BioTeSys

From Human and Non-Human Samples

Differences in Sample Preparation and Planning for Reliable Analytics

Physiological Samples from Human and Non-Human Sources

The analysis of human samples in clinical studies is well established and is performed worldwide using highly advanced and specialized measurement techniques, depending on the analyte. Human samples provide a standardized basis for the development and validation of analytical methods, as extensive data and reference values are available.

Differences Between Human and Non-Human Samples

Physiological samples from non-human sources are not identical to their human counterparts. Small but significant differences, such as viscosity and temperature dependence, have been described in the literature [1]. These differences can lead to changes in recovery rates, deviations in measurement results, or incompatibilities with analytical methods, depending on the measurement technique used. This is particularly critical for methods that require high sensitivity and reproducibility.

Therefore, sample preparation steps should be established and verified using an exactly matching sample matrix as the one to be analyzed later. Deviations in matrix composition, such as differences in protein, lipid, or water content, can significantly impact analytical results. In addition to physical properties, chemical factors also play an important role, as they can influence the stability and reactivity of target analytes.

Evolutionary Differences Between Species

Furthermore, physiological characteristics of different species are often strongly shaped by evolution. A well-known example is ubiquinones, which function as electron and proton carriers in the respiratory chain of living organisms. In this case, the primary active molecules in rodents and other mammals differ in the length of the isoprene side chain of the compounds (ubiquinones UQ-9 and UQ-10) [2]. These differences have direct consequences for the interpretation of biochemical analyses and require specific adaptations of methods to the species being studied.

Additionally, differences in metabolic pathways, enzyme activities, and cell compositions can make it difficult to directly transfer analytical results to humans. Therefore, a precise understanding of the target matrix and its specific physiological parameters is essential.

Methodological Requirements

To address these challenges, methods for determining analytes in physiological samples must be established, validated, and calibrated using an identical blank matrix or, if necessary, a suitable surrogate. This is particularly important in LC-MS/MS analyses, as this technique, despite the use of stable isotope-labeled internal standards, is susceptible to matrix effects. Matrix effects can lead to signal suppression or enhancement, thereby distorting quantification. Therefore, sample preparation and analysis should be designed to minimize these effects.

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Challenges Due to Limited Sample Material

Limited sample material also presents a challenge. The total blood volume of a mouse (<2 ml), a rat (<20 ml), and a human (~6000 ml) differ significantly [3]. These differences not only impact the available sample amounts but also necessitate specific adjustments to study designs. For example, the analysis of blood plasma uptake kinetics in small animals is only feasible if sample preparation is miniaturized.

If miniaturization of sample preparation is not possible, the demand for test animals increases, leading to both ethical and logistical challenges. However, modern developments in microfluidics and the use of highly sensitive analytical systems offer promising approaches to obtain meaningful results with minimal sample volumes.

Recommendations for Non-Human Sample Analytics

- **Establishment and Validation:** Analytical methods should be developed and validated considering the specific properties of the target matrix.
- **Matrix Effects:** Comprehensive investigation and minimization of matrix effects are essential, especially for LC-MS/MS-based methods.
- **Miniaturization:** Development of methods that work with minimal sample volumes to reduce the need for test animals and improve study efficiency.
- **Species-Specific Adaptation:** Consideration of biochemical and physiological differences between species to ensure accurate and reproducible results.

Conclusion

The analysis of physiological samples from non-human sources requires a precise understanding of the specific properties of the sample matrix and an adaptation of analytical methods. Through careful planning, validation, and the use of modern technologies, the challenges outlined can be successfully addressed. This enables reliable and reproducible analytics that yield meaningful results even under challenging conditions.

References

[1] Windberger et al: Haemorheological values in nine mammalian species. Exp Physiol 88.3 (2003).
[2] Mikael Turunen, Jerker Olsson and Gustav Dallner: Metabolism and function of coenzyme Q. BBA - Biomembranes Volume 1660 (2004).
[3] Animal Research Advisory Committee (ARAC) Guidelines: Guidelines for Survival Blood Collection in Mice and Rats, https://oacu.oir.nih.gov/animal-research-advisory-committee-arac-guidelines, 12/14/2022.

Do you have any further questions or are you interested in a collaboration? We would be happy to advise you on your individual request and find the optimal solution for your needs.

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