B109 DNA Storage Under Multiple Conditions

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Learning Overview: After attending this presentation, attendees will understand the long-term storage of DNA under multiple conditions that reflect those in a laboratory setting and those in field work.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by establishing guidelines in regard to DNA storage under multiple conditions that will still maintain functional integrity for downstream forensic analysis.

Extracted DNA stored in a Tris-EDTA (TE) buffer at ambient temperatures of 18–20°C will not show significant degradation in quality or concentration that would impede downstream forensic analysis. Tris-EDTA is a widely used buffer in DNA storage, and it is accepted that DNA can be stored short-term at 4°C and long-term at -20°C.[1] This method of storage is reliant on consistent access to a power source to maintain temperature in the refrigerators and freezers. This is manageable in many first-world countries, but may not be possible in developing countries where fieldwork may be performed due to an increase in sexual violence.[2] It is imperative that DNA be properly maintained in order to be considered legally admissible.[3]

Samples were extracted from a single-source blood donor and spun down in order to pellet the red blood cells. The buffy coat was removed and washed with Deionized (DI) water through repeated centrifugation and removal of the supernatant to concentrate the white blood cells, from which the DNA was extracted using QIAGEN® QiAmp® DNA Investigator.[4] Samples were then eluted into the QIAGEN® ATE storage buffer, DI water, and Invitrogen™ Tris-EDTA. The samples were then pooled by storage media and diluted 1:200, and samples stored at 4°C and -20°C were aliquoted to avoid repeated freeze-thaw cycles. Samples were stored at 4°C, -20°C, 18–20°C, and 35°C in Eppendorf® DNA LoBind tubes and quantified using Quantifiler® Trio every two weeks in order to track degradation trends through the degradation index and concentration.[5] The data obtained from these quantifications will be analyzed using IBM SPSS statistics v23. Once degradation became apparent, samples were amplified using Identifiler® Plus and run through Capillary Electrophoresis (CE) on an ABI® 3500 Genetic Analyzer in order to obtain a genetic profile.[6] As of August 1, 2019, 200 samples have been quantified and amplified, and 80 samples have been run through CE to obtain a genetic profile. Initial attempts to run CE had encountered a technical problem and the samples are being rerun.

The study, as of writing this, has completed 18 weeks of experimentation. DNA stored in DI water at 35°C has shown complete degradation and evaporation at ten weeks with no detected DNA and no detected degradation index. This sample has been discontinued from future quantifications and amplifications.

Table 1. Degradation index (DI) and concentration (ng/µL) for DNA stored under various conditions at 18 weeks

	-20°C		4°C		18°C		35°C	
	DI	Conc.	DI	Conc.	DI	Conc.	DI	Conc.
QIAGEN® ATE	0.85	4.7	1.15	4.65	1	3.65	1	4.45
Invitrogen™ TE	0.95	4.2	0.9	0.9	0.8	0.1	0.95	1.05
DI Water	1.1	3.4	0.9	2.5	1.8	0.8	N/A	0

The degradation index is measured between <1 (no degradation/inhibition unlikely), 1–10 (slight to moderate degradation/inhibition possible), and >10 or blank (significant degradation/inhibition possible).[5] The degradation index for all samples at these temperatures have not shown to significantly differ, and the slight levels were not enough to impede downstream analysis. Samples stored under these conditions did not show a significant decrease in concentration that would impede downstream amplification or analysis, or an increase in level of degradation, over the study thus far.

Reference(s):

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3. Lee, Henry C., and Carll Ladd. Preservation and collection of biological evidence. *Croatian Medical Journal* 42, no. 3 (2001): 225-228.
4. QIAGEN® *QIAamp® DNA Investigator*. https://www.qiagen.com/us/resources/resourcedetail?id=dcc5a995-3743-4219-914d-94d6a28e49b3&lang=en (accessed February 15, 2019).
5. Thermo Fisher. *Quantifiler™ HP and Trio DNA Quantification Kits User Kits*. http://tools.thermofisher.com/content/sfs/manuals/4485354.pdf (accessed February 20, 2019).
6. Life Technologies. *AmpFℓSTR™ Identifiler™ Plus PCR Amplification Kit User Guide*. http://tools.thermofisher.com/content/sfs/manuals/cms_076395.pdf (accessed April 4, 2019).

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