

# 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 integral 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  $\mu$ L 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

- Are all different types of bodily fluids including blood, plasma, saliva and urine suitable for use in Human Identification Studies?
- What is the minimal quantity of each bodily fluid sufficient for DNA-based analysis?
- Does the extraction method affect the sensitivity of the recovery and quality of DNA from different bodily fluids?
- 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# 45300) 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).

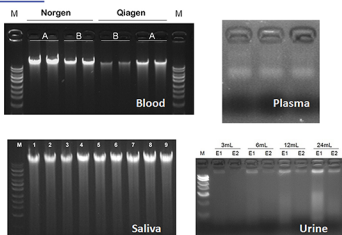
**Urine** Urine DNA was isolated using Norgen's Urine DNA Isolation Kit (Slurry Format) (Cat#45800) (silicon carbide slurry).

**End-point and Quantitative PCR for Genotyping or Detection of Human DNA** PCR was performed using Norgen's 2x PCR Master Mix (Cat# 26007) for various human genetic products as indicated in the Results.

### Human Identification using Short Tandem Repeats (STRs) Detection

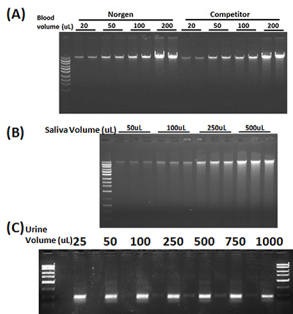
Isolated DNA was assayed for human STRs using Promega's PowerPlex<sup>®</sup> 1.1 and 2.1, PowerPlex<sup>®</sup> 16 HS System, as well as Promega's PowerPlex<sup>®</sup> Y23 System.

## Results



**Figure 4. DNA Profile and Quantity is Different in Various Human Bodily Fluids**

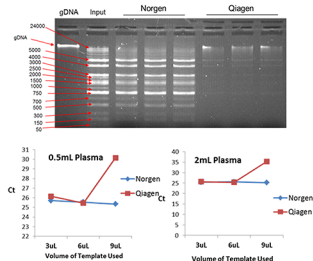
- High yield and highly intact genomic DNA (gDNA)** could be isolated from bodily fluids with higher cell counts such as blood and saliva. Consistently good recovery of DNA could be achieved using different extraction methods including silica column-based methods (shown here for Blood) or alcohol precipitation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

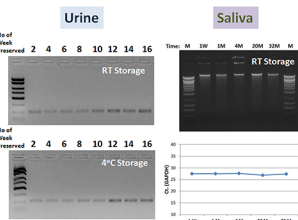
- Using silica column-based methods, Genomic DNA was effectively recovered from as little as **20  $\mu$ L of blood** (equivalent to a tiny blood spot) and 50  $\mu$ L of preserved saliva (equivalent to **25  $\mu$ L of saliva**) (Panel A and B).
- DNA was detected from as little as **25  $\mu$ L of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

- 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).
- 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)
- Norgen's plasma DNA isolation method recovered **much better quality of DNA**. As much as 9  $\mu$ L DNA could be used in a 20  $\mu$ L 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.

- 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.
- 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.

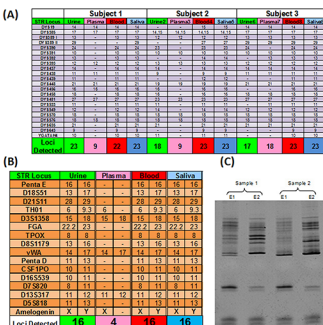
	PCR	SNP detection	Genotyping
<b>Blood</b>	100%	98.1%	92.3%
<b>Saliva</b>	100%	100%	96.1%
<b>Urine</b>	97.8%	87%	80%

**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.



**Figure 6. Urine showed Comparable Applicability to Blood or Saliva for DNA 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<sup>®</sup> 16 HS System (Panel A) for autosomal DNA, as well as Promega's PowerPlex<sup>®</sup> Y23 System for Y-DNA (Panel B). Additional test of Urine DNA was performed using the PowerAmp<sup>®</sup> 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 Identification. 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

- 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.
- 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  $\mu$ L of Blood, Saliva or Urine could be used in different PCR-based detection**.
- 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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