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Effects of Storage Temperature on the Recovery of DNA from Aged-Bloodstains

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Many DNA sample repositories or "DNA banks" exist, primarily for the support of epidemiological and genetic research or to enable identification of forensic evidence or human remains. Whole blood, plasma, and buccal epithelium are convenient and minimally intrusive sources of DNA for current DNA analysis technologies. Given that "banking" tissue below ambient temperature is expensive (equipment, space, and energy) and problematic (assuring continuous operation), banking facilities are evaluating sample types, storage media and temperature to ascertain the optimum conditions to successfully recover DNA information.

Artificial aging of samples may not be equivalent to natural aging. Therefore, it is desirable to evaluate proposed sample and storage conditions using naturally aged materials. Control samples prepared on untreated Schleiher & Schuell 903 paper (903) in 1994 were stored for over ten years at the following temperatures: ambient, -20°C, -80°C, and -150°C. The "release" of the DNA from all of these samples was followed with yield gels and slot-blots as well as with peak heights and areas of Short Tandem Repeat (STR) multiplex loci for the recovery of typeable DNA. To add to the results of the control samples, over 300 anonymous bloodstains, field collected on 903 and stored from 2 to 15 years at ambient temperature and humidity, were made available for study. Fifty similarly collected samples stored at -20°C for 6 years were available for direct comparison.

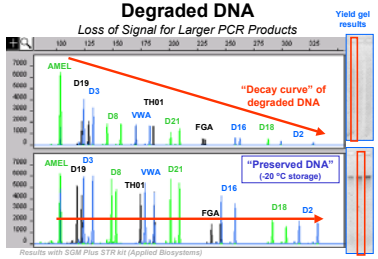
All of the field-collected samples yielded typeable DNA, although some loss of the larger STR loci occurred in the older and more degraded samples stored under ambient conditions. Yield gels show the DNA from these samples to be smears with fragment size ranging from approximately 100 base pairs (bp) to about 12 kilo bases (kb). The DNA extracted from the 6 y old samples stored at -20 °C appears intact (fragment size greater than 12 kb). No consistent differences in typeability as a function of extraction technique were observed. All control samples yielded typeable results, however differences were seen at the various storage temperatures. Examples of the analytical results will be presented with brief explanations.

The Initial Experimental Design

- A long term study on the stability of DNA recovered from a dried bloodstain stored on 903 paper was initiated July 1994 at the request of the Armed Forces DNA Identification Laboratory (AFDIL).
- Whole blood, as 20 µL aliquots, was spotted on 903 paper, and dried overnight in a vacuum desiccator at ambient temperature.
- Six dried bloodstains were punched into each cryogenic vial using a 6.3 mm hole punch.
- Sixteen vials were secured in each of four storage boxes.
- Boxes were stored at either ambient, -20° C, -80° C or Liquid Nitrogen (-150° C).
- One vial was removed from each box, extracted, amplified and analyzed at a variety of time periods to include 10 years.

Measurement Criteria: Typeable DNA

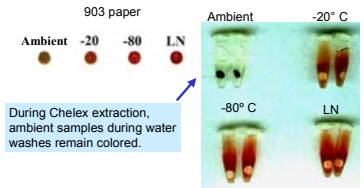
- In 1994 when the study was started Typeable DNA meant you could successfully Polymerase Chain Reaction (PCR) amplify loci of interest ranging from 300 bp to 700bp.
- In previous years DNA "fit for purpose" would have needed to range in size from 1000 bp to 20,000 bp for Restriction Fragment Length Polymorphisms analysis.
- **Today most Human Identity loci are less than 400 bp**



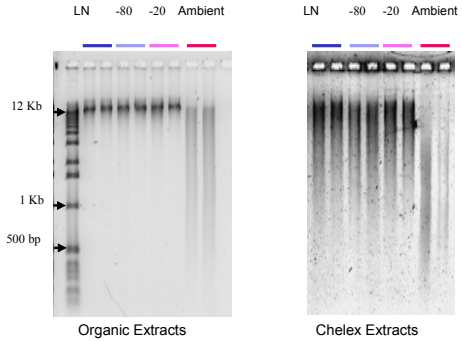
Initial Observations

- Visual observation of the samples:
  - all remained "red" as they were at the beginning except the ambient samples.
  - Ambient samples were turning a darker "brown" through the years.
- Chelex extractions performed on the samples indicated that the "heme" bound to the paper of ambient samples.

DNA Stability Study 6 y time point



Quality of the DNA Extracts from 7.5 y Control Samples



The color of the LN stored samples appear the same as a fresh bloodstain even after 10 y of storage. This probably indicates that the Heme has not been oxidized.

Untreated Paper Studies, S&S 903

Control samples

- NIST prepared
- Dried vacuum desiccator
- Sealed in tubes
- Stored at -150 ºC, -80 ºC, -20 ºC, and lab ambient
- Stored for 7.5 years
- 6 reps of 4 temperatures

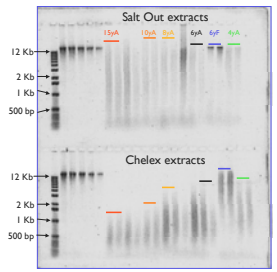
Field samples

- State Health Labs
- Dried ???
- Sealed ????
- Most stored at warehouse ambient, one set at -20 ºC for 6 years
- Stored for 2 to 15 years
- 1 rep of 318 samples

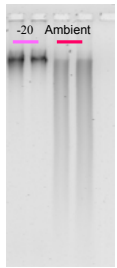
Field Samples Evaluated

Year Spotted (Code)	Storage Conditions	# Samples Received
1986 (15 yA)	ambient	51
1987 (14 yA)	ambient	51
1991 (10 yA)	ambient	25
1993 (8 yA)	ambient	26
1994 (7 yA)	ambient	50
1995 (6 yF)	-20°C	50
1995 (6 yA)	ambient	25
1997 (4 yA)	ambient	20
1999 (2 yA)	ambient	20
	Total	318

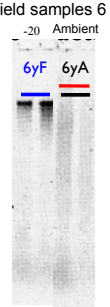
Quality of the Field DNA Extracts



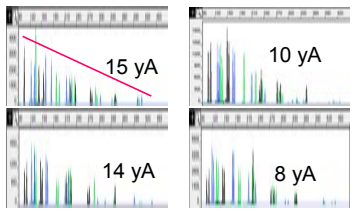
Controls 7.5 Y



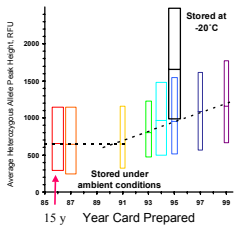
Field samples 6 Y



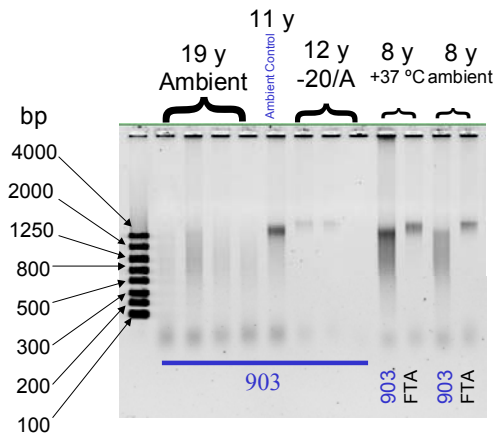
Ambient Stored Field Samples



Peak Heights Vs Years Stored



STR typing of the field samples DNA extracts: Sample profiles were analyzed for the presents or absence of alleles at all loci and the total relative fluorescence units (rfu's) obtained for each sample. The same amount of DNA extract was PCR amplified for each sample. The Peak Heights Vs Years Stored graph indicates that at ambient conditions there is an incremental decrease in the rfu's obtained from 0 to 10 years. At 10 years ambient storage the decrease in the rfu's seems to plateau. Also note that the samples stored at –20 ºC produce higher rfu's after PCR amplification.



Additional dry-storage media have been developed through the years to "protect" DNA from degradation at ambient temperature. One stabilization medium is FTA paper (Whatman, Hillsboro, OR), a high-purity cotton linter pulp that has been treated to tightly bind DNA and RNA while allowing selective removal of PCR inhibitors. This paper was designed to enable direct PCR amplification from a washed 1.2 mm punch of the stored stain. While this amplification method is functional, at times researchers want to remove the DNA from the media and quantify the purified DNA prior to further processing. We have published (Kline et al. Anal Chem. 2002, 74, 1863 – 1869) a modified Chelex extraction method for such purpose. GenVault (Carlsbad, CA) recently introduced a procedure for the extraction of DNA from FTA paper that yields "high quality, double stranded" material. As one step in our evaluation of this method, DNA extracts from aged-bloodstains were evaluated in the yield gel whose image is to the left.

From the smears on the gel image, the DNA of the 19 year-old ambient bloodstains stored on 903 paper is degraded. These stains did not totally "wash" – the processed samples were the same color before and after processing. We have no record of how these samples were dried prior to ambient storage.

The remaining samples did "wash" and provided relatively intact DNA of at least 4000 bp. While relatively little DNA was recovered from the three 12 y –20/A samples (first stored for 7 y at –20 ºC then removed to ambient), the DNA that was extracted appears intact. The 8 y paired samples were prepared at NIST on 903 paper and the FTA media; sets of these samples have been stored at ambient and +37 ºC. The DNA recovered from the samples stored on 903 paper is somewhat less intact than that recovered from those samples stored on FTA, indicating that the FTA media may indeed stabilize DNA.

Conclusions:

These stability studies have been focused on the ability to recover "fit for purpose" DNA that can be amplified for the STR loci commonly used by Human Identity forensic laboratories. The studies do not address other analytes that may be useful in the future. These study results also indicate that DNA stability is only a weak function of temperature.

Informative References:

Miller et al. (1988) Nucleic Acids Research, 16, 1215. (Saltout extraction procedure)

Walsh et al. (1991) BioTechniques, 10, 506-513. (Chelex extraction procedure)

Kline et al. Anal Chem. 2002, 74, 1863 – 1869 (Short-term DNA stability on different storage media)

Disclaimer

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