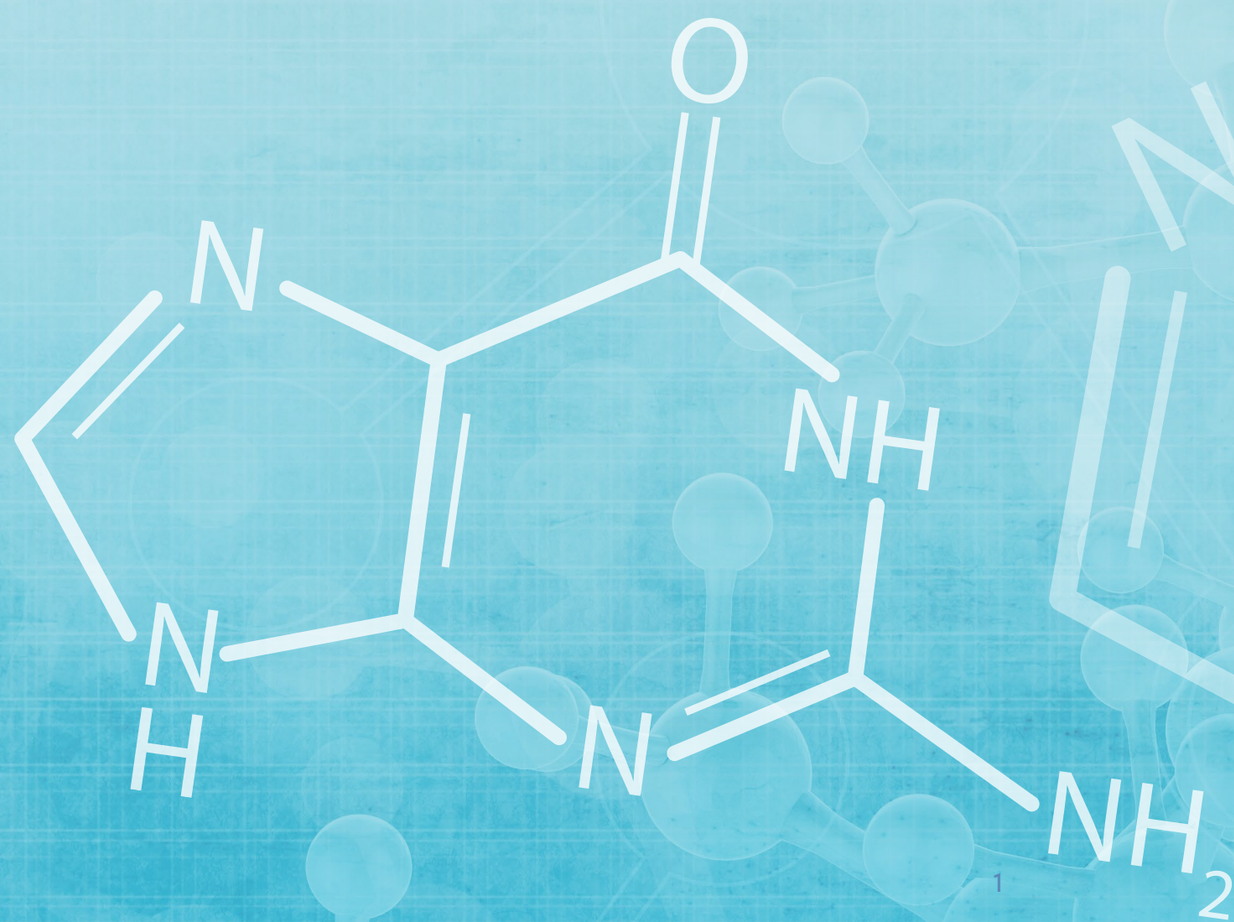
Controlling Preanalytical Variability in Biospecimen Collections

By Abdul Ally, *Area Director of Laboratory and Operations Science at Fisher BioServices*



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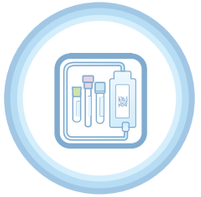

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About the Author

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By Abdul Ally

Abdul Ally is the Area Director of Laboratory and Operations Science at Fisher BioServices. He has more than 28 years of experience in molecular biology, laboratory management, and managing contract research operations for clinical research support. Mr. Ally advises clients on specimen collection and processing, and develops custom processes to meet client research specifications. He has published more than 18 articles in peer-reviewed publications, holds four US patents, and has been involved in such highly diverse activities as genomics research and development, managing contract research laboratories under FDA compliance, and developing and fitting out genomics and nucleic acid research core facilities for King Abdullah University for Science and Technology (KAUST) in Thuwal, KSA.

Overview

Introduction

Large, well-designed population studies and the interrelationships they reveal are the backbone of public health and serve as a foundation for medical research. The health and lifestyle information of participants, collected via questionnaire and linked to their biospecimen samples, allow investigators to examine the intricate relationships between genetics, physiology, behavior, environment, and disease.

However, in order to have confidence in both laboratory assay results and their associations with other data, the biospecimens must be correctly collected, processed, shipped, and stored. If these samples were subjected to conditions that compromised their molecular integrity—inappropriate temperatures, contaminated containers, and others—then the results of the study may also be questionable. This is an issue that must be thoroughly addressed in public health research—the possibility that the handling of specimens before laboratory testing (i.e., preanalytical variability) has skewed results.

For this reason, controlling preanalytical variability requires the same attention to detail as the design of questionnaires. This eBook is an introduction to some of the variables that must be considered when collecting biospecimens as part of a cohort study.

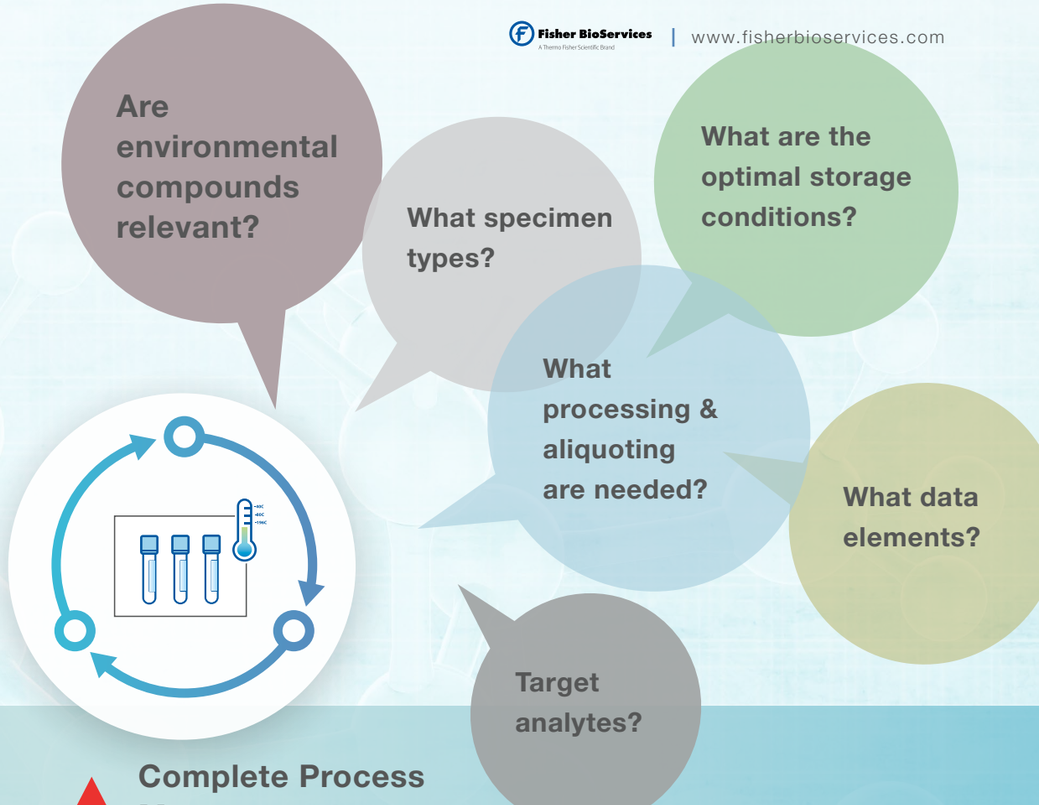
1 Complete Process Management

Complete Process Management

Control of the total process must begin at sample collection—the moment a specimen leaves the donor—and must continue until a specimen is analyzed. Any variation in how samples are collected, transported, processed, or handled can result in a decrease in specimen integrity.

Complete process management includes use of the same protocols across all study sites for collection, shipping, and storage. The protocols should ensure that all steps in the sample management process conform to best practices [1,2,3], and site staff should be well trained on uniform use of the protocols. In addition, laboratory processing of large numbers of samples should be automated where possible, to further minimize aliquoting variability across nested sets of aliquots from the same sample.

Preanalytical variability during sample collection and other events may account for up to 68 percent of erroneous results in the lab [4]. When planning collection of specimens for distant future use, there are many questions to consider, and some are less obvious than others. These include:

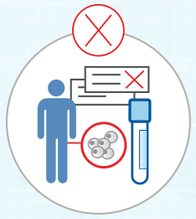


▲ Complete Process Management

- Are environmental compounds relevant, and how significant a role does the exposome and microbiome play?
- What specimen type is most appropriate for the study goal?
- What are the target analytes (cotinine in urine? microbial mix in stool samples?), and what container/additives should be used to preserve or stabilize these analytes?
- What processing (e.g., DNA/RNA extraction) and aliquoting are needed?
- What data elements should be appended to the sample?
- What are the optimal storage conditions, including temperature, for maintaining stability of molecules of interest (e.g. cell surface receptors, metabolites, molecular markers)?

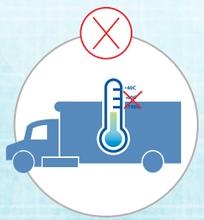
Complete Process Management

Below are some examples of common mistakes that can result in preanalytical variability in the samples or the data:



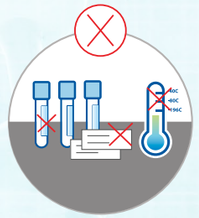
Collection

- Blood collected in vacutainer with incorrect anticoagulant for downstream processing
- Urine collected in containers which have not been pre-screened for interfering substances
- Mis-labeled sample
- Data error
- Leaking and loss of sample contents
- Contamination of samples with microorganisms or chemicals



Transport

- Incorrect sample shipping temperature
- Extended transit time



Processing Lab

- Vial labeling errors
- Insufficient volume collected for desired number of aliquots
- Inadequate control of freeze rate
- Incorrect processing temperature
- Poor aseptic technique
- Mismatch of parent/child aliquots
- Quality documentation incorrect or lacking
- Lack of validated/certified instruments and equipment

Storage

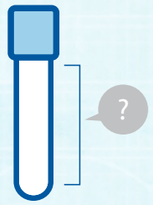
- Location errors
- Incorrect storage temperature
- Inadequately maintained or un-validated freezers, leading to temperature excursions
- Poor temperature control when pulling and shipping samples

Of all of the factors involved in sample management, inadequate temperature control is a major contributor to preanalytical variability. Samples are far more often subject to incorrect temperature during shipping and handling than during long term storage. By establishing and following a standard process from initial collection through downstream laboratory analysis—tested in a pilot study if applicable—you can ensure full compliance with correct temperature and other requirements.

2 Additional Sample Collection Considerations

Additional Sample Collection Considerations

Biospecimens collected in the context of a cohort study, especially if multiple samples are collected from participants over time, are particularly valuable for assessing environmental exposures, determining toxicity of exposures, assessing risk and epigenetic influences, and identifying biomarkers. To maximize the value of specimens for a wide range of health and medical investigations, some additional questions to consider include:

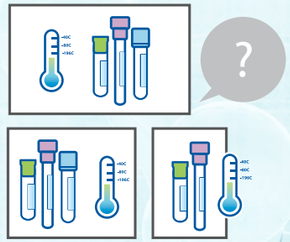


Sufficient Volume?

The amount of the biological sample available can limit the possible analyses; when the target analytes are unknown, collections should be planned to ensure an optimal number of aliquots for a variety of downstream assays.

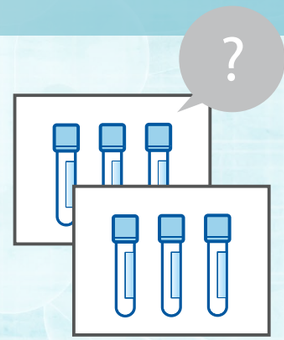
Non-Standard Samples?

Including additional “non-standard” samples such as hair, teeth, and adipose tissue will allow downstream analyses of cumulative environmental exposures and chronic conditions.



Unknown Handling or Storage Parameters

Emerging areas of research (e.g., the microbiome, the exposome, and others) call for the collection and storage of samples for which best practices have not yet been established. Biobanking of these materials may require consulting with an expert in biospecimen management.



Sufficient Sample Sets?

Biomarker discovery and examination of subtle interactions between multiple risk factors can require very large sample sets to control for confounding factors and generate statistically significant results.

3 Controlling Preanalytical Variability at Point of Collection

Controlling Preanalytical Variability at Point of Collection

Standardization and Quality Control

Standardization of sample collection improves efficiency and optimizes use of resources. For instance, poor phlebotomy practices leading to hemolysis is a major cause of waste and results in re-draws. Adopting a standard process to minimize hemolysis will improve efficiency as well as preserve sample integrity.

Standardization is partly achieved by providing collection kits, which ensures that the correct tubes and additives/preservatives are used, materials are well within their usable life, labeling of kit components is accurate and consistent, shipping issues are minimized by provision of an air bill, and compliance in all handling is supported by enclosed instructions. (For more information, see our eBook, ***Standardizing Biosample Management: Why Use Collection Kits***). However, a standard protocol is as critical as collection kits, and study sites should train staff in the specific process to ensure adherence to the protocol. These two steps—devising a uniform collection process and standardized collection kits—are especially important when collecting samples for which best practices have not been as well defined (i.e., the microbiome and others) as other time honored biosamples (blood, urine, etc).

Controlling Preanalytical Variability at Point of Collection

Documentation

In addition to standardization, capturing data related to the sample collection should be a central element of the protocol. The data accompanying the sample is critical to its value for future research, particularly with regard to samples for which standard procedures are not defined. For instance, the BRISQ recommendations [5] list the following as the minimum data that should be collected along with the specimen:

- Collection mechanism and parameters (i.e., fine-needle aspiration, pre-operative blood draw),
- Collection container,
- Mechanism of stabilization,
- Time between collection and stabilization of the specimen, and
- Temperature of the specimen during this time period.

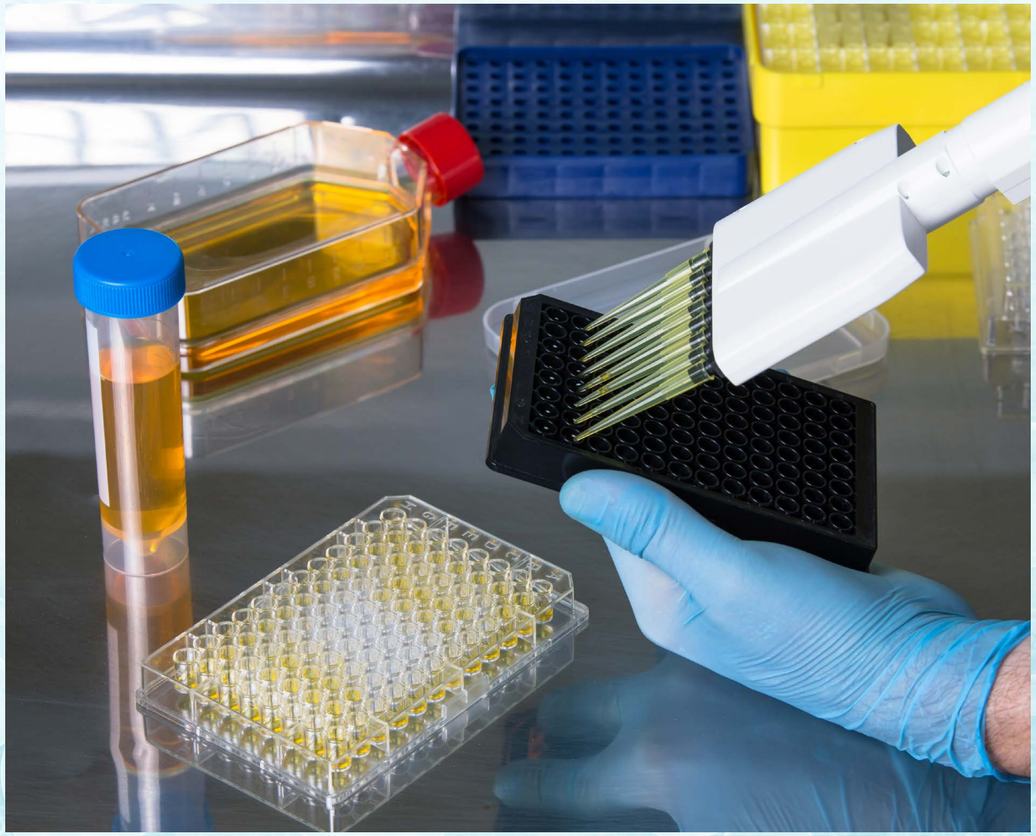
The BRISQ prioritizes sample-related data into three tiers, with the most important data designated as Tier 1, less critical as Tier 2, and so forth. In addition to the above list, Tier 1 data includes organ of origin or site of blood draw, relevant clinical characteristics of the donor (stage of cancer, pre- vs. post-menopausal, etc), type of long-term preservation, shipping temperature, and others. Any variable that could influence the quality of a particular specimen should be captured as Tier 1. Note that these data are also likely to be required for publication.

4 Specimen Processing

Specimen Processing

Processing of specimens for future use, whether extracting serum or DNA from whole blood or simply aliquoting to minimize freeze-thaw cycles, should not introduce variability into the sample. Standardization and documentation is just as critical in processing samples for storage, and includes:

- Processing of samples soon after collection, to preserve integrity (generally 24 hours, but varies with the specimen);
- Processing by trained, experienced technicians following proven, well-established, standard operating procedures (SOPs);
- Documentation of reagents and materials used in sample processing, capturing lot specifics, ensuring storage containers are selected based on target volumes, storage temperature and intended analysis;
- Including reference and QC samples when applicable;
- Ensuring laboratory equipment is validated, calibrated, and maintained as appropriate;
- Ensuring standardized forms are in place for documenting procedures and results;
- Using controlled, step-down freezing of cells to preserve viability and/or other processes as applicable, to prepare samples for long-term storage; and
- Storing processed samples in a SBS format or other sample size or container that is downstream assay-ready.



5 The Storage Container

The Storage Container

Ideally, samples are aliquoted and otherwise processed before being placed in storage, to minimize freeze-thaw cycles and preserve their integrity. Selection of both an acceptable primary collection container and a post-processing storage container is critical. There are many choices of vial, with associated pros and cons. For instance,

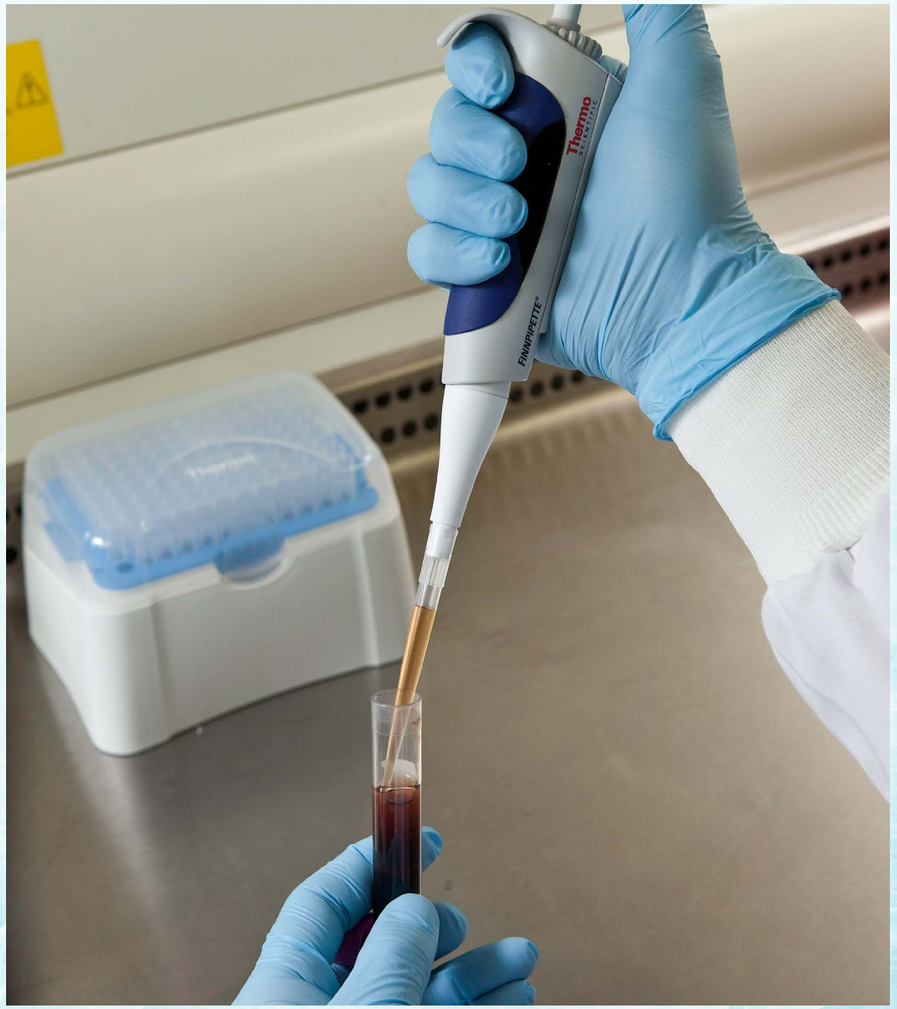
Sterile or free of specific contaminants:

Containers, equipment, and laboratory surfaces are sources of contaminants that can skew assay results. Consider the need for sterile containers for urine samples, cleaning to eliminate RNAses, etc.

Shape: A V-bottom tube allows the very last drop of a sample to be recovered; U-bottom tubes can allow greater visibility of precipitate.

Volume: Plan for minimal head space. The small space between the sample and the cap is a source of contamination with CO_2, water, and other air-borne materials.

Closure: Internal threads can be a source of contamination; external threads protect against contamination but create more headspace.



Automation/high throughput ready:

Processing samples directly into -96 or -384 matrix plates for high throughput will reduce freeze-thaw cycles and save time.

6 Storage and Risk Mitigation

Storage and Risk Mitigation

Biobanked samples spend the great majority of their time in a freezer, and freezer failure is a valid concern. Preserving molecular integrity means implementing a risk mitigation infrastructure to preserve samples in the event of an emergency. This should include:

- Validation of equipment, to ensure it performs according to manufacturer specifications;
- A preventive maintenance, repair, and replacement program;
- Validated and 21 CFR part 11 compliant temperature monitoring systems and trained staff members to respond to after-hours alarms;
- Storage systems that allow rapid transfer of samples in bulk;
- Back-up generators;
- Facility security systems;
- Fire suppression systems; and
- Other infrastructure.

These systems are addressed in detail in our eBook, *Planning and Design Considerations for Biological Specimen and Biopharmaceutical Risk Mitigation*.

7 Shipping

Shipping

Specimens used in research may travel between multiple points within a clinical site, between the clinical site and biorepository storage, from the biorepository to research laboratories, and back to the biorepository. All of these steps include handling the specimen, if only for a moment, in ambient temperature conditions. Reducing these ambient events to the smallest interval possible (or eliminating them) is critical to preserving sample integrity. This is where expertise in biorepository science is critical.

Shipping of biological materials must conform to a spectrum of regulatory standards for the safety of those that handle the packages. Shipping must also conform to standards that protect the integrity of the specimen. Research specimens should not only be transported in a validated shipper, but also packaged according to a standard configuration that has been tested and shown to reliably maintain the correct temperature under typical transit conditions. For additional information, see our eBook, ***Cold Chain Qualification: 5 Questions You Must Ask When Shipping Biologics.***

Conclusion and References

The scope of public health research is rapidly expanding in new directions. This is particularly the case for research into the microbiome and the exposome, which includes every environmental compound to which an individual is exposed between conception and death. In planning sample collection to support today's cohort studies as well as for assays that do not yet exist, it's imperative that quality is at the forefront.

Much of the life of a sample is hidden from the investigators who use it for research, yet what happens to a sample over its lifespan can profoundly influence assay results. To ensure that biobanked specimens will be suitable for use, regardless of new avenues of research, preanalytical variability must be carefully considered and controlled, to prevent irreproducible results and waste of resources.

References

1. National Cancer Institute. (2016). NCI Best Practices for Biospecimen Resources. Biorepositories and Biospecimen Research Branch, NCI. [http://biospecimens.cancer.gov/bestpractices/2016-NCIBestPractices.pdf]

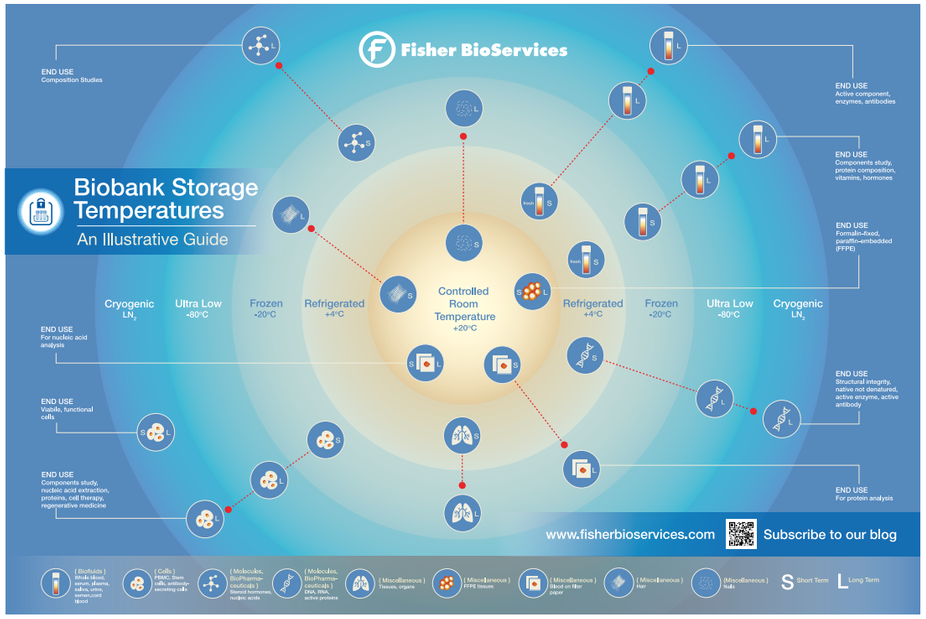
2. International Society for Biological and Environmental Repositories. (2011). Best Practices for Repositories: Collection, Storage, Retrieval and Distribution of Biological Material for Research, Third Edition. ISBER, [http://c.ymcdn.com/sites/www.isber.org/resource/resmgr/Files/ISBER_ Best_Practices _3rd_Edi.pdf]

3. National Cancer Institute. (2016). NCI Best Practices for Biospecimen Resources. [http:// biospecimens.cancer.gov/bestpractices/2016-NCIBestPractices.pdf]

4. Kalra J. (2004). Medical errors: impact on clinical laboratories and other critical areas. Clinica Biochemica, 37, 1052–1062.

5. Moore, H.; Kelly. A.; Jewell, S.D.; McShane, L.M. & Clark, D.P. et al. (2011). Biospecimen reporting for improved study quality. Biopreservation and Biobanking, 9, 57–70. DOI: 10.1089/bio.2010.0036

Additional Resources



This InfoPoster illustrates the complexity of various biospecimen storage temperatures.

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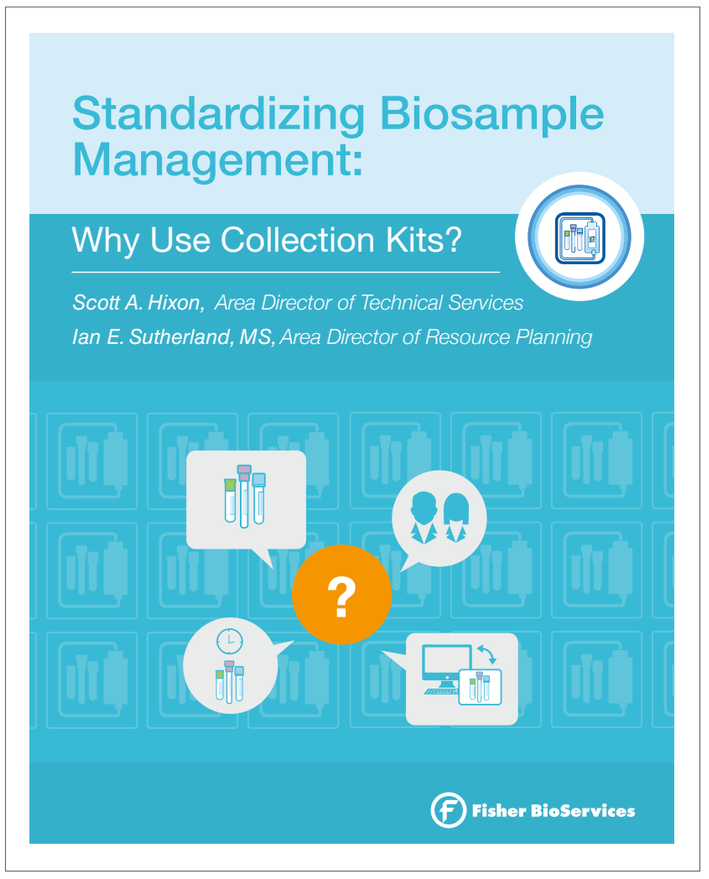

This eBook provides some basic tips on optimizing sample collection and preserving sample integrity.

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