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DNA Science Update

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Disclaimers

Funding: Interagency Agreement 2008-DN-R-121 between the National Institute of Justice and NIST Office of Law Enforcement Standards.

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Preservation of DNA What are the issues?

- How much can you recover?
- Is the material degraded?
- Is it typeable:
 - Can you obtain an STR profile?
 - Are there new technologies that help obtain STR profiles?
- What about the storage of DNA extracts?

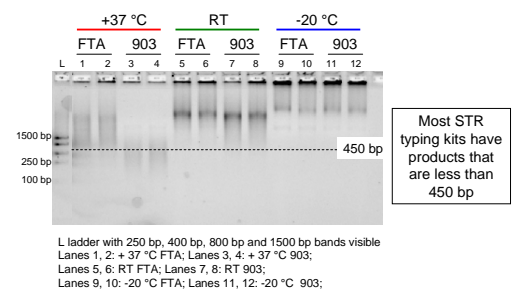
Bloodstain Storage Study

11 year FTA_903 Study

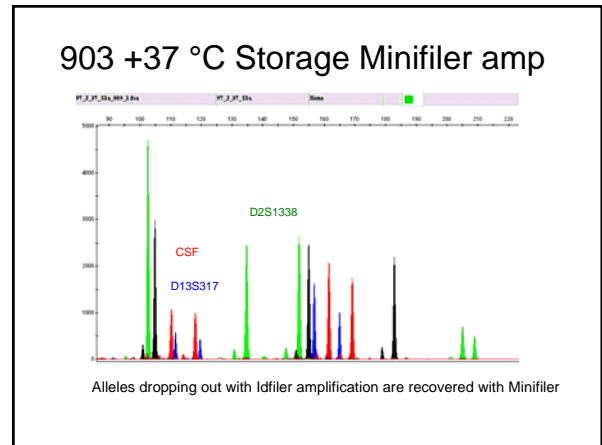
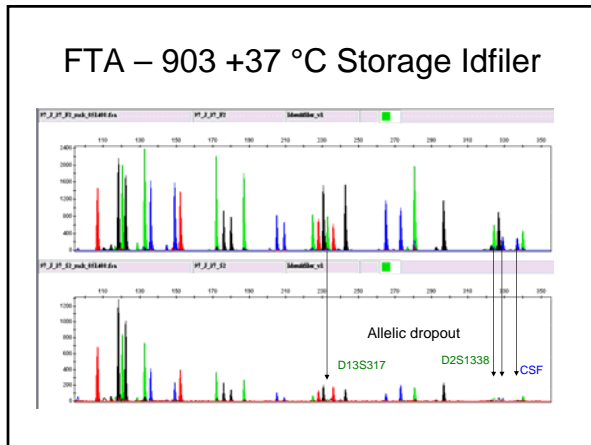
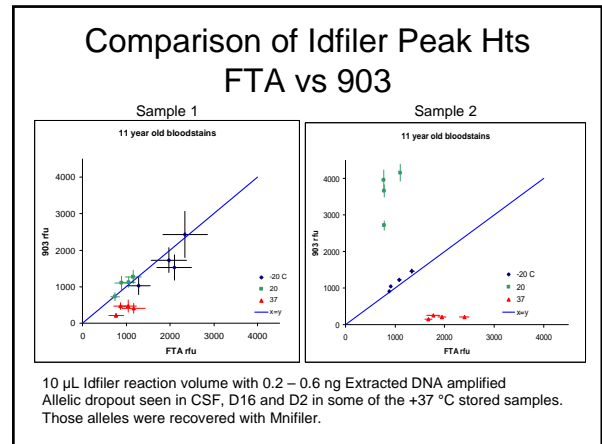
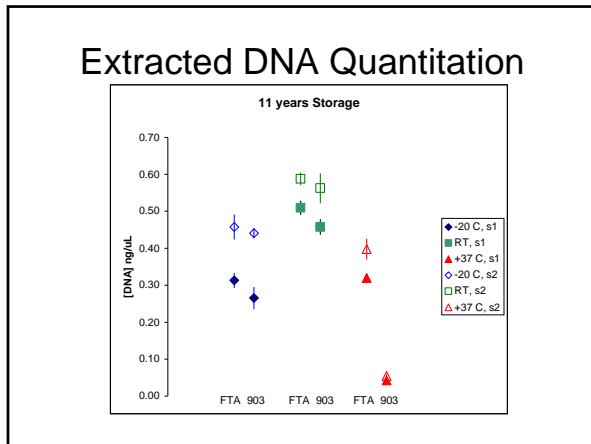
Results as of 13 August 2008

- Bloodstains prepared 09/97 on FTA and 903 stain cards.
- Stains dried and vacuum sealed in Mylar bags.
- Stains stored at -20 °C, RT, and +37 °C.
- Duplicate stains extracted with DNA IQ.
- Extract analyzed on a Flashgel to check degradation.
- Extracts quantified with Quantifiler Human qPCR kit.
- Quantified extracts amplified with Identifier.
- If allele dropout observed, samples amplified with Minifiler.

Quality of the Extracted DNA



After 11 years of storage at 37 °C both FTA and 903 show signs of degradation, the FTA samples exhibit DNA with slightly higher molecular weight than the 903 samples.



FTA-903 Controlled Study Summary

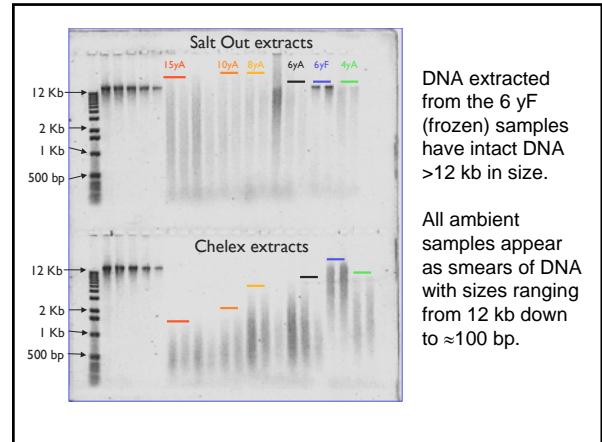
- After 11 years at 37 °C, the FTA samples yield a full profile with Identifiler while the 903 samples have allelic dropout due to the DNA degradation. However, the loci lost with Identifiler can be recovered with the use of miniSTRs.
- There is no difference in DNA recovery between ambient and -20 °C storage.

903 Bloodstain Study

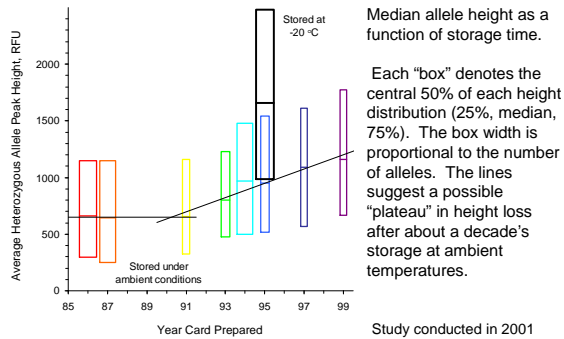
Stains obtained from outside sources.

“Uncontrolled Bloodstain Stability”

Examined over 300 anonymous bloodstains:
 Stored on untreated Schleicher & Schuell 903™ paper (S&S® 903)
 2 - 15 years at ambient temperature, no humidity control.
 Control samples stored at -20 °C for 6 years.
 Different methods of extraction:
 Chelex® and Salt Out
 Evaluation of the quality of the recovered DNA:
 Yield gel and STR-typeability



STR analysis results: Average Heterozygosity Peak Heights



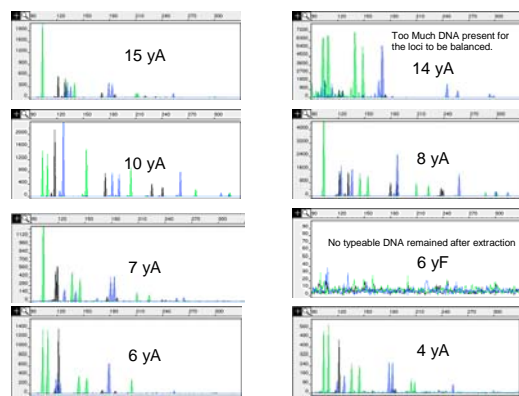
But how efficient is the extraction?

- Observations
 - The longer the bloodstain is stored at room temperature the harder it is to wash the heme away.
 - Heme washes away easily from stains stored at -20 °C.
- Theory:
 - If protein (i.e. heme) sticks to the paper what stops DNA from sticking?

What’s left on the paper?

- Take bloodstain that has been Chelex extracted
- Wash with a Tris buffer
- Take a 1.2 mm punch of the washed stain
- Place the washed 1.2 mm punch in a PCR tube and amplify.

Amplification of the 903 spot after Chelex extraction. Sample Size: 1.2 mm



Summary “Uncontrolled” Bloodstain Stability

- All samples gave typeable DNA.
- Loss of some larger STR loci noted in some of the older, more degraded samples.
- Chelex extracts typed as well as the “salt out” extracts.
- DNA is more tightly bound to 903 paper the longer it is stored at ambient; the number of “dropout” alleles increases with storage time.
- More DNA available from stains stored for 6 years at -20 °C than from those stored for 2+ years at ambient.
- DNA from the -20 °C stored samples was readily Chelex extracted from the 903 paper.
- Bound DNA can be amplified directly from 903 paper stored at ambient temperature after Chelex extraction.

New Amplification technologies

- **PowerPlex 16HS** and direct PCR
 - Samples from year 1986, 1987, 1991, 1993, 1994 were selected.
 - One 1.2 mm punch of a 903 stain placed into each of two PCR tubes for each sample.
 - 8 sample tubes per year were amplified.
 - No extraction of 16 to 24 year old samples stored at ambient.

Results of the Direct amplification

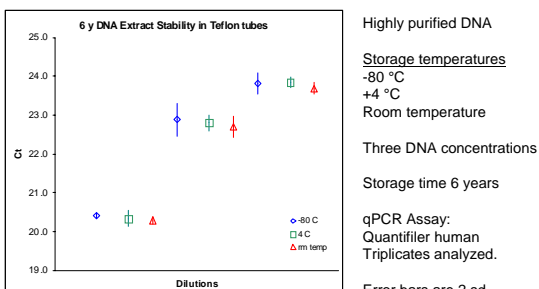
- One of the duplicate samples from 1987 lost one allele (Penta E locus)
- All other samples yielded full STR Profiles.

- Another direct amplification kit (AB Identifiler direct) has not been tested.

Stability of Extracted DNA

Liquid extract or dry storage

Extracted DNA Stability in Teflon Tubes



No differences between storage temperatures regardless of DNA concentration.
(But Teflon tubes are not cheap!)

Biomatrix SampleGard Plate experiments 208 days of Shipping/Stressing

NIST experiments conducted by
Margaret Kline

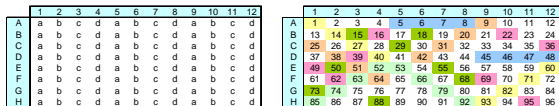
Study Design

- Well characterized DNA (highly purified) of known concentration was diluted in Teflon containers to target concentrations of:
 - 1.0 ng/μL (a)
 - 0.25 ng/μL (b)
 - 0.05 ng/μL (c)
 - TE⁻⁴ buffer was used as the fourth sample set. (d)
- 20 μL of prepared solutions were aliquoted in 4 different Biomatrica SampleGard 96 well plates.
- DNA solutions remaining in Teflon containers were stored at 4 °C as a cold storage control.

Study Design Continued

- SampleGard plates air dried in a laminar flow hood overnight.
- Plates labeled A, B, C, and D.
- Plates A and C stayed at NIST
- Plates B and D shipped/stressed .
- Temperature and humidity dataloggers stored with the plates.

Biomatrica Plate set-up



Sample wells highlighted in colors have been tested. Each color represents a different analysis time point.

Four Biomatrica SampleGard plates were prepared using a well - characterized and purified genomic DNA solution listed as follows:

- a = 20 μL of a DNA solution at 1 ng/μL.
- b = 20 μL of a DNA solution at 0.25 ng/μL.
- c = 20 μL of a DNA solution at 0.05 ng/μL.
- d = 20 μL of TE⁻⁴ to serve as a negative control.

Two prepared plates were stored at lab ambient temperatures, while the remaining two plates were "shipped" cross-country and exposed to harsh environmental conditions prior storage at lab ambient temperatures.

Study Data analysis

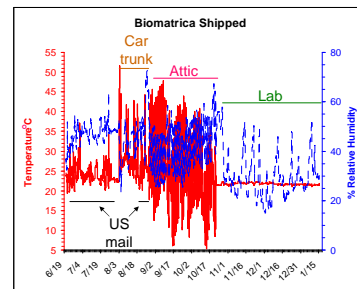
- 2 wells of each DNA concentration, one from a "shipped/stressed" plate and one a Lab ambient control plate, re-hydrated with 20 μL of DI Water.
- Plates containing the re-hydrated wells were slowly rotated for 45 min.
- Immediately following rotation, samples quantified with a Quantifiler Human qPCR kit along with 4 °C Teflon-stored DNA solutions.

Study Data analysis con't

- Re-hydrated materials and Teflon controls amplified with Identifiler.
- Volumes used for amplification based on the [DNA] applied.
 - 12.5 μL total reaction volume

[DNA] ng/μL	μL used for Idfiler	ng DNA amplified
1	1	1
0.25	2	0.5
0.05	5	0.25
TE ⁻⁴	5	0

"Shipped/Stressed" Temperature & % Relative Humidity Profile, 208 days



Max: 51.6 °C, 73 % RH Median: 22.1 °C, 40 % RH
 Min: 5.3 °C, 15 % RH Avg: 23.6 °C, 39 % RH

Two Biomatrica SampleGard plates were "shipped" back and forth between MD and CA during the Summer of 2007.

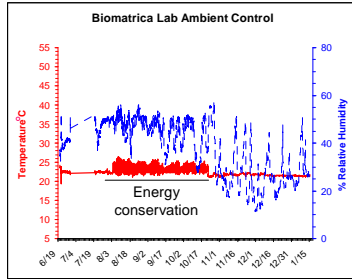
After 6 cross country trips the plates were placed in a car trunk for 14 days.

Two more cross country trips.

Exposed to ambient attic temperatures for 56 days.

Finally plates were placed at lab ambient conditions.

Lab Ambient Temperature & % Relative Humidity Profile, 208 days



Two Biomatrica SampleGard plates stored in a office/lab during the Summer of 2007. Materials were transferred to the lab when it became apparent that summer energy conservation efforts were measurable.

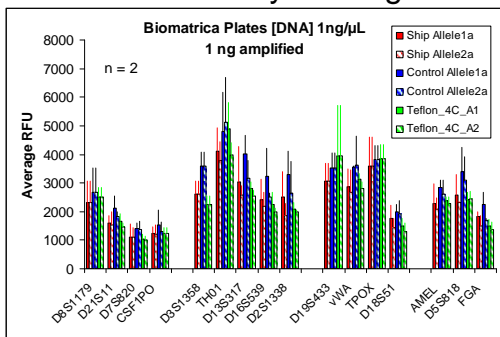
Max: 26.4 °C, 58 % RH
 Min: 19.4 °C, 11 % RH
 Median: 22.2 °C, 41 % RH
 Avg: 22.4 °C, 38 % RH

208 Day Quantitation Results

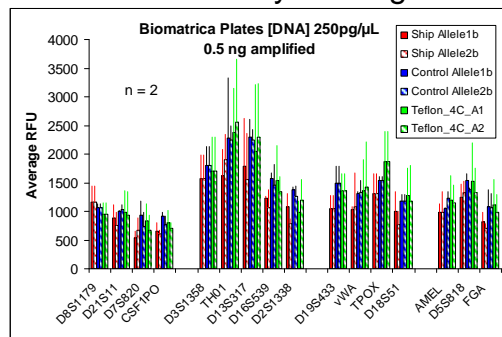
n=4	[DNA] ng/μL	Shipped Stressed	Lab ambient	4 °C Teflon
a	1	0.65 ± 0.06	0.69 ± 0.03	1.01 ± 0.02
b	0.25	0.18 ± 0.03	0.20 ± 0.01	0.30 ± 0.01
c	0.05	0.04 ± 0.00	0.04 ± 0.06	0.05 ± 0.00

QFiler results run on a 7900 HT qPCR instrument SRM 2372 Component A used to establish the calibration curve. The decrease in the DNA concentration of the materials added to the Biomatrica SampleGard plates had been noted earlier, based on genotyping results obtained from Identifier. This quantitation data **may be** influenced by the color present in the Biomatrica samples.

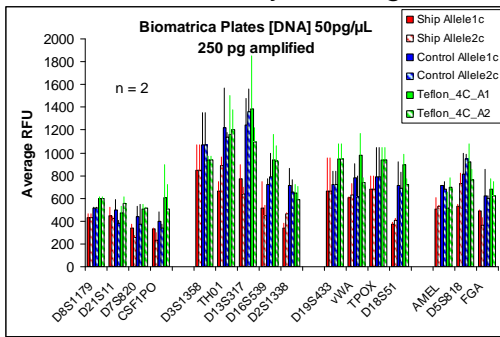
Identifier results [DNA] 1 ng/μL after 208 days storage



Identifier results [DNA] 0.25 ng/μL after 208 days storage



Identifier results [DNA] 0.05 ng/μL after 208 days storage



Identifier results

- Data from Lab ambient control SampleGard plate is in agreement with the data obtained from the 4 °C Teflon controls at 208 days.
- Data obtained from Shipped/Stressed SampleGard plate is similar to, but with slightly lower rfu's, than the data from the Lab ambient control SampleGard plate at 208 days.
- All samples gave full Identifier profiles above 200 rfu's even for the 250 pg samples amplified.

Additional information Teflon extracts

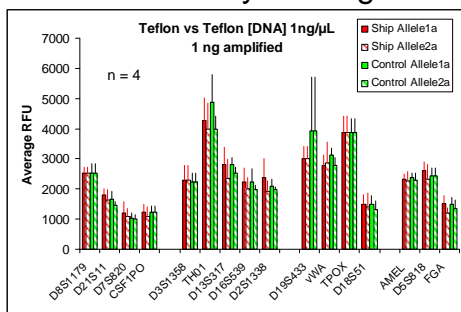
- On August 26, 2007, 100 μL aliquots from the Control 4 °C Teflon containers were removed and placed in sterile labeled Teflon vials.
- These vials stored with the “shipped/stressed” Biomatrixa SampleGard plates.
- The “shipped/stress” box placed in an attic for 8 weeks then moved to Lab ambient temperature.
- At analysis time the Teflon vials were stressed for 147 days out of the total 208 days.

147 Day Quantitation Results

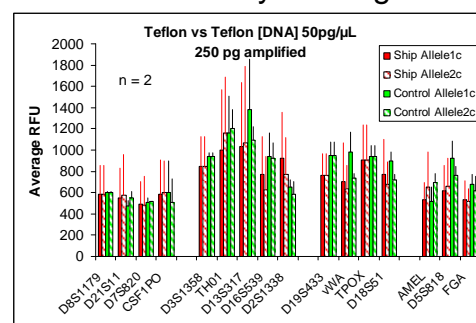
	[DNA] ng/ μL	Shipped Stressed	Lab ambient	Shipped Teflon (147 days)	4 °C Teflon
a	1	0.65 \pm 0.06	0.69 \pm 0.03	1.00 \pm 0.02	1.01 \pm 0.02
b	0.25	0.18 \pm 0.03	0.20 \pm 0.01	0.25 \pm 0.02	0.30 \pm 0.01
c	0.05	0.04 \pm 0.00	0.04 \pm 0.06	0.04 \pm 0.01	0.05 \pm 0.00

QFiler results from a 7900 HT qPCR instrument
 SRM 2372 Component A used to establish the calibration curve.

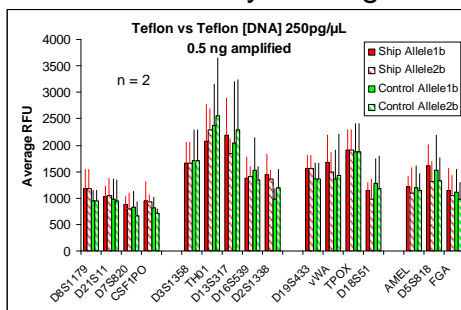
Identifiler results [DNA] 1 ng/ μL after 147 days storage.



Identifiler results [DNA] 0.05 ng/ μL after 147 days storage



Identifiler results [DNA] 0.25 ng/ μL after 147 days storage



Teflon vs Teflon results

- The shipped/stressed DNA in the Teflon vials after 147 days quantified and amplified the same as the 4 °C Teflon containers that were stored for 208 days.
- Highly purified DNA seems to be extremely stable.



Thank You for Your Attention...

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