THE INTEGRITY STUDY: MEASURING THE IMPACT OF SAMPLE PREPARATION TECHNIQUES AND STORAGE TEMPERATURES ON DNA AND RNA SAMPLE INTEGRITY.

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STATEMENT OF THE ISSUE

As the premier provider of sample management services supporting all aspects of maximizing critical scientific assets, BioStorage Technologies (BST) is frequently asked to provide recommendations regarding sample preparation techniques and best-practice storage conditions. In general, the clinical research industry recognizes that sample stability is defined during R&D efforts by the target assay methodology or per accepted blood product component handling techniques (i.e., EDTA as a preservative for serum via whole blood collected in a purple-top vial). This 24-month longitudinal comparative research study will enable BST to provide data-driven nucleic acid sample preparation and storage comparators to the bioscience industry.

HYPOTHESIS

- Sample storage conditions and temperature may affect sample integrity
- Sample manipulation/preparation may affect sample integrity

METHODOLOGY

This study includes two arms: the human DNA comparator and the human RNA comparator. Each will analyze the impact on sample integrity when different preparation, storage conditions, temperature and time are applied. The study will compare: fresh samples, frozen pre-extracted samples, and extracted nucleic acids which require freeze/thaw cycles for processing. Various storage temperatures and aliquoting strategies will be evaluated over a 24-month period subsequent to a single patient collection event via whole blood preserved collection vial.

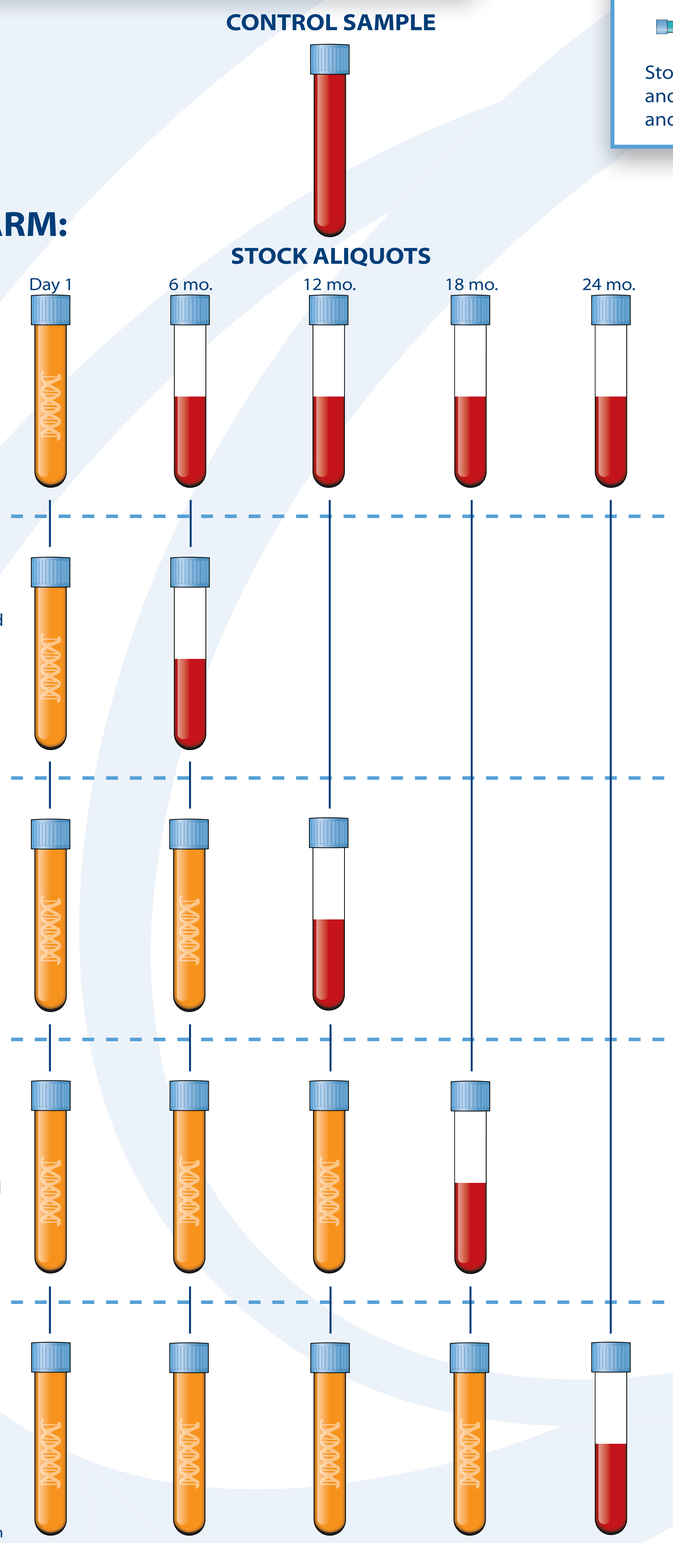
Whole blood

Resultant DNA

Resultant RNA

Stock aliquots generated per time period and stored at 4–6°C, -20°C, -70°C, -80°C and LN2.

DNA COMPARATOR STUDY ARM:



DAY OF COLLECTION: Whole blood sample will be aliquoted (one per each future time point) for each storage temperature (proposed: 4–6°C, -20°C, -70°C, -80°C, LN2). Aliquots will be placed into storage for future extraction and verification. One sample per temperature will be extracted and yield verified at ambient conditions of collection, manipulation, extraction and verification. Resultant DNA will be placed in storage at defined temperature(s).

6 MONTHS: Control sample resultant DNA sample will be removed from storage, thawed and re-verified for current yield **plus** one stock aliquot from each temperature will be removed from storage, extracted and verified for yield. Samples will be placed back into storage.

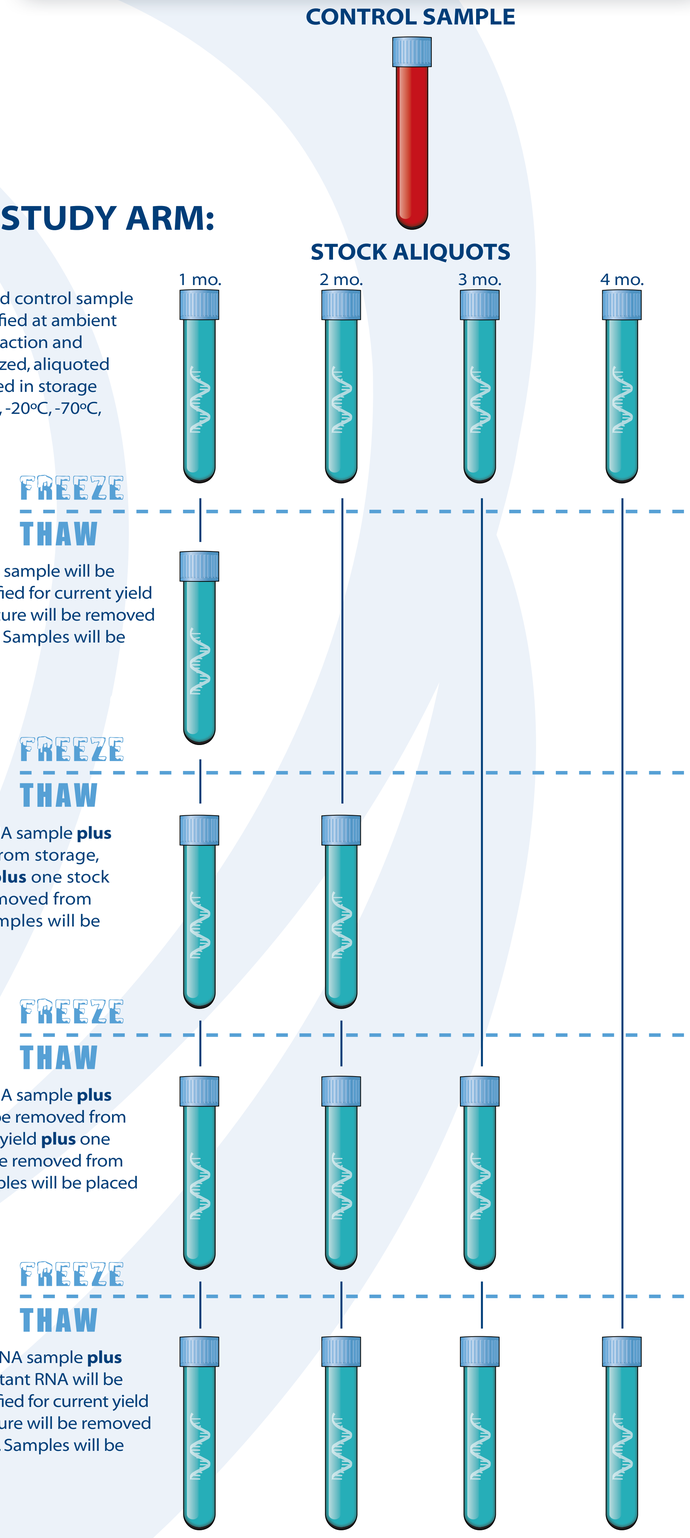
12 MONTHS: Control sample resultant DNA sample **plus** 6-month resultant DNA will be removed from storage, thawed and re-verified for current yield **plus** one stock aliquot from each temperature will be removed from storage, extracted and verified for yield. Samples will be placed back into storage.

18 MONTHS: Control sample resultant DNA sample **plus** 6-month **plus** 12-month resultant DNA will be removed from storage, thawed and re-verified for current yield **plus** one stock aliquot from each temperature will be removed from storage, extracted and verified for yield. Samples will be placed back into storage.

24 MONTHS: Control sample resultant DNA sample **plus** 6-month **plus** 12-month **plus** 18-month resultant DNA will be removed from storage, thawed and re-verified for current yield **plus** one stock aliquot from each temperature will be removed from storage, extracted and verified for yield. Samples will be placed back into storage.

Note: If sample integrity/degradation information is valued, study may continue beyond 24 months with subsequent thaw, verification and refreeze actions.

RNA COMPARATOR STUDY ARM:



DAY OF COLLECTION: The whole blood control sample will have the RNA extracted and yield verified at ambient conditions of collection, manipulation, extraction and verification. Resultant RNA will be normalized, aliquoted (one per each future time point) and placed in storage at defined temperatures (proposed: 4–6°C, -20°C, -70°C, -80°C, LN2).

1 MONTH: Control sample resultant RNA sample will be removed from storage, thawed and re-verified for current yield **plus** one stock aliquot from each temperature will be removed from storage, thawed and verified for yield. Samples will be placed back into storage.

2 MONTHS: Control sample resultant RNA sample **plus** 1-month resultant RNA will be removed from storage, thawed and re-verified for current yield **plus** one stock aliquot from each temperature will be removed from storage, thawed and verified for yield. Samples will be placed back into storage.

3 MONTHS: Control sample resultant RNA sample **plus** 1-month **plus** 2-month resultant RNA will be removed from storage, thawed and re-verified for current yield **plus** one stock aliquot from each temperature will be removed from storage, thawed and verified for yield. Samples will be placed back into storage.

4 MONTHS*: Control sample resultant RNA sample **plus** 1-month **plus** 2-month **plus** 3-month resultant RNA will be removed from storage, thawed and re-verified for current yield **plus** one stock aliquot from each temperature will be removed from storage, thawed and verified for yield. Samples will be placed back into storage.

Notes:
- *If sample integrity/degradation information is valued, study may continue beyond four months with subsequent thaw, verification and refreeze actions.
- If applicable, a further end point may be defined in addition to yield verification across freeze/thaw cycles. That is, potential exists for a defined protein yield marker to also be used as a definition of residual integrity of RNA.

CONCLUSION

During drug development, clinical trial samples must be securely managed and properly stored to support prospective or retrospective nucleic acid analysis. The goal of the Integrity Study is to determine the best method for preparing whole blood samples for nucleic acid extraction and the best temperature to store samples for future research.

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