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Short Communication

Standardized protocol for the isolation of DNA from human saliva- a pilot study

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Abstract

This study aimed to standardize the methodology for the isolation of DNA from human saliva. The present study was conducted with 20 healthy control subjects with no history of smoking habits and other oral complications. The subjects were recruited from Chettinad Dental College and Research Institute (CDCRI) with the study protocol's approval from Institutional Human Ethics Committee (392/IHEC/10-17). Their age of the subjects was ranged between 30-45 years. Approximately 5 ml of human saliva was collected in a fresh tube with informed consent from healthy controls by informing the current research study by the donors. Genomic DNA was prepared from human saliva sample by ammonium acetate method. The quantification process was determined by a UV-visible spectrophotometer (UV-1800 Shimadzu spectrophotometer) to determine the purity and concentration of DNA. The average concentration of DNA from a saliva sample was found at1.48mg/ml.

Keywords: DNA, human saliva, isolation, spectrophotometer, forensic serology.

Introduction

Forensic science is the application of science to criminal investigation governed by legal standards[1]. Through the progress of an investigation, forensic scientists work to collect, preserve, and analyze scientific proof. The scope of work is divided into two parts, some scientists visit the crime scene to analyze and gather forensic evidence, while some perform laboratory examination on the gathered evidence. Moreover, scientists hold responsibility as an expert witness in criminal and civil cases and work for either prosecution or defense[2].

Any field may hold forensic aspects; however, some have eventually developed to consist of a specific division under forensics. In forensic science branches, the serology field plays an important role to identify, classify, and study of bodily fluids such as blood, saliva, sperm, urine, sweat, etc[3]. In sexual assault cases, the most common evidence found blood, sperm, and saliva. In the serology branch, forensic experts do the preliminary and confirmatory test for the examination of serological samples[4]. Collection of biological from the crime scene is a crucial job for the forensic expert because it's very sensitive. If any problem occurs especially while collecting then there will not able to find any clue from the sample[5].

Human contains more than millions of nucleated cells in every part of the body[6]. Every nucleated cell has DNA to represent the genetic structure of humans and its unique nature for every individual.

Inside the DNA there are building blocks of genes which are having unique codons to determine the genotypic and phenotypic features[7-9]. To analyze the DNA it's possible to find out ethnicity[10] and it plays a major role in identical twin determination[11] for sexual assault cases. There is a recent study published based on the phenotypic identification of hair and eye color[12] from DNA fingerprinting by next-generation sequencing technique[13]. Usually, blood, sperm, and saliva were used for isolating the DNA. Because these three are a good source for isolating the DNA to prove the guilt of an offender[14].

There are some of the techniques that have been standardized for isolating the DNA from blood and sperm. The latest technique for isolation of DNA from blood is Magnetic bead extraction to get the good concentration and purity of the DNA[15]. The Microfluid method is the best novel technique to isolate DNA from sperm[16]. But for the saliva preliminary test only was standardized to examine molecular composition. The most common preliminary test was done in the saliva is an alpha-amylase test[17] also identified as the Phadebas Test[18].

It was reported that the novel identification of saliva from reverse transcription-loop-mediated isothermal amplification[19]. For saliva, there was research conducted and mentioned five different protocols for the separation of DNA from the saliva sample. In this research, the author has isolated a DNA from saliva employing the ammonium acetate method[20]. After the DNA is isolated from the saliva the concentration and purity of the DNA are quantified through the spectrophotometer analysis.

Materials and methods

Selection of Subjects: The present study consisted of 20 healthy control subjects with no history of smoking habits and other oral complications were recruited from, Chettinad Dental College and Research Institute (CDCRI). Their ages ranged between 30-45 years.

Specimen Collection: Approximately 5ml of human saliva samples were collected in a fresh tube with informed consent from both patients and controls making sure about the adequate understanding of the current research study by the donors [Institutional Human Ethics Committee Clearance (392/IHEC/10-17)].

Chemicals: Preparation of Extraction Buffer: 0.121g of TrisHcl, 0.186g of EDTA was dissolved in 0.55% of SDS with a pH of 7.8.

Preparation of 10M Ammonium acetate: 10M of ammonium acetate was prepared by adding 77g of ammonium acetate with 70mL of distilled water.

Preparation of Proteinase K: Measure 5ml of 200 Mm Tris Buffer, add 1.66 mg of 3 Mm calcium chloride ($CaCl_2$) to the Tris Buffer, and dissolve completely. Measure 500µl of Tris $CaCl_2$ buffer (pH 8.0) into a centrifuge tube. Add 20mg of Proteinase k. Fill to a final volume of 500µl of glycerol. Mix until completely dissolved and stored at -20 ºC.

Preparation of TE buffer: 0.0788g of Tris-Hcl and 0.015g of EDTA was dissolved in distilled water and autoclaved with the pH of 7.8.

Data Collection: First, the respondents were briefed about the aim of this research, and consent was obtained. The subjects were asked to sit comfortably and a 50ml centrifuge tube with water was given to the subjects and asked them to gargle the water for 1 minute then spit the water into the same centrifuge tube. The researcher labeled the centrifuge tube and kept it in an icebox to avoid contamination.

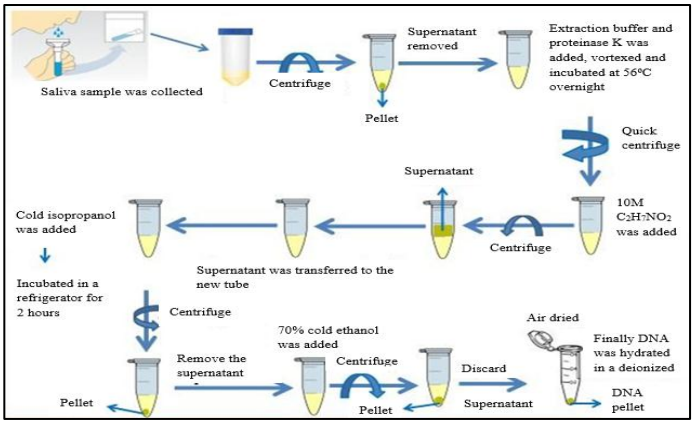

Figure-1: Isolation of DNA from Human Saliva Sample by using Ammonium Acetate Method.

Genomic DNA Preparation: Figure-1 showed that Genomic DNA was prepared from the human saliva sample by the ammonium acetate method[20]. The subjects were advised to gargle with water for some time and the saliva was collected along with gargle water around 35ml in a 50ml centrifuge tube and the saliva samples were subjected to centrifugation at 10,000rpm for 5 minutes. Then the supernatant was removed, and the pellets were resuspended with 1 ml of water transferred to a 1.5ml microfuge tube and resuspended followed by subjected to centrifugation at 10,000rpm for 5 minutes. After centrifugation, pellets were resuspended in 1ml of extraction buffer. Then 5µl of proteinase k was added, followed by vortexed and incubated overnight at 56˚C. Following the incubation, centrifuged quickly and 500µl of 10 M ammonium acetate solution was added. Then centrifuged at 21,000 rpm for 15 minutes and 500µl of supernatant was collected along with 540µl of cold isopropanol was added and vortexed for 15 minutes and incubated for 2h at 4ºC. Then centrifuged at 10,000 rpm for 20 minutes and pellets were dissolved in 1ml of 70% cold ethanol and resuspended, followed by centrifuging at 10,000 rpm for 5minutes and pellets were resuspended in 50µl of deionized water and incubated in a water bath for 56˚C overnight and 500µl of 10 M ammonium acetate solution was added. Followed by centrifuged at 21,000 rpm for 15 minutes and 500µl of this supernatant was transferred to a new tube. Then 540µl of cold isopropanol was added followed by 15minutes vortexing. The samples were placed in a refrigerator for 2h and centrifuged at 10,000rpm for 20 minutes. Finally, 1mL of 70% cold ethanol was added and centrifuged at 10,000 rpm for 5 minutes then 50µl of deionized water was added. Finally, the quantity and purity of DNA were determined using spectrophotometrically and calculated using the formula mentioned below.

Quantification of DNA: Genomic DNA was quantified using a UV-visible spectrophotometer (UV-1800 Shimadzu spectrophotometer) to check the purity and concentration of DNA[18].

$$\textbf{DNA concentration} = \frac{\text{OD at 260nm} \times \text{Dilutionfactor} \times 50\mu g/ml}{1000}$$

$$\textbf{DNA purity} = \frac{\text{OD at 260nm}}{\text{OD at 280nm}}$$

Results and discussion

Quantification of DNA: The Table-1 represents the concentration and purity of the DNA and it indicates that the DNA concentration and purity were found to be moderate. The highest concentration and purity of DNA from saliva sample.

Mean and Standard deviation: The Table-2 represents the mean and standard deviation of 20 subjects and found that the mean score for the concentration of the DNA is 1.48mg/ml (SD=0.227) and purity is 1.21 (SD=0.122).

Table-1: Concentration and Purity of DNA from human saliva.

Age	Sample sex	OD 260 (nm)	OD 280 (nm)	DNA concentration (µg/ml)	DNA purity
31	Female	0.65	0.41	1.63	1.59
30	Female	0.44	0.33	1.10	1.33
32	Male	0.67	0.56	1.68	1.20
39	Male	0.72	0.65	1.80	1.11
42	Male	0.67	0.54	1.68	1.24
37	Male	0.48	0.35	1.20	1.37
38	Male	0.69	0.59	1.73	1.17
32	Male	0.71	0.53	1.78	1.34
31	Female	0.56	0.41	1.40	1.37
30	Female	0.67	0.63	1.68	1.06
40	Male	0.54	0.44	1.35	1.23
41	Male	0.66	0.58	1.65	1.14
42	Female	0.76	0.55	1.90	1.38
43	Male	0.45	0.34	1.13	1.32
39	Female	0.55	0.41	1.38	1.34
37	Male	0.64	0.51	1.60	1.25
36	Female	0.68	0.57	1.70	1.19
33	Male	0.58	0.46	1.45	1.26
33	Male	0.65	0.58	1.63	1.12
43	Female	0.58	0.47	1.45	1.23

Table-2: Mean and Standard Deviation of quantified DNA.

	Mean	Standard Deviation
OD 260 nm	0.59	0.091
OD 280 nm	0.48	0.098
DNA concentration	1.48	0.227
DNA purity	1.21	0.122

Discussion: This pilot study was conducted to standardize the protocol for the isolation of DNA from the saliva sample. Fewer samples only have been taken for this study. Moreover, the saliva samples were collected from the participants directly not from the objector any other sources. So the limitation of this protocol is only for a whole saliva sample. For further research, it is suggested that the saliva samples should be collected from different conditions like wet/dry, and more number of samples must be tested in the same protocol for the isolation of DNA to get a better result.

Conclusion

The purpose of this study to provide a novel technique to isolate a perfect concentration of DNA for forensic purposes. In the crime scene if the biological samples are collected it will directly go for preliminary assay. In case of sexual assault cases is essential to know the identification of the culprit. By collecting the biological samples which are available at the crime scene must be tested for DNA isolation to prove the guilt of a person. So this protocol would be helpful for forensic experts to compare the DNA of the suspects. In this study, DNA was isolated from the human saliva sample, and found out that the average concentration and purity of the DNA of 20 subjects was found to be 1.48mg/ml. Hence this study will be helpful for the forensic serology field to analyze the saliva samples for sexual assault cases.

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