B13 Comparison of DNA Storage Methods

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After attending this presentation, attendees will understand the best methods of DNA storage.

This presentation will impact the forensic community and/or humanity by enabling the forensic laboratories to store their samples in such a manner that they are assured that there will be no DNA retention to the plastic.

DNA storage is a critical issue in forensic, epidemiological, clinical and genetic database laboratories. [1]In forensic DNA laboratories, there is always the possibility that cases may be re-opened and samples may need to be re-tested. This is especially important when the amount of DNA is limited. In addition to sample quantity, intrinsic differences in sample types resulting in differences in quality, and extrinsic differences in the storage buffers (e.g., ionic strength), tube surface type, exposure to UV and temperature of storage may lead to differences in the ability to recover and re-test the sample.

Laboratories utilize different methods to extract DNA depending on the sample type. The composition of the final solvent in the different methods and the inherent properties of the casework samples may impart differences in ionic strength. Casework DNA samples are usually dissolved in TE-4 and kept in plastic tubes. The commercial tubes that are utilized to store the DNA come from different sources and are composed of different types of plastics.

It has been observed that DNA can bind to polypropylene tube surfaces and these surfaces cause the DNA to denature.[2] Alternate polymers (polyallomer vs polypropylene) appear to reduce the retention of DNA.[2] Non ionic detergents have been found to be effective in preventing DNA adhesion at concentrations that do not inhibit PCR.[3] In addition some of the tubes may contain nucleases and chemical contaminants that may digest and/or denature the DNA.[2]

In this study, a comparison of storage of DNA samples at varying concentrations, in different buffers over varying amounts of time, at different temperatures in different tube types, will be performed. Utilization of the most efficient storage method (buffer, tube, and temperature) may prove critical in the ability re-test samples.

Interand intra-lot tube variation will first be evaluated using control DNA. Glass tubes will be used as a control. Any variation in storage temperatures and humidity will be evaluated and monitored using NIST certified digital thermometers. Tubes containing replicate samples at 0.5, 1.0 and 10ng of DNA in 30ul volume for each time point and each temperature and each tube type will be stored in the dark and covered in aluminum foil to avoid exposure to UV.

Samples will be stored at –20°C, 4°C, and room temperature. Aliquots will be analyzed at the start, 1 day, 1 week, 1 month, 3 months, 6 months and 1 year (a 3 year and 10 year time point set will also be made). Samples will be in TE storage buffer and Chelex storage buffer.

Quantification will be performed by UV spectrophotometry and a subset will be analyzed using agarose gel electrophoresis. These values will be compared to the original sample values to determine if there has been any loss/retention of the DNA sample based on storage method or length of time. Data in triplicate for each sample type, storage tube and temperature will be analyzed for standard deviation and coefficient of variance. In addition, amplification of STRs from a subset of the samples will be performed.

References:

1. Steinberg K, *et al*., “DNA banking for epidemiologic studies: a review of current practices.” Epidemiology 14, no 2 (2003): 254-5.
2. Gaillard C. and Strauss F. “Eliminating DNA loss and denaturation during storage in plastic microtubes.” Technical Tips Online (1998)
3. Rensen G. Buoncristiani M. and Orrego C. “Assessment of DNA retention on plastic surfaces of commercially available microcentrifuge tubes.” (2002): available from http://www.promega.com/geneticidproc/ussymp13proc/abstracts/73_buon cristiani.pdf

DNA, Storage, Polypropylene

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