Laboratory Protocols For Sample Processing and Storage to Ensure Sample Integrity

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Abstract
For obtaining accurate and dependable findings in the nutritional status randomized controlled trials (RCTs), it is crucial to meticulously design and execute the collection and management of clinical data. This article is a comprehensive guide to the most effective methods for collecting and analyzing biospecimens, as well as the essential principles of managing clinical data. It covers important topics such as preparing for and initiating a research, collecting and entering data, ensuring data accuracy, and finalizing the database. The reader is advised to consult other resources for information that might aid in the preparation and execution of human randomized controlled trials (RCTs). The techniques and tactics outlined are anticipated to enhance the caliber of data generated in human nutrition research, which may subsequently be used to bolster food and nutrition policy.

Keywords: Dietary treatments, human nutrition, clinical trials, best practices, biospecimens, laboratory procedures, data management.

1. Introduction

The cornerstone of effective clinical nutrition research is a thorough and meticulous strategy that outlines the study's structure (1), documentation, and regulatory protocols (2), as well as the collection and management of data (covered in this article). Ensuring data integrity is crucial for producing accurate and replicable outcomes, which are crucial for using research findings to create evidence-based dietary recommendations. When data is compromised, it severely undermines the ability to make meaningful scientific interpretations. By implementing meticulous planning, comprehensive training, and effective supervision, it is possible to significantly reduce the likelihood of mistakes in data collecting, cleaning, and analysis. This article examines the most effective methods for maintaining the quality of biospecimens and offers a comprehensive analysis of the proper administration of clinical data in human nutrition randomized controlled trials (RCTs). The topics covered include preparation and study initiation, data collection, entry, cleaning, authentication, and database lock.

2. Preservation of Biospecimen Quality

The reliability of a human nutrition randomized controlled trial (RCT) largely relies on the implementation of high-quality quality control (QC) methods for biospecimens. Prior to commencing a human nutrition randomized controlled trial (RCT), it is essential to thoroughly contemplate each stage of the procedure and establish a comprehensive strategy for the collection, processing, transportation, storage, and analysis of biospecimens. The success of this strategy relies on accurately predicting and preparing for all necessary actions and potential problems, while also considering the practical requirements and constraints of the study's design, participants, infrastructure, and expenses. Standardizing processes for "chain-of-custody" is crucial in order to reduce result variability in both single-center and multicenter investigations.

Due to the high cost of conducting research with human participants, it is crucial to foresee possible future applications and include strategies and extra resources to ensure the long-term preservation of biospecimens while preserving their integrity. It is crucial to highlight that the planning of all stages related to biospecimens takes substantial time and preparation. Therefore, it is advisable to finish these preparations well in advance of recruiting the first participant. Any disruption at any stage of the process might diminish the accuracy or result in the loss of crucial data. The National Institutes of Health (NIH) has issued guidelines for the storage, tracking, sharing, and disposal of human biospecimens (3). All individuals participating in the collection or analysis of biospecimens must undergo the necessary institutional safety training on universal precautions for bloodborne infections.

3. Assays

When developing a procedure for the collection, storage, transportation, and analysis of biospecimens, it is important to consider the desired laboratory output, namely the findings of the assays. The specific needs of the assay will determine the appropriate processes for collecting, processing, transporting, and storing the samples. It is crucial to evaluate the needs for each experiment individually. The following information mostly pertains to whole blood, serum, and plasma. However, several additional biospecimens are often obtained for particular assay needs. These include blood spots, buffy coats, spot and 24-hour urine samples, stool samples, cerebrospinal fluid, saliva, biopsies, and buccal cells, among others. Online sources from national clinical labs (4-9) and university medical facilities (10) provide reliable assay requirements for several analytes. When tests are not performed in clinical labs, doing literature searches may help locate references that provide information on suitable assays to utilize, as well as particular needs for sample collection and storage. Commercial assay kits often include instructions on the proper methods for sample collection and storage.

4. Quantitative measurement or magnitude

While each assay is designed to work with a specified minimal volume or quantity of a biospecimen, it is necessary to deliver a larger volume to the laboratory conducting the test. This is attributed to the losses incurred during the transfer of samples before and during an assay, as well as the dead volumes present inside automated systems. Additionally, there is the potential need to repeat an assay if there is an issue with the measurement, or to do duplicate or triple runs. Occasionally, assays may need to be downsized to fit limited sample availability, even when they are originally designed for certain volumes or quantities. Prior to running real research samples, it is necessary to verify the scaled down assay to ensure that any changes in methods provide valid findings, meaning they are both exact and accurate. To avoid the negative effects of repeated freeze/thaw cycles, it may be essential to use numerous aliquots when doing various experiments. Having extra aliquots on hand is advantageous in case the assay has to be repeated, which often occurs when the data is examined and there is uncertainty regarding the accuracy of an outlier.

5. Fresh, refrigerated, and frozen

Certain analytes exhibit sensitivity to the process of freezing and thawing, necessitating the use of fresh samples that have not been previously frozen. An excellent illustration is the comprehensive blood count, which includes measurements of the quantities of intact red blood cells and white blood cells, which will rupture upon undergoing the process of freezing and thawing. If an analyte has to be tested without cryopreservation, but not immediately (i.e., within hours to days), cooling or temporary storage on ice may be required. However, it is important to note that there are time constraints on how long the sample remains viable. The majority of analytes may be preserved by cryopreservation, however the specific temperature can be a significant factor to consider.

The usual norm for storage is at temperatures ranging from -70°C to -80°C. Certain analytes can be preserved at a temperature of -20°C, however their viability may be compromised if held for a lengthy period of time. Another crucial factor to consider is that some samples, although being held at a temperature of -80°C, may degrade with time, while others may remain stable. Preservatives may be added to certain analytes to increase their viability. For example, metaphosphoric acid is used to stabilize serum that will be tested for vitamin C, while a solution comprising sulfamic acid and a surfactant is used as a preservative for urine samples to prevent the loss of mercury (8). In addition, some substances, such as plasma fatty acids, degrade when subjected to repeated freezing and thawing cycles. (13-16)

6. Data validation and cleansing

The function of clinical data management is crucial in guaranteeing the precision and uniformity of the trial database. This encompasses the process of generating and monitoring data inquiries. Queries or data clarification forms are created for multiple purposes, such as verifying that each participant fulfilled the trial entry criteria, ensuring proper documentation for any protocol deviations/violations, providing sufficient documentation for missing data, ensuring the completeness of study procedures (e.g., reviewing all adverse events with appropriate documentation of any necessary treatment or follow-up), and identifying outlier values that seem implausible or suspicious. Information obtained from sources other than Case Report Forms (CRFs) must also be assessed for its comprehensiveness, precision, and the existence of improbable or duplicated data points. Queries are created and sent to the research site to solicit more material if necessary. It is necessary to monitor these in order to establish a record of activities with sufficient documentation for any modifications made to the database, and to guarantee that any inquiries for data clarification are acknowledged and answered.

Data managers use a mix of automated tests and human assessment to detect improbable or extreme statistics. Descriptive statistics are used to detect outlier values that are located at the extremes of the observed distribution. For instance, if the average alteration in body weight from the moment of randomization to a specific trial visit is 0.2 kg, a number indicating a modification of 20 kg may be marked for confirmation. This amount might have arisen due to a mistake in entering weight using incorrect units, swapping digits, or making an error when transcribing the data from the original document (17).

During the data validation and cleaning process, calculations related to compliance with the research intervention, such as the proportion of predicted servings of the study product ingested or the number of supplement tablets returned, are confirmed. Data managers also verify items to evaluate internal coherence; for instance, if an adverse event has a specified therapy, this treatment should also be included in the list of concurrent drugs administered. In addition, if a negative incident is mentioned, but it falls within a category that had already been documented in the participant's medical records, the data manager may raise a question to confirm if the incident should be classified as a genuine adverse event. If the

adverse event that was recorded is determined to be a pre-existing condition rather than a deterioration, it may need reclassification. One aspect of the data management process involves ensuring that adverse events, such as serious adverse events and adverse events of special interest, are reported to the relevant parties, such as the Institutional Review Board (IRB) or the Data and Safety Monitoring Board, within the specified time frame.

Data management plays a crucial role in ensuring that all clinical choices made throughout a study are well recorded. Hence, queries can be created to gather supplementary data to verify the existence of documentation pertaining to the individual responsible for the clinical decision, the timing of the decision, the reasoning behind the decision, the suggested course of action, and any subsequent interactions with the study participant and their healthcare provider(s), as deemed necessary, until the issue that prompted the clinical decision is resolved (18-20).

7. Conclusion

This article provides a concise overview of the essential procedures involved in collecting and analyzing biospecimens, as well as managing clinical data for randomized controlled trials (RCTs) focused on human nutrition. In order to maintain the accuracy and reliability of the data, it is crucial to have a comprehensive strategy before starting the trial. This plan should include all the requirements for collecting, processing, transporting, storing, and analyzing all biospecimens. The procedures for verifying the accuracy of laboratory and other data collection, recording, and assessment are also outlined to enable the establishment of a reliable and precise research database for statistical analysis. The article presents best practices that guarantee data integrity and quality in human nutrition RCTs. These standards ensure that the findings may be confidently utilized to support the creation of clinical public health recommendations.

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