Analysis of Old Biological Samples: A Study on the Feasibility of Obtaining Body Fluid Identification and DNA Typing Results

T. Spear and N. Khoshkebari, California Criminalistics Institute (CCI), Sacramento, CA

Purpose

In an effort to ascertain the feasibility of successfully analyzing old biological samples from cases that could be more than 10 years old in the "Cold Hit" program (a program funded to find and type biological evidence from suspectless cases in California), we requested that crime laboratories in California send us any old biological samples that they could spare and provide a description of the sample and how it had been stored. Most of the samples that were sent to CCI had been obtained from laboratory staff members. These samples had been used as standards, training samples or experimental samples. Since these samples were not originally designed for use in this study, the information on the storage conditions is somewhat incomplete. For example, standards or training samples which were described as being held frozen would probably have been taken in and out of laboratory freezers numerous times. In addition, there is obviously some uncertainty about the storage conditions of samples which were described as being held frozen, refrigerated and at room temperature since there is no record of how long the samples were in each of these three different storage conditions. This is an inherent limitation of this type of study but may reflect some of the conditions that evidence samples experience.

Samples obtained from crime labs in California were extracted and amplified for Profiler Plus loci in Spring 2001 by CCI staff. The following details the findings of this study.

Sample Description

1) Sample description, storage conditions and preparation:

a) Samples were obtained from California crime laboratories. Typically, these samples were obtained from staff members and had been used for standards in tests, experiments or training samples.

b) The range of samples included: blood, neat and mixed semen stains, aspermic semen stains, saliva, and semen free vaginal swabs

c) Storage conditions included: (1) frozen, (2) combination frozen, refrigerated and room temperature, (3) room temperature, and (4) outside in Redding, California (shielded from the sun)

d) A ½ cm X 1 cm cutting was taken from each of these samples and extracted with 100 ul of deionized water for a minimum of 30 minutes. The substrate was then placed into a spin basket and centrifuged for 5 minutes to obtain the sample used for body fluid identification tests. This same cutting was used in a standard phenol-chloroform extraction for DNA.

Table #1. This table displays the types of samples that were tested with the body fluid identification tests and then typed with the Profiler Plus reagent kit:

Sample Identifier	Body Fluid Type	Substrate	Stain Made	Storage
C-1	Blood	Gauze	4/23/1975	Freezer/Refrig/RT
C-2	Blood	Gauze	4/23/1975	Freezer/Refrig/RT
C-3	Blood	Gauze	4/23/1975	Freezer/Refrig/RT
C-10	Mixed Semen	Cloth	Aug-85	Freezer
C-11	Mixed Semen	Cloth	Jun-81	Freezer
R-7	Saliva	Gauze	Jul-88	Freezer
R-8	Saliva	Gauze	Feb-92	Freezer
R-9	Semen	Gauze	Dec-85	Van/variable
R-10	Semen	Stain Card	April-89	Van/variable
S-2	Semen	Tissue paper	1992	In & out Freezer
S-3	Semen	Cloth	1990	In & out Freezer
F-1	Semen-free Vag. Swabs	Swab	12/9/1993	Freezer
F-2	Semen	Filter Paper	6/13/1990	Freezer
A-1	Semen	Cloth	Dec.-1952	R.T.
A-2	Semen	Cloth	Nov.-1979	R.T.
A-3	Aspermic Semen	Gauze	Jun-99	Freezer
Fr-1	Blood/mixed	Cloth	10/2/1974	R.T.
Fr-2	Blood	Cloth	10/2/1974	R.T.
Fr-3	Semen	Cloth	3/12/1975	Frozen or Refrig.
Fr-4	Aspermic Semen	Cloth	7/13/1982	Frozen or Refrig.
Red-1	Blood	Stain Card	2/26/1990	Outside/Redding
Red-2	Blood	Gauze	Jan-1988	Frozen

Table #2. This table shows samples that were only analyzed by body fluid identification tests since there was little question that they would yield DNA typing results:

Sample Identifier	Body Fluid Type	Substrate	Stain Made	Storage
C-4	Blood	Stain Card	May 1992	Freezer
C-5	Blood	Stain Card	May 1992	Freezer
C-6	Blood	Stain Card	May 1992	Freezer
R-1	Blood	Gauze	July 1999	Freezer
R-2	Blood	Gauze	Sept 1999	Freezer
R-3	Blood	Gauze	March 2000	Freezer
R-4	Saliva	Gauze	March 2001	Freezer
R-5	Saliva	Gauze	Nov. 1996	Freezer
R-6	Saliva	Gauze	March 1997	Freezer
S-1	Semen	Cotton Cloth	1992	In & out of freezer
S-4	Semen	Cloth	1999	In & out of freezer

R.T. = Room Temperature

2) Body Fluid Identification Test Results

a) Blood

i) Presumptive chemical tests: All the bloodstains in this study gave positive results with a leucomalachite test reagent when the stains were tested **directly**. The older bloodstains in this study (C-1, C-2, C-3 and Fr-2) were very insoluble and produced very weak or **negative** Hemastix results.

ii) Species tests by cross-over electrophoresis: Negative tests results were obtained for the following five bloodstains (which were more than twenty-five years old): C-1, C-2, C-3, Fr-1 and Fr-2. In general, species tests conducted by cross-over electrophoresis produced more positive results than by the double diffusion (Ouchterlony) method.

b) Semen

i) Acid phosphatase and P30 tests: Of the 14 semen stains tested, 12 out of 14 stains (86%) produced strong, positive test results for acid phosphatase (including a semen stain almost 50 years old!). These same stains also produced a precipitin band in a P30 crossover immunological test. The two semen stains that gave a very weak (inconclusive) AP result and no clear P30 precipitin band were the Fr-3 stain which had been made in 1975 and stored frozen or refrigerated and the R-10 stain which had been maintained outdoors in a van and then stored under variable conditions.

c) Saliva

i) Amylase diffusion test results: All five of the saliva stains (R-4, R-5, R-6, R-7 and R-8) produced a cleared circle with a diameter that exceeded the 1:100 saliva standard dilution.

3) DNA typing results

a. **Full profiles (10 loci) were obtained from 18 of the 22 samples (82%) (e.g. blood, saliva, semen and vaginal samples) as follows:** C-1, C-2, C-10, C-11, R-7, R-8, R-9,R-10, S-2, S-3, F-1, F-2, A-2, A-3, Fr-2, Fr-4, Red-1 and Red-2.

b. **Partial profiles [types were obtained at most loci] were obtained from 2 of the 22 samples (9%) (e.g. one semen and one bloodstain):** (a) Fr-1 [bloodstain made in 1974 and held at room temperature] and (b) Fr-3 [one semen stain made in 1975 and held under a variety of conditions].

c. **Only the amelogenin locus, which is the smallest locus of this multiplex, was noted in 2 of the 22 samples (9%):** (a) A-1 [semen stain made in 1952 and held at room temperature] and (b) C-3 [bloodstain made in 1975 and held frozen/refrigerated or at room temperature].

Discussion

1. Most samples [82%] from this study yielded full profiles. Given the range of ages of these stains and the various storage conditions in which these stains were maintained, it is clear that DNA is a very stable molecule and it is quite possible to obtain a complete DNA profile from samples that are quite old. Full profiles (10 loci) were obtained on samples that were more than 25 years old and which were described as being stored at room temperature.

Of the 18 stains that showed a complete profile, 8 stains (44%) did **not** display a reduction in the amount of DNA in the larger loci ("wedged shaped" profile). These samples were: C-10, C-11, R-8, F-1, F-2, A-3, Fr-4 and Red-2. These stains ranged in age from 8 to 20 years old. **All eight of these samples had been held frozen.** This was an indication that frozen storage preserved biological samples better than a combination of storage conditions (frozen, refrigeration & room temperature) or holding a dry biological sample at room temperature.

The other ten stains (C-1, C-2, R-7, R-8, R-9, S-3, A-2, Fr-2, Fr-3, and Red-1] all showed a reduction in the amount of DNA at the larger loci. This group included a semen stain (S-3 from 1992) and a saliva stain (R-7 from 1988) that had been used as standards/training samples and **had** been taken in and out of the freezer. A pronounced decrease of DNA at the large loci was also noted in the following samples: three bloodstains (C-1, C-2, and Fr-2). Finally, a bloodstain (Red-1 from 1990), which had been held outside in Redding and a semen stain (A-2 from 1979) held at room temperature showed an obvious reduction in the amount of DNA at the larger loci.

2. Partial profiles were obtained from 2 of the 20 samples studied. These two samples (Fr-1 and Fr-3) displayed information at **most** of the loci and both profiles were still quite informative. It is interesting that partial profiles were obtained from one sample in a group of similar samples (described as being stored in an identical fashion) that yielded full profiles. Thus, for example, there were three bloodstains made in 1975 that were described as being held frozen, refrigerated and at room temperature. Two of those three samples yielded full profiles while the third bloodstain only showed one locus (amelogenin).

3. Only the amelogenin locus was obtained from 2 of the 20 analyzed in this study. These 2 samples (A-1 and C-3) showed information at only the smallest locus in the multiplex. The A-1 sample was a semen stain from 1952 that was maintained at room temperature. The C-3 sample was a bloodstain made in 1974 and held in a combination of storage conditions (e.g. frozen, refrigerated, and room temperature).
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Summary

On **most** samples, it was still possible to obtain positive body fluid identification test results using various body fluid identification tests (e.g. presumptive tests for blood and semen, immunological tests and amylase diffusion). Two semen stains (R-10/made in 1989 and Fr-3/made in1975) did not give a positive AP or P30 test. A few of the very old bloodstains (25 years or older) gave weak or negative Hemastix test results. The most problematic test (i.e. the test producing the greatest number of false negative test results) in this study was the species test. The failure to obtain positive species results on some of the bloodstains tested was likely a result of the fact that these samples were very old and thus very insoluble. It is possible that had the stain extraction time been extended, more positive results would have been obtained.

The majority of the stains in this study yielded very informative DNA typing profiles when amplified by the Profiler Plus reagent kit. Most of the samples analyzed in this study had been stored for at least some time period in the freezer. It was clear that **samples did not have to be stored frozen in order to obtain a DNA result.** [Given, that a full profile was obtained on a bloodstain held at room temperature for more than 25 years.] **However, there was also some information to indicate that samples benefited from frozen storage since the only samples that did not display a decrease in the DNA at the larger loci were the samples that had been held frozen.** All the samples that displayed a wedge shaped profile (e.g. a decrease in DNA at the large loci) also showed very degraded DNA when run on a yield gel **and yielded a relatively minimal amount of extracted DNA** (compared to similar sized samples of the same body fluid type). It is not surprising that samples that showed degraded DNA (and a wedged-shaped STR typing profile) would also yield less total DNA. This would mean that samples which are not well preserved would be more difficult samples to analyzed for at least 2 reasons: (1) possible loss of typing signal at the larger loci and (2) minimal amount of DNA yield making additional or repeat testing difficult. It is clear that DNA that is present in minimal amounts and degraded would complicate the interpretation of evidence stains that frequently contain more than one source of DNA.

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