalized and maintained as continuously growing cell lines, stocked and distributed to researchers as done by ATCC, to study changes in genes or proteins that are responsible for the initiation and progression of cancers in India. A few spontaneous cell lines have been derived from highly proliferative breast and brain tumors.

Patient samples, data, and biobank's expertise in cell and molecular biology are used to develop better clinical diagnostics

to benefit patients. A test combining the determination of CYP2C19 genotype and functional response to antiplatelet therapy for percutaneous coronary intervention-stent patients has been developed and launched in 2013.^[9] Validation of non-invasive screening tests for cervical (urine-based) and oral (mouth rinse-based) cancers is under discussion to bring the power of biotechnology into preventive health screening paradigms, to catch cancer early enough for cure [Figure 3].

Core facilities including cell line repository and validation, digital pathology, TMA for cross-lab quality validation, etc., could be established in partnership with DBT-CSIR-ICMR labs to maximize the use of biobank's richly curated sample set and make it widely available to the research community globally.

References

1. Park A. 8. Biobanks: 10 Ideas Changing the World Right Now. Time; 2009.
2. Baker M. Biorepositories: Building better biobanks. Nature 2012;486:141-6.
3. Vaught J, Rogers J, Carolin T, Compton C. Biobankonomics: Developing a sustainable business model approach for the formation of a human tissue biobank. J Natl Cancer Inst Monogr 2011;42:24-31.
4. Priyadarshini S. India Needs Gen-Next Cancer Biobank. Nature India; 2013. p. 103.
5. Saini KS, Saini ML, Marbaix E. Biobanking in the era of precision oncology. Indian J Med Paediatr Oncol 2015;36:1-2.
6. Available from: http://www.icmr.nic.in/ethical_guidelines.pdf. 2006.
7. Available at [http://www.nabl-india.org/nabl/file_download.php?filename=201210170522-NABL-112-doc%20\(1\).pdf](http://www.nabl-india.org/nabl/file_download.php?filename=201210170522-NABL-112-doc%20(1).pdf).
8. Sapien Partnership with Oncostem: Press Release; 2015. Available at http://www.business-standard.com/article/companies/oncostem-ties-up-with-sapien-for-breast-cancer-recurrence-diagnostic-test-115022300551_1.html.
9. Rath PC, Chidambaram S, Rath P, Dikshit B, Naik S, Sahoo PK *et al.* A study on the impact of CYP2C19 genotype and platelet reactivity assay on patients undergoing PCI. Indian Heart J 2015;67:114-21.

How to cite this article: Jain J, Natarajan S, Chatterjee S. Using human medical waste to build biobanks. Int J Mol Immuno Oncol 2016;1:24-27.

Source of Support: Nil. **Conflict of Interest:** None declared.