Sensitivity of DNA Extraction Methods from Different Bodily Fluids for Human Identification

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Abstract

Biological evidence found at a crime scene can provide critical genetic information for crime scene reconstruction by determining if there is a link between sample donors and actual criminal acts. Extracted DNA coupled with detection using different short tandem repeat (STR) systems have become an integrated part of crime scene investigation. Many biological samples are present in the form of biological fluids including blood, saliva, urine, semen and vaginal fluid. The applicability of each of these fluids to human identification could vary greatly. While fluids such as blood contains relatively large amount of cells and hence nucleic acid for genetic identification, some fluids such as urine has very diluted amount of genetic materials for analysis. Moreover, the quality of nucleic acid depends on the degree of degradation of the sample upon sample collection and whether the sample collected is properly preserved, prior to nucleic acid extraction. Finally, different DNA extraction methods may result in different quantity and quality of DNA for analysis. In this study, we attempted to address a number of questions regarding sample collection/preservation as well sample extraction for use of DNA-based human identification including (1) What is the minimal quantity of each bodily fluid sufficient for DNA-based analysis? (2) Which extraction method could provide the most sensitive recovery of DNA from different bodily fluids? (3) Is there a benefit with sample preservation for the bodily fluids collected? DNA was extracted from different volumes of blood, saliva and urine (both preserved and not preserved) using different procedure including both column-based protocol and alcohol precipitation protocol. The extracted DNA was subjected to standard STR analysis (including Promega's PowerPlex systems). While the amount of DNA recovered per volume input was much lower from urine compared to blood and saliva, using a column procedure, successful STR analysis could be achieved with as little as 50 uL of urine input. Both column-based and alcohol precipitation extraction worked well for samples with high DNA content such as blood and saliva. However, the use of resin/-column-based protocol provided much better sensitivity for urine. Finally, the requirement sample preservation is generally preferred, in particular for input rich in nucleases like saliva.

Purpose of the Study

1. Are all different types of bodily fluids including blood, plasma, saliva and urine suitable for use in Human Identification Studies?

2. What is the minimal quantity of each bodily fluid sufficient for DNA-based analysis?

3. Does the extraction method affect the sensitivity of the recovery and quality of DNA from different bodily fluids?

4. Is there a benefit with sample preservation for the bodily fluids collected?

Materials and Methods

Sample Collection and Preservation

Blood Human blood was collected in standard EDTA blood tubes.

Plasma Human blood was collected in standard EDTA blood tubes. Plasma was separated immediately from cellular components by standard centrifugation.

Saliva Human saliva was collected using Norgen's Saliva DNA Collection, Preservation and Isolation Kit (Cat# 35700). All preserved samples were stored at Room Temperature until use.

Urine Human urine was collected using Norgen's Urine Collection and Preservation Tubes (Cat# 18111). All preserved samples were stored at Room Temperature until use.

DNA Isolation

Blood Blood DNA was isolated using either Norgen's Blood Genomic DNA Isolation Mini Kit (Cat# 46300) or an equivalent product from Qiagen (silica spin columns).

Plasma Plasma DNA was isolated using Norgen's Plasma/Serum Circulating DNA Purification Mini Kit (Slurry Format) (Cat# 50600) (silicon carbide slurry).

Saliva Saliva DNA was isolated using Norgen's Saliva DNA Collection, Preservation and Isolation Kit (Cat# 35700) (alcohol precipitation).

Urine Urine DNA was isolated using Norgen's Urine DNA Isolation Kit (Slurry Format) (Cat#48800) (silicon carbide slurry).

End-point and Quantitative PCR for Genotyping or Detection of Human DNA

PCR was performed using Norgen's 2x PCR Master Mix (Cat# 28007) for various human gene products as indicated in the Results

Human Identification using Short Tandam Repeats (STRs) Detection

Isolated DNA was assayed for human STRs using Promega's PowerPlex® 1.1 and 2.1, PowerPlex® 16 HS System, as well as Promega's PowerPlex® Y23 System.

Results

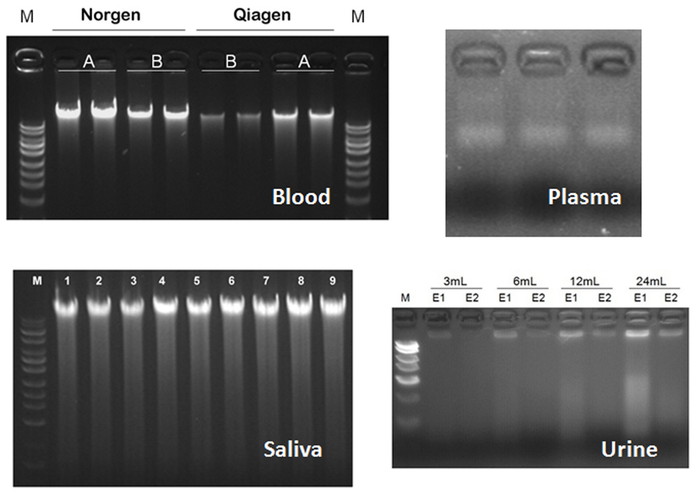

Figure 1. DNA Profile and Quantity is Different in Various Human Bodily Fluids

1. High yield and highly intact genomic DNA (gDNA) could be isolated from bodily fluids with higher cell counts such as blood and saliva. Consistently good recovery of DNA could be achieved using different extraction methods including silica column-based methods (shown here for Blood) or alcohol precipitation (shown here for Saliva).

2. Plasma is deprived of cells and hence DNA isolated from plasma is of low quantity and of low molecular weight (circulating DNA, size ~ 1000 bp)

3. Urine DNA consists of both large gDNA (from cells) as well as low molecular weight DNA (circulating DNA, size ~ 1000 bp).

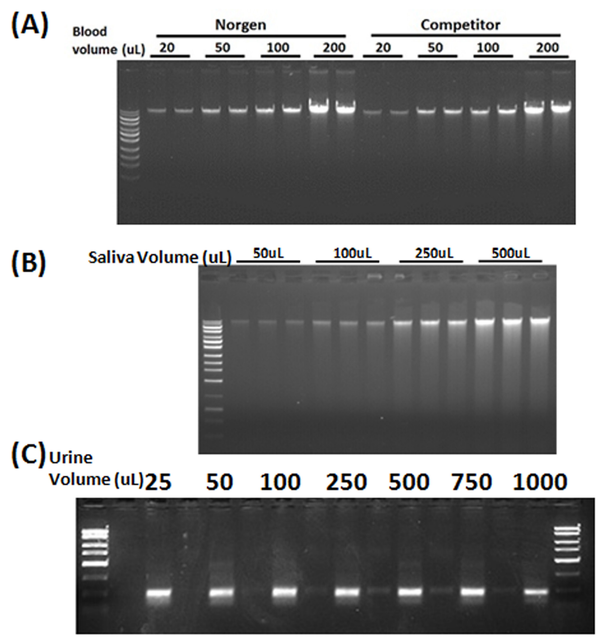

Figure 2. Effective Recovery of High Quality Human DNA from Small Input of Bodily Fluids

Using an appropriate DNA isolation method, a good amount and a good quality DNA could be recovered:

1. Using silica column-based methods, Genomic DNA was effectively recovered from as little as 20 µL of blood (equivalent to a tiny blood spot) and 50 µL of preserved saliva (equivalent to 25 µL of saliva) (**Panel A and B**).

2. DNA was detected from as little as 25 µL of urine using an optimized extraction method that utilized Norgen's proprietary Silicon Carbide columns. The results presented here was a PCR reaction using Y-chromosome-specific primers. (**Panel C**).

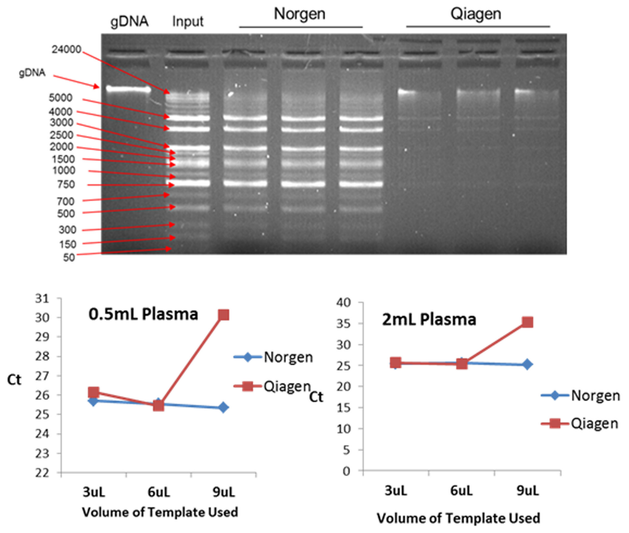

Figure 3. High Recovery of Plasma DNA with Full Size Diversity requires the use of Specialized Method of Norgen's Silicon Carbide Technology

Plasma is drastically different from other bodily fluids due to the lack of cells. The majority of the DNA present in plasma is circulating DNA which is usually small in molecular weight. Hence, an optimized method is required to effectively recover DNA of all sizes.

1. Two commercially available kits, Norgen (using Silicon Carbide slurry) and Qiagen (using standard silica columns) were compared for their ability to recover all sizes of DNA fragments from plasma, using a plasma sample spiked with two DNA ladders. Norgen's kit was found to recover all sizes of DNA (even as low as 50 bps), while Qiagen's kit only successfully recovered high molecular weight bands (4000 bps and above).

2. The use of Norgen's Silicon Carbide in the form of slurry resin also results in an advantage of being able to scale up to large input volume (up to 5 mL plasma)

3. Norgen's plasma DNA isolation method recovered much better quality of DNA. As much as 9 µL DNA could be used in a 20 µL qPCR reaction (5S rRNA) without showing any inhibition.

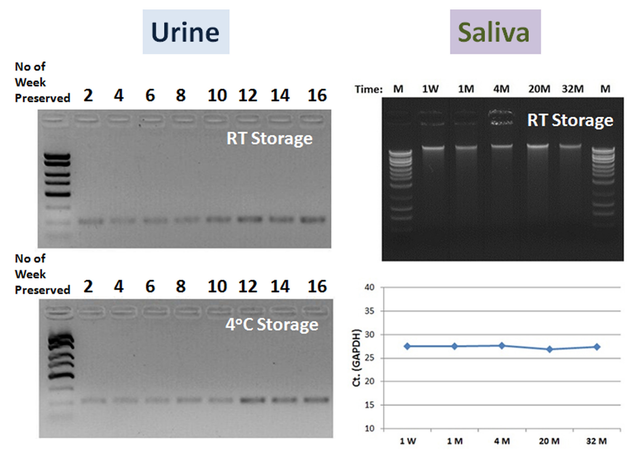

Figure 4. High Yield and Quality DNA recovered from Preserved Bodily Fluids

Bodily fluids are easily degradable due to an abundance of nucleases. The use of preservatives allows proper condition for sample or forensic evidence archiving.

1. Urine collected using Norgen's Urine Collection and Preservation Tubes could be stored either at Room Temperature or 4°C for up to 16 weeks without showing any deficiency of detection of a specific PCR amplicon.

2. Saliva collected using Saliva DNA Collection, Preservation and Isolation Kit could be stored at Room Temperature up to 32 months without showing any gDNA degradation nor any deficiency of detection of a specific PCR amplicon.

	PCR	SNP detection	Genotyping
Blood	100%	98.1%	92.3%
Saliva	100%	100%	96.1%
Urine	97.8%	87%	80%

Figure 5. Urine showed Comparable Applicability to Blood or Saliva for Genotyping or DNA-Based Identification

Blood, Saliva and Urine are collected from 52 individuals. DNA was extracted from each bodily fluids using DNA extraction products from Norgen as described above. The DNA was then subjected to standard PCR detection of the Human CYP2C9 gene (PCR), qPCR detection of the CYP2C9*2 single nucleotide polymorphism (SNP detection), as well as the standard bi-directional sequencing of the CYP2C9*2 region (Genotyping).

1. As expected, both Blood and Saliva DNA showed high success rate of detection. This showed that Saliva, which is collected in a true non-invasive manner, is a highly applicable source of bodily fluid for DNA extraction for human identification.

2. Even with the low cell number and lower DNA yield with respect to Blood or Saliva, Urine can still be used effectively in DNA-Based Identification. In this population study, over 80% of the individuals tested could be accurately genotyped.

(A)

	Subject 1				Subject 2				Subject 3			
STR Locus	Urine	Plasma	Blood	Saliva	Urine2	Plasma3	Blood4	Saliva5	Urine6	Plasma7	Blood8	Saliva9
DYS19	14	14	14	14	-	-	15	15	14	14	14	14
DYS385	17	17	17	17	14,15	14,15	14,15	14,15	17	17	17	17
DYS389 I	13	-	13	13	12	12	12	12	13	13	13	13
DY S389 II	29	-	-	29	-	-	27	27	29	29	29	29
DYS390	24	-	24	24	23	-	23	23	24	-	24	24
DYS391	10	-	10	10	10	10	10	10	10	10	10	10
DYS392	13	-	13	13	-	-	14	14	-	13	13	13
DYS393	12	12	12	12	13	13	13	13	12	12	12	12
DYS437	14	-	14	14	14	-	14	14	14	14	14	14
DYS438	11	11	11	11	9	-	9	9	11	11	11	11
DYS439	11	-	11	11	-	-	12	12	-	-	11	11
DYS448	21	21	21	21	19	19	19	19	21	21	21	21
DYS456	16	16	16	16	16	-	16	16	-	-	16	16
DYS458	18	-	18	18	16	-	16	16	18	18	18	18
DYS481	27	27	27	27	23	23	23	23	27	27	27	27
DYS533	11	-	11	11	11	-	11	11	-	11	11	11
DYS549	12	-	12	12	14	-	14	14	12	12	12	12
DYS570	18	-	18	18	18	18	18	18	18	18	18	18
DYS576	18	18	18	18	18	18	18	18	18	18	18	18
DYS635	21	-	21	21	21	-	21	21	21	21	21	21
DYS643	9	-	9	9	-	-	9	9	-	-	9	9
YGATAH4	10	-	10	10	11	-	11	11	-	-	10	10
Loci Detected	23	9	22	23	18	9	23	23	17	18	23	23

(B)

STR Locus	Urine		Plasma		Blood		Saliva	
Penta E	16	16	-	-	16	16	16	16
D18S51	13	17	-	-	13	17	13	17
D21S11	28	29	-	-	28	29	28	29
TH01	6	9.3	6	-	6	9.3	6	9.3
D3S1358	15	18	15	18	15	18	15	18
FGA	22.2	23	-	-	22.2	23	22.2	23
TPOX	8	8	-	-	8	8	8	8
D8S1179	13	16	-	-	13	16	13	16
vWA	14	17	14	17	14	17	14	17
Penta D	11	13	-	-	11	13	11	13
CSF1PO	10	11	-	-	10	11	10	11
D16S539	10	11	-	-	10	11	10	11
D7S820	8	11	-	-	8	11	8	11
D13S317	11	12	11	12	11	12	11	12
D5S818	11	13	-	-	11	13	11	13
Amelogenin	X	Y	X	-	X	Y	X	Y
Loci Detected	16		4		16		16	

(C)

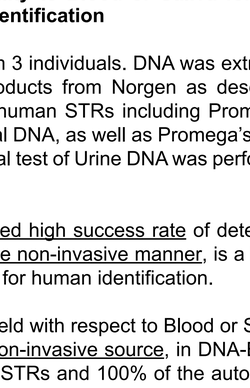

Figure 6. Urine showed Comparable Applicability to Blood or Saliva for DNA Short Tandem Repeats (STRs)-based Human Identification

Blood, Plasma, Saliva and Urine are collected from 3 individuals. DNA was extracted from each bodily fluids using DNA extraction products from Norgen as described above. The DNA was then applied in assays for human STRs including Promega's PowerPlex® 16 HS System (Panel A) for autosomal DNA, as well as Promega's PowerPlex® Y23 System for Y-DNA (Panel B). Additional test of Urine DNA was performed using the PowerPlex® 1.1 and 2.1 (Panel C).

1. As expected, both Blood and Saliva DNA showed high success rate of detection. This showed that Saliva, which is collected in a true non-invasive manner, is a highly applicable source of bodily fluid for DNA extraction for human identification.

2. Even with the low cell number and lower DNA yield with respect to Blood or Saliva, Urine can still be used effectively, also as a true non-invasive source, in DNA-Based Idenfication. In this study, over 80% of the Y-DNA STRs and 100% of the autosomal STRs could be accurately genotyped.

3. On the other hand, due to its cell-free nature, plasma may not be a good input for DNA for Human Identification

Summary

1. Blood, Saliva and Urine could all be used effectively in DNA-based Human Identification assays including STR analysis as well as other genotyping tests. However, the success of the test is highly dependent on the method of DNA extraction. For example, the use of Norgen's silicon carbide technology significantly improved detection using Urine DNA.

2. With the appropriate sample preparation method, DNA-based Human Identification tests could be carried out using a minimal amount of bodily fluids input. For example, DNA isolated from as little as 25 µL of Blood, Saliva or Urine could be used in different PCR-based detection.

3. Sample preservation is critical for archiving and protecting valuable biological samples. This is particularly important for bodily fluids with high nuclease contents (such as saliva).

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