

Saliva: An Expert Witness

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Abstract: *The recent advances in molecular biology have revolutionized all aspects of medical fields. DNA, the language of life yields information beyond our imagination, both in health or disease. DNA is an excellent means for identification of unidentified human remains. It is frequently used to acquire information from biological material to aid investigations associated with criminal offences, disaster victim identification and missing persons. The technical advances in molecular biology have propelled the analysis of the DNA into routine usage in crime laboratories for rapid and early diagnosis. DNA analysis is important for different types of research in biomedical and forensic science, in which DNA isolation is the foremost and a mandatory step. Although DNA may have to be isolated from different sources for forensic purposes such as semen, stains, hair, bones and blood samples, but saliva has long been known for its diagnostic value in several diseases and is a non invasive method which has also a potential to be used in forensic science. So, this paper aims for DNA based analysis from human saliva for identification in the field of forensics*

Keywords: Saliva, DNA, forensic medicine, human identification

1. Introduction

DNA is known as language of life. DNA contains all the information required to copy itself, resulting in genetic information being passed on to the next generation of cells. It contains all the genetic Information of biological material which help to aid in investigations such as criminal offences, paternity testing, disaster victim identification & missing persons.¹

One of the main advantages of DNA use in forensic sciences lies in the fact that it can be extracted from different sources such as blood samples, saliva, mouth mucosa cells (on cigarettes, envelopes, etc.), bone, teeth, tissues, organs, hair strands, semen, urine, faeces, sweat, prints and other biological materials.² DNA isolation is an important first step in DNA analysis for biomedical and forensic purposes.

Saliva is a very significant sample in many forensic science cases example:-

- Traces of saliva on the dress
- Envelope
- Crime spot
- Cigarette butts
- Human skin through licking, biting, kissing³

Saliva is very good source of human DNA, as the mean number of epithelial cells per 1 mL of saliva is about 4.3×10^5 . Moreover, the turnover of epithelial cells is quite extensive in the mouth as the surface layer of epithelial cells is replaced, on average, every 2.7 hr, suggesting that there is likely to be intact genomic DNA in saliva samples.⁴ The major advantages of saliva over blood when used for diagnostic purposes include easy access, non invasive Collection and better patient/subject compliance.⁵

The conventional nucleic acid isolation techniques have the drawbacks of

- The starting amount of sample
- Sample specificity of a kit

- Yield of DNA

The kit GENEI pure isolation kit requires minimum amount of sample for extraction of DNA from saliva, buccal swab and other saliva stained samples.

So, this present study aims to extract, isolate & visualize the DNA from human saliva for the identification in the field of forensics.

2. Materials and Methods

This study was conducted at the Department of Oral and Maxillofacial Pathology at Manav Rachna Dental College, Faridabad, Haryana.

Saliva samples were collected from 10 non related volunteers after informed consent and approval from Hospital Ethics Committee of Manav Rachna Dental College, Faridabad, Haryana. Saliva was collected by asking the subjects to spit in a sterile test tube, 2 ml of this saliva was transferred to sterile vials, using a sterile pipette, and stored at -20°C until further use. (fig 1)



Figure 1: Saliva sample

The materials required for the following procedure is as follows:-

- 1)Gloves
- 2)Face mask
- 3) **Genei Pure Isolation kit (fig 2)**
- 4) Centrifuge machine (fig 3)
- 5)Dry/Water bath
- 6)Vortex

Absolute ethanol (96-100%)

- 8) Proteinase k
- 9) Distilled water
- 10) 1.5 ml vials, 10ml centrifuge tube
- 11) Micropipette & tips

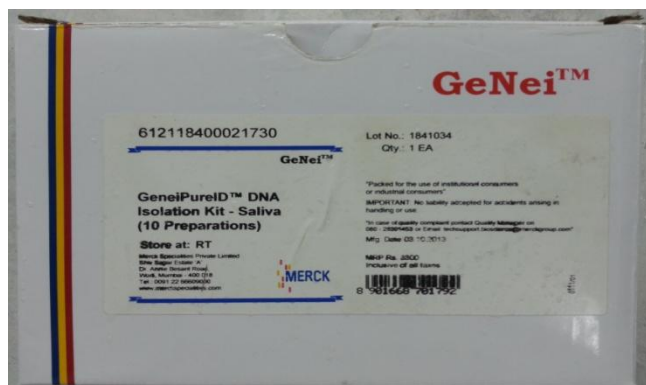


Figure 2: Genei pure DNA isolation kit

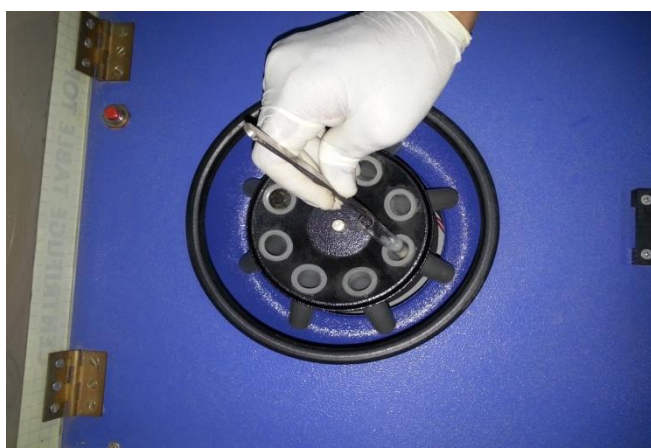


Figure 3: Centrifuge machine
 Genei pure DNA isolation kit includes:

Materials	Quantity
Lysis buffer-I	50ml
Lysis buffer- II	7ml
Wash buffer (concentrate)	2ml
Elution buffer	1.5ml
Spin columns	10 nos
Collection tubes (2ml)	10 nos

The following steps are involved in the DNA analysis and they are as follows:-

- 1) DNA isolation and extraction
- 2) VISUALIZATION
- 3) AMPLIFICATION
- 4) ANALYSIS (Detection & interpretation)

3. Procedure

- 1) **Lysis:** sample+ 5ml lysis buffer I + 700 µl lysis buffer II + 50 µl proteinase k, incubate at 58 ° C for 45 min
- 2) **Binding:** mix with equal volume of ethanol, load to Genei pure spin column, centrifuge at 10000 rpm for 1min
- 3) **Wash:** 700 µl diluted wash buffer, centrifuge at 10000 rpm for 1 min at 25-28° C.
- 4) **Wash:** centrifuge at 10000 rpm for 2 min at 25-28° C.
- 5) **Elution:** 150 µl pre warmed elution buffer, centrifuge at 10000 rpm for 1 min.

After this procedure either DNA finger printing assay or polymerase chain reaction (PCR) can be done for interpretation.

Before interpretation detection of human DNA is done by loading the wells of agarose gel electrophoresis. (fig 4) Human DNA is identified under UV visualization. (fig 5)

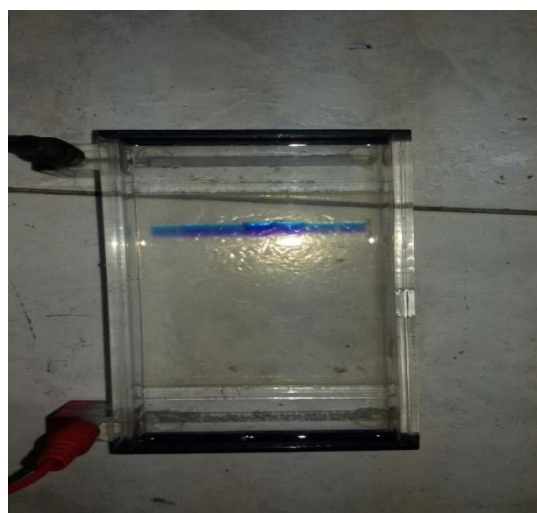


Figure 4: AGAROSE GEL ELECTROPHORESIS



Figure 5: UV visualization

4. Results & Observations

According to molecular weight DNA is classified as:

- High

- Low

High molecular weight DNA is suggestive of human and Low molecular weight DNA is suggestive of Bacterial or viral. In our study the bands which we had analyzed under UV visualization suggestive of high molecular weight which signifies it as a human DNA.

Till UV visualization can be done in a normal lab or college set up, but further, PCR is used to amplify the DNA and with electropherogram the interpretation is done for the matching which is very expensive and specialists are required with well equipped laboratory.⁶

5. Discussion

Forensic science was 1st coined in 1936. In 1868, a biologist named Freidreich Meischer carried out research which indicated that the nucleus of cells contains a material which he called nuclein. It was not until much later, in the 1940s, that deoxyribonucleic acid (DNA) was recognised as the carrier of the genetic code. The DNA structure was determined by James Watson and Francis Crick in 1953. The Watson and Crick model is often described as the DNA ladder.⁷

DNA Fingerprinting was given by Alec Jeffrey's in 1984 and was first used in 1986. DNA fingerprinting is widely used in the human identification. The field of DNA forensics is ever increasing. The uses of DNA in forensics are in Paternity/maternity testing, Linkage of suspects to crime scenes, Identification of Individuals, Missing persons and casualties.⁸

The use of saliva and mouth swabs as sources of DNA shows some technical advantages over the use of blood. Collection is easier and painless, especially considering that it can be done on babies, children and elderly subjects, and does not have the religious implications of using blood. Also, there are higher potential risks of contamination when using blood, especially from hepatitis and AIDS, due to the use of sharp objects such as needles⁹. Forensic studies have demonstrated that DNA analysis using the PCR of saliva deposited in prints, bite marks, cigarette butts, marks and prints left on stamps and other objects may help in individual identification. Exfoliated buccal epithelial cells and other cells found in saliva are a very promising alternative source of DNA.¹⁰ Saliva contains cellular material and thus can be typed by DNA analysis. This can be an alternative to gathering controls by venipuncture. The purpose of this study is to extract, isolate & visualize the DNA from human saliva for the identification in the field of forensics.¹¹ Results of our study show that DNA could be successfully extracted from saliva on the basis of molecular weight we could analyze the human DNA.^{12,13}

This study was a pilot project using a small sample size in order to assess the feasibility of utilizing salivary samples for DNA based genetic analysis, the results show that DNA obtained from salivary samples could be successfully utilized for gene amplification and visualized for human DNA. Hence, saliva probably has a huge potential as a source of DNA evidence in forensics, though improvements

in handling technique and isolation methods are required to increase the yield and quality of DNA from salivary samples.

6. Conclusion

Saliva is a unique fluid which can be utilized for gene amplification and has a optimal source of DNA. Hence, saliva is proved to be a good source of DNA and may be successfully used for forensic purposes.

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