To Freeze or Not to Freeze?: Science-Based Guidance for Preserving Biological Evidence

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*National Institute of Standards and Technology Gaithersburg, MD
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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

Inaugural meeting took place in August 2010

Broad goal to “establish proper collection, storage, and preservation techniques throughout the forensic science disciplines”
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.
20 TWGBEP Members

Sue Ballou, Program Manager, Forensics Program, NIST
Phylis Bamberger, Retired Judge, Taskforce for Wrongful Convictions, New York State Bar Association
Rebecca Brown, Police Advocate, Innocence Project
Larry Brown, Property Manager, Los Gatos/Monte Sereno PD
Dennis Davenport, Senior Crime Scene Investigator, Commerce City PD
Rock Harmon, Consultant, DNARock
Cynthia Jones, Associate Professor of Law, American University
Ralph Keaton, Executive Director, ASCLD/LAB
William Kiley, IAPE
Margaret Kline, Research Biologist, NIST
Karen Lanning, Unit Chief, Evidence Control Unit, FBI

Gerry Laporte, Program Manager, Office of Investigative and Forensic Sciences, NIJ
Joe Latta, Exec. Director, IAPE
Linda Ledray, Director, Resource Center, SANE-SART
Randy Nagy, VP Sales and Marketing, Bode Technology
Brian Ostrom, Criminalist, Portland Metro Forensic Laboratory
Yvette Burney, Commanding Officer, Scientific Investigation Division, LAPD
Lisa Schwind, Unit Head, Forensic Service and Education, Office of the Public Defender, State of Delaware
Stephanie Stoiloff, Commander, Forensic Services Bureau, Miami-Dade Police Department
Shannan Williams, Associate, Forensics Program, NIST/OLES
Initial Observations

Evidence can be found in a variety of places e.g.:

- Law Enforcement Property/Evidence Rooms
- Laboratory Property/Evidence Rooms
- Court Property/Evidence Rooms
- Prosecutor’s Offices, Detective’s Desk drawer,…
- Hospitals

Finding:
- Better methods need to be established to be able to track evidence across the different agencies.
Handbook on Biological Evidence Preservation

Audience

– All handlers of biological evidence (emphasizing property and evidence custodians)
– Challenge to encompass small to large agencies

Major Sections

– Packaging and Storage
– Tracking and Chain of Custody
– Disposition
Handbook on Biological Evidence Preservation

Packaging and Storage Section

• Identifies methods and procedures for the proper packaging and storage of biological evidence

• Specifies storage conditions for temporary and long term storage of all types of biological evidence (wet, dry, etc.)

• Findings are based in scientific studies and collective expertise of the working group
Handbook on Biological Evidence Preservation

Tracking and Chain of Custody Section

- Explains the importance of chain of custody
- Describes the basic requirements of an effective tracking system
Disposition Section

- Provides guidance for the length of retention of evidence based on crime categories and cases statuses (open, charges filed, adjudicated, unfounded/refused/denied)
- Explains step-by-step guide for evidence disposition and purging
- *Disposition* is the ongoing process of determining what to do with evidence in a case. The process includes retention, destruction, auction, or return to owner.
## Temporary Storage

<table>
<thead>
<tr>
<th>Type of Evidence</th>
<th>Frozen</th>
<th>Refrigerated</th>
<th>Temperature Controlled</th>
<th>Room Temp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid Blood</td>
<td>Never</td>
<td>Best</td>
<td>≤24 hours</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>Best</td>
<td>≤24 hours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry Biological Stained Items</td>
<td>Best</td>
<td>Acceptable</td>
<td></td>
<td>Acceptable</td>
</tr>
<tr>
<td>Wet Bloody Items (dry ASAP)</td>
<td>Best</td>
<td>Acceptable</td>
<td>≤24 hours</td>
<td></td>
</tr>
<tr>
<td>Bones</td>
<td>Best</td>
<td>Acceptable</td>
<td></td>
<td>Acceptable</td>
</tr>
<tr>
<td>Hair</td>
<td>Best</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swabs with Biological Material</td>
<td>Best (wet)</td>
<td>Best (dry)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal Smears</td>
<td>Best</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feces</td>
<td>Best</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buccal Swabs</td>
<td>Best</td>
<td></td>
<td>≤24 hours</td>
<td></td>
</tr>
<tr>
<td>DNA Extracts</td>
<td>Best</td>
<td></td>
<td>Acceptable (dry)</td>
<td></td>
</tr>
</tbody>
</table>

**DEFINITIONS:**

**Temporary Storage:** refers to the period between the time when an officer submits an item with evidentiary value into a locker or other facility, and the time that it is removed and documented as received into the property room by property room personnel.

**Frozen:** Stored by freezing at a constant temperature at or below –10°C (14°F)

**Refrigerated:** Stored between 2°C (35°F) and 8°C (46°F) with less than 25% humidity

**Temperature Controlled:** Stored between 15.5°C (60°F) and 24°C (75°F) with less than 60% humidity

**Room Temperature:** No humidity control

**Dry:** Evidence that has been fully dried so that no liquid (blood, semen, etc.) can drip from the object that it exists upon.
# Long Term Storage

<table>
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<tr>
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<td>Best</td>
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<td></td>
<td></td>
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<td>Best(liq) → Acceptable(liq)</td>
<td>Acceptable (dry)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## DEFINITIONS

**Long-Term Storage of Biological Evidence:** A long-term storage location must be designated to secure all biological evidence or property items in the custody of the agency for the duration of the time it is held in the property room, until the items are diverted, sold, released, or destroyed.

**Frozen:** Stored by freezing at a constant temperature at or below –10°C (14°F)

**Refrigerated:** Stored between 2°C (35°F) and 8°C (46°F) with less than 25% humidity

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**Room Temperature:** No humidity control
Evidence for the Storage conditions

• Extensive literature search
• Review of the “newer” preservation materials, advances in technology
• Room temperature stability studies maturing
• On going experiments by the NIST Human Identity Project Team members
Partial Reference list


DNA recovered from 10 year old bloodstains

Three different extraction methods used duplicated samples
Stains on 903 paper

<table>
<thead>
<tr>
<th>Sample Storage</th>
<th>Extraction Method A</th>
<th>Extraction Method B</th>
<th>Extraction Method C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab Ambient</td>
<td>52 ng</td>
<td>75 ng</td>
<td>11 ng</td>
</tr>
<tr>
<td>-20 °C</td>
<td>49 ng</td>
<td>43 ng</td>
<td>13 ng</td>
</tr>
<tr>
<td>-80 °C</td>
<td>42 ng</td>
<td>45 ng</td>
<td>9 ng</td>
</tr>
<tr>
<td>Liq N₂</td>
<td>43 ng</td>
<td>37 ng</td>
<td>11 ng</td>
</tr>
</tbody>
</table>
# Quality of the Extracted DNA

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Type</th>
<th>Lanes</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>+37°C</td>
<td>FTA</td>
<td>1, 2</td>
<td>37°C FTA</td>
</tr>
<tr>
<td></td>
<td>903</td>
<td>3, 4</td>
<td>903</td>
</tr>
<tr>
<td>RT</td>
<td>FTA</td>
<td>5, 6</td>
<td>RT FTA</td>
</tr>
<tr>
<td></td>
<td>903</td>
<td>7, 8</td>
<td>RT 903</td>
</tr>
<tr>
<td>-20°C</td>
<td>FTA</td>
<td>9, 10</td>
<td>-20°C FTA</td>
</tr>
<tr>
<td></td>
<td>903</td>
<td>11, 12</td>
<td>-20°C 903</td>
</tr>
</tbody>
</table>

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;

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.

Most STR typing kits have products that are less than 450 bp.
FTA – 903 +37 C Storage Idfiler

Allelic dropout

D13S317

D2S1338

CSF
Alleles dropping out with Idfiler amplification are recovered with Minifiler
25 year old Bloodstain (1986)

PowerPlex 18D (903 paper)

Single 1.2 mm punch stored at room temperature

No Extraction

Data from Pete Vallone and Erica Butts NIST Human Identity Project Team members
Data from DNA extracts stored in PFA tubes at -80 °C, 4 °C, and Lab ambient temperature for 6 years. Each storage temperature had three DNA concentrations: neat, 1→5 dilution, and 1→10 dilution. qPCR results of triplicate aliquots are displayed with error bars representing 2 sd. There is no difference as a result of temperature storage after 6 years.
Thank you for your Attention!!

Acknowledgments

NIST Law Enforcement Standards Office
NIJ Office of Investigative & Forensic Sciences
TWGBEP members

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