Comparison of DNA Storage Methods

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Abstract:

The methods used to extract, store and type DNA vary from laboratory to laboratory. Although each lab may have different methods of extraction, amplification and statistical reporting they all must keep accurate records and samples from past cases (ref-DAB guidelines/ASCLD lab guidelines).

Paramount in forensic DNA, epidemiological, clinical and virtually any genetic database laboratory is the issue of storage of samples of DNA. In forensic laboratories there is always the possibility that cases may be re-opened and any stored DNA sample may need to be re-tested. This is especially important when the amount of sample is limited. In addition to sample quantity, intrinsic differences in sample types resulting in differences in quality, extrinsic differences in the storage buffers especially 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.

Methods:
Comparison of the DNA recovery from different plastic tubes of control DNA (both 9947a and K562) will be used to establish a baseline. Glass tubes will also be used as a control. Any variation in storage temperatures will be evaluated and monitored using NIST certified digital thermometers. Inter- and intra-lot tube variation will be evaluated using control DNA. Tubes containing samples will be stored in the dark and covered in aluminum foil to avoid and exposure to UV. Samples will be stored at –20C, 4C, and Room temperature and aliquots will be analyzed at start, 1 day, 1 week, 1 month, 3 months, 6 months and 1 year. All samples will be in the same ionic concentration of storage buffer. An additional control will be samples of the DNA stored on FTA paper.

Previous studies:
It has been observed that DNA can bind to polypropylene tube surfaces and these surfaces cause the DNA to denature (3). Rensen et al 2002 demonstrated that Furthermore, some of the tubes may contain nucleases and chemical contaminants that may digest and/or denature the DNA (3). Utilization of the most efficient storage method (buffer, tube and temperature) may prove critical in the ability re-test samples. It has also been shown that exposure to UV lighting can cause a false positive result in PCR testing (2). DNA stored dried on paper has been shown very effective for extraction over long periods of time. (5)

Effect of Tube Types:

Based on past research, it has been shown that storing DNA in polypropylene tubes can render the DNA useless because of denaturatization. The amount of denaturatization

varies depending on tube type (3). To study the effects of time, temperature and type of tube I will be taking known quantities of DNA and storing them at various temperatures and in different tubes and measuring the quantity of DNA present after varying lengths of time. Quantification will be preformed by UV Spectrophotometry at 260nm & 280nm and for a small number of the samples by yield agarose gel electrophoresis. Comparison of these values to the original sample values will be preformed to determine if there has been any denaturing 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 accordance with NIST standards.

It is my hope that this study will enable forensic laboratories to store their samples in a tube of certain qualities and the DNA in a form that will allow it to be kept for future reference without the possibility of denaturization.

References:
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2) Moore, J. UV irradiation causing thymine dimmers of DNA to adhere to surface- Biotechniques
3) Galliard, et al. – Intro statement on quality of tubes vis a vis obvious defects include leaking, do not withstand centrifugation,, contamination of plastics with chemicals used during manufacturing (ref 1 from Galliard). Another problem is loss of sample on the tube wall. Since DNA is hydrophilic and polypropylene is hydrophobic, the interactions of DNA with these tubes are unexpected. In point of fact, DNA has been shown to bind to polypropylene as interactions of DNA with the tube walls causes changes in conformation which can go as far as complere denaturation with strand separation (3-6). Buoncristiani- CA DOJ ionic strength effect on stability of storage- Promega abstract The adherance of the DNA is particularly noticeable with short DNA fragments and at high ionic strength.
4)Steinberg, et al.- DNA banking for epidemiological studies: a review of current practices. Statements from the study of blood spots.
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