Stability Studies for DNA in Bloodstains and as Extracted Material

Margaret Kline
Research Biologist, Applied Genetics Group

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Why do we care about the stability of DNA?

There’s two sides of that coin

Find the guilty

Protect the innocent
Cold-case unit: DNA solves 1991 homicide: American Canyon Eagle napavalleyregister.com/.../cold-case...dna-solves.../article_8eb2e0d0-...
Jun 29, 2012 – DNA evidence has solved a “cold” case — a 1991 homicide in which ... Lantz was identified by DNA found on the handle of the knife used to kill ...

DNA solves 13-year-old cold case murder mystery | ksl.com
www.ksl.com/?nid=148&sid=18133538
Nov 17, 2011
DNA solves 13-year-old cold case murder mystery. By Sandra Yi.
November 17th, 2011 @ 10:07pm. This ...

More videos for cold cases solved through dna »

Cold Case Investigations and Forensic DNA | National Institute of ...
nij.gov/topics/forensics/investigations/cold-case/
Jul 16, 2012 – Overview of Using DNA to Solve Cold Cases. Experience has shown that cold case programs can solve a substantial number of violent crime ...

40 Years Later, LBPD Solves City’s Oldest Cold Case Homicide
www.lbpost.com › News
by Sarah Bennett - More by Sarah Bennett
Aug 28, 2012 – Police said that the 40 year-old murder is the Department’s oldest cold case homicide solved to date. Using modern DNA-testing methods paid ...

55 years

1957 cold case solved, man convicted for murder of 7-year-old
www.heraldextra.com › News › Latest National News
Sep 15, 2012 – 1957 cold case solved, man convicted for murder of 7-year-old ... him in one of the oldest unsolved crimes to make it to trial in the U.S. ...
DNA Frees Innocent Man. But What About Eyewitnesses...
news.discovery.com › Human News
May 7, 2010 – An innocent man was recently freed by DNA testing; but what about the people who saw him do it?

DNA evidence frees innocent man 24 years later - New York Daily...
articles.nydailynews.com/.../33406831_1_nina-morrison-dna-eviden...
Aug 26, 2012 – A Texas man who spent half his life behind bars for a crime he did not commit is finally free. David Lee Wiggins, 48, was released from prison ...

New DNA testing frees convicted Colorado rapist, killer - U.S. News
usnews.nbcnews.com/.../11466476-new-dna-testing-frees-convicted...
Apr 30, 2012 – Update: A man sentenced to life in prison for the rape and killing of a Colorado woman was freed on Monday based on advanced DNA testing ...

Innocent man accused of rape freed by DNA after 20 years | NOLA ...
www.nola.com › ... › Breaking News
Apr 27, 2012 – It was the first exoneration under the Orleans Parish Post-Conviction DNA Evidence Project in its two-year existence.

DNA frees innocent man after 30 years | News24
www.news24.com/.../DNA-frees-innocent-man-after-30-years-20110...
Jan 4, 2011 – Dallas - Prosecutors declared a Texas man innocent on Monday of a rape and robbery that put him in prison for 30 years, more than any other ...

Innocent Man Free After 35 Years - DNA evidence clears him of rape ...
Dec 17, 2009 – (Newser) – James Bain left prison today after serving 35 years for a crime he didn’t commit. The 54-year-old Florida man, cleared by DNA ...

Michael Morton Goes Free After Nearly 25 Years in Prison...
abcnews.go.com › US
Oct 4, 2011 – Man Shot in the Head Awakens From Coma. ... Michael Morton Set Free After Spending Nearly 25 Years in Prison, Exonerated by DNA Evidence for His ... The
Considerations for DNA Stability studies?

• What is the form of your DNA?
  – Bloodstain or Buccal swab
  – Extracted liquid in a tube
  – Extracted and dried in a tube or as a stain
  – Stabilized with a matrix/additive
Considerations for DNA stability studies?

• What are the best conditions for storage?
  – Room temperature?
  – Refrigerated?
  – Frozen?

• How long you can keep a sample under specified storage conditions?
  – Days?
  – Months?
  – Years?
Considerations for DNA stability studies?

• What environmental factors influence stability?
  – Temperature excursions
    • Power failures
    • Shipping/Transporting the samples

• Does the sample need to stay pristine, or can you tolerate some degradation?

• What are you measuring and how do you rate the results, DNA Quality/Quantity or both?
So what metric are you going to use?

Since our bottom line is can you get an STR profile?

We can take the electropherogram and plot allele 1 vs allele 2 peak heights.

Or the results of matrix 1 vs matrix 2.
Dried Bloodstains

• Blood from 2 different donors

• Storage Media
  ✓ FTA paper
  ✓ 903 paper

• Environmental factors
  ✓ 37 °C (some places this is ambient)
  ✓ Laboratory Room Temperature
  ✓ -20 °C.
DNA/Bloodstain Storage Papers

903
- high-purity cotton linter pulp
- no chemicals added
- DNA not bound to paper

FTA
- high-purity cotton linter pulp
- chemically treated with several compounds designed to kill pathogens and resist bacterial growth and DNA degradation Tris, EDTA, SDS, and uric acid
- DNA binds to paper
Quality of the Extracted DNA

L ladder with 250 bp, 400 bp, 800 bp and 1500 bp bands visible
Lanes 1, 2: +37°C FTA; Lanes 3, 4: +37°C 903;
Lanes 5, 6: RT FTA; Lanes 7, 8: RT 903;
Lanes 9, 10: -20°C FTA; Lanes 11, 12: -20°C 903;

Most STR typing kits have products that are less than 450 bp

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 +37  C Storage
Normal length amplicon typing kit
903 +37 C Storage
Minimum length amplicon typing kit

Alleles dropping out before are recovered.
Comparison of the Peak Heights FTA vs 903

Sample 1

Sample 2

11 year old bloodstains

FTA rfu

903 rfu

-20 C

20

37

x=y

Allelic dropout seen in some of the +37 C stored samples. Those alleles were recovered with minimum length amplicons.
25 year old Bloodstain (1986)
Direct amp kit (903 paper)

Single 1.2 mm punch stored at room temperature
No Extraction
Data from Pete Vallone and Erica Butts
Extracted DNA

• Factors:
  – Liquid or Dried
    • Storage Media
      – Tube type (liquid)
      – Storage Paper-Matrix (dried)
    • Storage temperature
    • Environmental excursions (planned or unplanned)
DNA sticking and releasing from tube walls

Each line represents an SRM 2391b component and the points along the lines (open circles) correspond to DNA concentrations obtained in this analysis. Several components (2, 8, 9, and 10) were approaching the original nominal DNA concentration of 1 ng/µL at the last time point of 4 days.

DNA stored in tubes for 5 years at -20 °C then moved to 4 °C
6 year Extracted DNA Stability in PFA Tubes

Data from DNA extracts stored in PFA tubes at -80 °C, 4 °C and room temperature for 6 years.

Each storage temperature had had 3 DNA concentrations neat, 1→5, 1→10 dilutions.

Results are the qPCR Cts. Error bars representing 2 sd.

There is no difference as a result of temperature storage after 6 years.
Study Design with Additive

• Stored DNA extracts with preservation additive. Four 96 well plates prepared
  – 3 different concentrations
  – 1 blank (no DNA)

  • Plates were labeled A, B, C, and D.
  • Plates A and C stay at NIST
  • Plates B and D are shipped/stressed.
  • Temperature and Humidity dataloggers are stored with the plates.

• Control DNA without additive stored in the PFA containers at 4°C.
“Shipped/Stressed” Temperature & % Relative Humidity Profile, 208 days

Two plates with additive 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.

Followed by exposure to ambient attic temperatures for 56 days.

Finally plates were placed at lab ambient conditions.

Max: 51.6 °C, 73 % RH  Median: 22.1 °C, 40 % RH  Min: 5.3 °C, 15 % RH  Avg: 23.6 °C, 39 % RH
## 208 Day Quantitation Results

<table>
<thead>
<tr>
<th>[DNA] ng/µL</th>
<th>Shipped</th>
<th>Stressed</th>
<th>Lab ambient</th>
<th>Shipped PFA (147 days)</th>
<th>4 C PFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>1</td>
<td>0.65 0.06</td>
<td>0.69 0.03</td>
<td>1.00 0.02</td>
<td>1.01 0.02</td>
</tr>
<tr>
<td>b</td>
<td>0.25</td>
<td>0.18 0.03</td>
<td>0.20 0.01</td>
<td>0.25 0.02</td>
<td>0.30 0.01</td>
</tr>
<tr>
<td>c</td>
<td>0.05</td>
<td>0.04 0.00</td>
<td>0.04 0.06</td>
<td>0.04 0.01</td>
<td>0.05 0.00</td>
</tr>
</tbody>
</table>

The decrease in the DNA concentration of the materials added to the plates with additive had been noted earlier, based on the genotyping results obtained the quantification data **may be** influenced by the color present in the additive.
PFA Stored at 4°C versus PFA Shipped

Blue symbols are from 4°C.

Red Symbols from harsh “ambient” conditions.
So you want harsher conditions?

Or DNA extracts dried on paper?

OK
X Ray screening of DNA Samples?

X Ray Package screening machines are found in more places than airport security lines these days. It’s “rumored” that shipping companies such as UPS and FedEx randomly screen shipped packages.

Could shipping DNA/evidence to other laboratories be a problem?

When we are conducting interlaboratory studies are the results bias due to shipping conditions?
Study High Dose Irradiation (Sterilization)

- 5 MeV industrial x-ray beam
- Alanine dosimeter
  - (NIST reference class)
- Magazine Container
  - Dose (91 ±4) kGy
  - Max temp (57.5 ±1) °C
- Letter Mail Container
  - Dose (87 ±4) kGy
  - Max temp (62.5 ±1) °C

Sample Packages were at these temperatures for 20 to 30 minutes.
Experiment Parameters

• DNA extracts at 2 concentrations
  ✓ with and without stabilizer
• Stored in 3 different Tubes types
  ✓ with and without stabilizer
  ✓ Stored as liquid or dried
• Stored as “Stains” on paper:
  ✓ FTA:
  ✓ 903: with and without stabilizer
One set of Irradiation Study samples
Treatments

- **Control**: Stored 4 °C
- **Lab Ambient**: Stayed in the lab until processed
- **Shipped**: Shipped UPS, returned UPS (diagnostic x-rayed) 15 days in transit to and from (Washington State Patrol)
- **X Rayed**: Shipped UPS, X-rayed when received, returned UPS 7 days in transit (Evidence Control Unit FBI, VA)
- **High-Dose Sterilization X Ray**: U.S. Postal Service Government Mail
Flash Gel Results
Post Treatment

Tube A

Tube B

Tube C
The peak heights of allele 1 are plotted on the X-axis.
The peak heights of allele 2 are plotted on the Y-axis.
Error bars span from the minimum to the maximum.
The large symbols represent the average over all loci.
Tube B Sample Results

14% volume loss
Tube B, Lab Ambient

33% volume loss
Tube B, Shipped

15% volume loss
Tube B, X-rayed

The peak heights of allele 1 are plotted on the X-axis.
The peak heights of allele 2 are plotted on the Y-axis.
Error bars span from the minimum to the maximum.
The large symbols represent the average over all loci.
The peak heights of allele 1 are plotted on the X-axis.
The peak heights of allele 2 are plotted on the Y-axis.
Error bars span from the minimum to the maximum.
The large symbols represent the average over all loci.
High Irradiation Sample Results

The peak heights of allele 1 are plotted on the X-axis. The peak heights of allele 2 are plotted on the Y-axis. Error bars span from the minimum to the maximum. The large symbols represent the average over all loci.

No full profiles were obtained - only a few scattered alleles amplified.
Extracted DNA on paper

FTA: 30 µL of 2 ng/µL extracted DNA spotted

903: 30 µL of 2 ng/µL extracted DNA spotted

903-BM: 30 µL of 2 ng/µL extracted DNA spotted with stabilizer added

2 mm punches (≈1.6 ng) amplified in 25 µL reaction volume.

Error bars span from the minimum to the maximum

The large symbols represent the average over all loci
Bottomline

- Tube packaging makes a difference
- Stabilizer doesn’t hurt, may help
- Getting a typing result from paper stain requires more DNA than you think
- Low dose X Ray doesn’t hurt
- High dose X Ray destroys
Technical Working Group on Biological Evidence Preservation

A partnership between the National Institute of Standards and Technology, Law Enforcement Standards Office and the National Institute of Justice, Office of Investigative and Forensic Sciences
The NIST/NIJ Technical Working Group on Biological Evidence Preservation (TWGBEP) is charged with: creating best practices and guidance to ensure the integrity, prevent the loss, and reduce the premature destruction of biological evidence after collection through post-conviction proceedings.
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NIST Team for This Work

Group Leader

John Butler  Becky Hill  Erica Butts  Pete Vallone  Dave Duewer

Contact Info:

margaret.kline@nist.gov
301-975-3134

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