Received: 18th February 2015 *Accepted: 19th May 2015* *Conflicts of Interest: None*

Source of Support: Nil

Original Research

Utility of Saliva and Stains of Saliva in Deoxyribonucleic Acid Fingerprinting: Application in Forensic Odontology

Eapen Cherian[1], K Rajkumar[2], Babu Cherian[3], Ebin Thuruthiyil Mathai[4], Tony Michael[5], Ramya Malini[6]

Contributors:

[1]Reader, Department of Oral Pathology, St. Gregorios Dental College, Ernakulam, Kerala, India; [2]Professor and Head, Department of Oral Pathology, SRM Dental College, Chennai, Tamil Nadu, India; [3]Professor, Department of Prosthodontics, Mar Baselios Dental College, Ernakulam, Kerala, India; [4]Senior Lecturer, Department of Pedodontics and Preventive Dentistry, St. Gregorios Dental College, Ernakulam, Kerala, India; [5]Reader, Department of Orthodontics and Dentofacial Orthopedics, St. Gregorios Dental College, Kerala, India; [6]Reader, Department of Oral Pathology, SRM Dental College, Chennai, Tamil Nadu, India.

Correspondence:

Dr. Cherian E. Department of Oral Pathology, St. Gregorios Dental college, Ernakulam, Kerala, India. E-mail: dreapencherian@gmail.com

How to cite the article:

Cherian E, Rajkumar K, Cherian B, Mathai ET, Michael T, Malini R. Utility of saliva and stains of saliva in deoxyribonucleic acid fingerprinting: Application in forensic odontology. J Int Oral Health 2015;7(12):66-71.

***Abstract*:**

Background: To isolate deoxyribonucleic acid (DNA) from blood, saliva, buccal swab, and betel quid (BQ) by phenol-chloroform method and Chelex method, and compare the efficacy of both the methods. To carry out restriction digestion of the DNA samples isolated from above mentioned sources using the restriction enzyme EcoRI (G|AATTC) and identify individuals based on the pattern of restriction banding and to ascertain the applicability of the restriction digestion in forensics.

Materials and Methods: Blood, saliva, BQ, and buccal swab were collected from 15 patients, and DNA isolation was done by phenol-chloroform method and Chelex method. DNA fingerprinting was carried out using EcoRI restriction enzyme.

Results: DNA could be extracted from residues of saliva, DNA fingerprinting done with the isolated DNA was able to match with those of individuals. Chelex method was found to be more efficient than the phenol-chloroform method.

Conclusion: The study proves to establish a decisive method for DNA extraction from samples collected from different sources of saliva and from traces of salivary stains comparable with that of blood. Identification of individual from samples collected from different sources of saliva and from traces of salivary stains can also be done by DNA fingerprinting or profiling which is based on the fact that although most of the DNA is common to all human beings some areas differ from individual to individual as is evident from the banding pattern obtained in the present study.

Key Words: Betel quid, Chelex method, deoxyribonucleic acid, deoxyribonucleic acid fingerprinting, phenol chloroform method

Introduction

Deoxyribonucleic acid (DNA) fingerprinting has ascertained an increasingly imperative role toward decision making in the judiciary. DNA tests have helped convict suspects, to exonerate suspects or overturned previous convictions. Scientific evidences such as fingerprints, blood, semen, shreds of clothing, hair, weapons, tire tracks, and other physical evidence at the crime scene can be a more riveting to a tribunal than the testimony of an eyewitness. DNA is more suitable because DNA remains scatheless in challenging environments where such evidence is found. The DNA molecule holds an impressive dependability to withstand time.[1]

DNA profiling compares the DNA fragment lengths and patterns. The isolated DNA from the samples is fragmented using a restriction enzyme. Then, the length of the resulting fragments is determined by electrophoresis and compared by a visual interpretation of the pattern of DNA bands.[2]

DNA can be sourced from fresh blood, fresh or dried human buccal swabs, soft tissue, saliva, and salivary stains. Optimizing the methodology in DNA extraction from various sources has been tried by many studies. Minute quantities of saliva allow establishing DNA profile.[3] DNA has been proven to be isolated from cell samples from objects that was in contact with the body and from sources such as chewing gums, cigarettes, bite marks in foods, among others.

Restriction fragment length polymorphism analysis provides details of the DNA which is referred as a DNA fingerprint. As DNA is unique to every individual, analyzing the sequence helps in identification of specific patterns of each individual. DNA profile is considered as valid evidence in the court of law for paternity disputes and human identification. Standardization of DNA extraction technique will improve the reliability and speed up sample processing time.[4-6]

Limited availability of biological samples in a crime scene challenges the procedure of extraction, characterization, and analysis of DNA. Furthermore, difficulty arises in retrieving DNA from stains and degraded samples, which provide contaminated or poor quality DNA. Hence, the purification of DNA from samples is still a significant step in obtaining useful genotypes. Notwithstanding, tremendous advances have been made in the recent times in DNA testing.[7]

Chewed betel quid (BQ) stains are encountered frequently on crime scenes in Southeast Asian countries. Though the quid presents as important biological evidence, the forensic analysis using BQ as evidence has been impeded due to difficulty in extraction of human DNA. Hence, constituting a definite method for extracting DNA from chewed BQ residues is of paramount importance.[8]

Saliva found on victims of several violent crimes is a potential source of DNA. They can be recovered from bite marks, cigarette butts, BQ, postage stamps, envelopes, and other objects. However, salivary stains usually dry up easily becoming invisible, making recognition and collection difficult.

Among the various biological sources available, salivary analysis has great discriminatory power and can be incorporated into a criminal investigation. Improvisation of DNA extraction procedures will improve its reliability and also help to expedite the process. The present study aims to isolate DNA from blood, saliva (under different conditions) by phenol chloroform method and Chelex method, and to find the efficacy of these methods in extraction of DNA from traces of saliva.[9,10]

Materials and Methods

A total of 15 patients were included in the study. Informed consent was obtained from each individual taking part in the study, and data sheet were completed detailing the name, age, sex, relevant medical history, habits, etc.

Samples were grouped in six different categories as blood, saliva, buccal swab, BQ, the saliva which was stored at −20°C and saliva, which was left at room temperature (Table 1).

Table 1: Details of the samples taken and the method used.

Parameters	Sample number	Phenol-chloroform method (µg/ml)	Chelex method (µg/ml)
DNA from fresh blood	1	10	12
	2	11	12
	3	8	7
	4	9	10
	5	8	9
DNA obtained from fresh saliva	1	7	8
	2	9	9
	3	5	8
	4	9	10
	5	8	9
DNA obtained from saliva stored–20°C	1	6	9
	2	8	11
	3	10	10
	4	11	9
	5	8	11
DNA obtained from saliva stored at 37°C for 24 h	1	6	8
	2	8	9
	3	9	9
	4	10	11
	5	9	10
DNA obtained from buccal swab	6	8	10
	7	9	10
	8	9	11
	9	11	11
	10	8	11
DNA obtained from BQ	11	3	3
	12	5	4
	13	4	5
	14	4	5
	15	4	5

BQ: Betel quid, DNA: Deoxyribonucleic acid

Isolation of DNA from blood and saliva by phenol chloroform method

The DNA was extracted with an equal volume of phenol:chloroform:isoamyl alcohol. This mixture was centrifuged at 10,000 rpm for 5 min. The aqueous phase was collected and extracted with chloroform:isoamyl alcohol mixture and centrifuged at 10,000 rpm for 5 min. The supernatant was transferred to a new microfuge tube, and 0.6 volume of isopropanol was added. The spongy white precipitate was transferred to a microfuge tube, and added an equal volume of ethanol was added. Then, it was centrifuged at 10,000 rpm at room temperature for 10 min. The supernatant was drained and to the pellet 100 µl of TE buffer was added stored at 4°C.

Isolation of DNA from blood and saliva by Chelex method

A volume of 0.5 ml of whole blood was collected in 2 ml tube, and the cells are harvested by centrifugation at 3000 rpm for 3 min at 4°C. The supernatant was discarded. A volume of 0.8 ml tributylphosphine buffer was added to the collection tube, vortexed gently then centrifuged at 3000 rpm for 3 min, the supernatant was discarded. The next step was continued if the blood pellet looks mauve 0.5 ml of TBM buffer was added to the tube, and vortexed followed by addition of 3 µl of proteinase K and incubated at 55°C for 30 min. The sample was centrifuged for 2 min at 5000 rpm, and the supernatant saved to 2 ml tube and then added 260 µl of absolute ethanol. The mixture was applied to EZ-10 column, centrifuged at 8000 rpm for 1 min; discarded the flow in the collection tube. A volume of 500 µl of wash solution was added and centrifuged at 8000 rpm for 1 min. This step was repeated spin at 8000 rpm for an additional minute to remove the residual amount of wash solution. The column was placed into a clean 1.5 ml microfuge tube, and 30 µl of elution buffer was added to the center part of the membrane. The tube was incubated at 50°C for 2 min centrifuged at 10,000 rpm for 1 min to elute the DNA from the column.

Isolation of DNA from BQ by Chelex method

BQ was collected, and the residue was dried on filter paper (Whatman No. 1) at room temperature for 1-week. The residue was suspended in 1 ml of sterile water and centrifuged at 12,000 rpm.

The supernatant was discarded, and the cell pellet was used for extraction of DNA. Isolation was done by Chelex method.

DNA quantification

Preparation of DNA samples

The standards and samples were removed from the freezer and thawed. In a separate sterile 1.5 ml microfuge tube for each standard/sample, 10 µl of DNA was mixed with 990 µl of D.I. water and vortexed. The solution was allowed to stand for 10 min to ensure the complete diffusion of DNA throughout the solution. This represents a 1:100 dilution of the standards and the DNA samples.

DNA quantification

The DNA sample was briefly vortexed, and the solution was transferred to the cuvette of the spectrophotometer with care not to create bubbles. The cuvette is inserted into the spec ensuring the correct face of the cuvette is in line the light beam. An absorbance reading appears on the screen. Reading is continued until all standards and samples have been quantified. The concentration of DNA in the sample is determined according to conversion factor (A_{260} of 1.0 = 50 µg/ml DNA). The concentration of DNA in the sample can be read as µg/ml using the conversion factor and dilution factor.

Restriction digestion

Restriction enzyme buffer was vortexed before pipetting to ensure that it was well-mixed and was added to the tube. An appropriate amount of DNA to be cut was vortexed before pipetting to ensure that it was well-mixed and was added to the tube. After vortexing, the enzyme to ensure that it was well-mixed 1 µl of enzyme EcoRI was added. The mixture is placed in a thermal cycler (Eppendorf) for 2-3 h incubation at 37°C. To heat inactivate the enzyme, the mixture is maintained at 80°C for 20 min. The mixture is kept at 4°C until the reaction mixture is out of the thermal cycler.

Agarose gel electrophoresis protocol

Preparation of the agarose gel

A volume of 1.25 g agarose powder was taken in 500 ml flask, and 125 ml of tris-acetate-EDTA (TAE) buffer was added to it. The mixture is melted in hot water bath till a clear solution forms. The solution is allowed to cool to a temperature of 50-55°C by periodic swirling to achieve even cooling. To it ethidium bromide solution was added. The ends of the casting tray are sealed with two layers of tape. The combs are placed in the gel casting tray. The melted agarose solution was poured into the casting tray and allowed to cool until it is solid. The comb and the tape are removed carefully. The gel is placed in the electrophoresis chamber. A length of 2-3 mm of TAE buffer is added over the gel.

Loading the gel

A volume of 6 µl of ×6 sample loading buffer is added to each DNA sample containing tubes. 20 µl of each sample is pipetted into separate wells in the gel. 10 µl of the DNA ladder standard is pipetted into one well of each row on the gel.

Running the gel

The lid is placed on the gel box; the electrode wires are connected to the power supply. The power supply is turned on to about 100 V. To ensure the correct direction of the current, the movement of the blue loading dye is checked. The power supply is continued till the blue dye approaches the end of the gel. The wires are disconnected from the power supply. The lid is removed from the electrophoresis chamber. Using gloves, the gel is carefully removed and observed in a transilluminator for the DNA bands.

Results

Isolation of DNA was done from blood, fresh saliva, saliva stored at −20°C, saliva stored at 37°C for 24 h, buccal swab, and BQ by both the phenol-chloroform method and the Chelex method. Gel electrophoresis of the isolated genomic DNA was carried out on 0.8% agarose gel (Figure 1).

After restriction digestion electrophoresis gel is prepared to run and to identify the number of bands. DNA samples obtained from blood were labeled as A_b and subsequently as B_b, C_b, D_b, E_b as shown in Table 2. DNA obtained from fresh saliva was labeled as A_s, B_s, C_s, D_s, E_s. DNA obtained from saliva stored at −20° was labeled as A_{fs}, B_{fs}, C_{fs}, D_{fs}, E_{fs}. DNA obtained from saliva stored at room temperature was labeled as A_{ds}, B_{ds}, C_{ds}, D_{ds}, E_{ds}.

DNA obtained from blood of five individuals was made to run in the well-marked 1-5 in a uniform manner, i.e., DNA obtained from the first individual named as A_b, was made to run in well number 1. DNA obtained from a second individual named as B_b was made to run in well number 2. DNA obtained from a third individual named as C_b was made to run in well number 3. DNA obtained from a fourth individual named as D_b was made to run in as well number 4. DNA obtained from a fifth individual named E_b was made to run in well number 5 (Table 2).

However, while running DNA obtained from the saliva of different sources the order was changed randomly. For

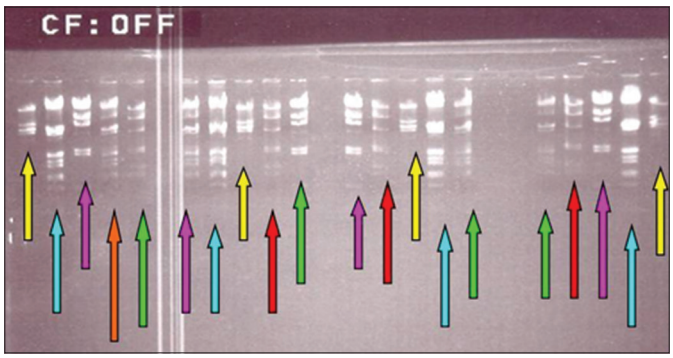

Figure 1: Restriction banding pattern from blood, fresh saliva, saliva stored at −20°C, saliva stored at 37°C for 24 h.

example, DNA isolated from fresh saliva for the first individual (A_s) instead of being run in the first well, i.e., well number 6 was made to run in the third well (well number 8); and DNA isolated from saliva stored at −20° for the first individual (A_{fs}) instead of being run in the first well, i.e. well number 11 was made to run in the third well (well number 13); and DNA isolated from saliva stored at room temperature for the first individual (A_{ds}) instead of being run in the first well, i.e., well number 16 was made to run in the fifth well (well number 20). Likewise, DNA isolated from different sources of saliva of different individuals made to run in different wells, and the number of bands produced is identified.

From the Figure 1, it could be identified that the well number 1, 8, 13, 20 corresponding to DNA isolated from the first individual from various sources named $A_bA_sA_{fs}A_{ds}$ identified by the yellow arrow has three bands uniformly.

For the well number 2, 7, 14, 19 corresponding to DNA isolated from the second individual from various sources named $B_bB_sB_{fs}B_{ds}$ identified by the blue arrow has six bands uniformly.

For the well number 3, 6, 11, 18 corresponding to DNA isolated from the third individual from various sources named $C_bC_sC_{fs}C_{ds}$ identified by the pink arrow has five bands uniformly.

For the well number 4, 9, 12, 17 corresponding to DNA isolated from the fourth individual from various sources named $D_bD_sD_{fs}D_{ds}$ identified by the red arrow has seven bands uniformly.

For the well number 5, 10, 15, 16 corresponding to DNA isolated from the fifth individual from various.

DNA isolated from the fifth individual from various sources named $E_bE_sE_{fs}E_{ds}$ identified by the green arrow has four bands uniformly. From the Figure 1, it could be identified that the well number 1, 10 corresponding to DNA isolated from different source for the first individual named $A_{b,}$ A_{bS}, identified by the yellow arrow has four bands uniformly.

For the well number 2 and 6 corresponding to DNA isolated from a second individual from blood and buccal swab named B_bB_{bS}l identified by the blue arrow has six bands uniformly. For the well number 3 and 7 corresponding to DNA isolated from a third individual from blood and buccal swab named C_bC_{bS} identified by the red arrow has five bands uniformly. For the well number 4 and 8 corresponding to DNA isolated from fourth individual from blood and buccal swab named D_bD_{bs} identified by the aqua arrow has seven bands uniformly.

For the well number 5and 9 corresponding to DNA isolated from a fifth individual from blood and buccal swab named E_bE_{bs}E identified by the green arrow has eight bands uniformly.

This shows that DNA obtained from an individual from blood, and buccal swab produces the uniform banding pattern. This shows that DNA obtained from an individual from the various source produce uniform banding pattern. Identification of individual from traces of saliva, which could be used for forensic application – The extraction of DNA from a buccal swab. Restriction digestion with Ecor-1 from extracted DNA obtained from above-mentioned source has been done for identifying individuals. Blood was used as a control and compared with DNA bands from a buccal swab. A total of 10 wells were created. DNA obtained from blood were labeled as A_b, B_b, C_b, D_b, E_b as shown in Table 3. DNA obtained from buccal swab was labeled as A_{bs}, B_{bs}, C_{bs}, D_{bs}, E_{bs}. DNA obtained from blood from 5 individuals was made to run in the well-marked 1-5 in a uniform manner.

However, while running DNA obtained from buccal swab the order was changed randomly. For example, DNA isolated from buccal swab for the first individual (A_{bs}) instead of being run in the first well, i.e., well number 6 was made to run in the fifth well (well number 10). Likewise, DNA isolated from a buccal swab of different individuals was made to run in different wells, and the number of bands produced is identified.

Different methods of DNA extraction were followed in that, most widely used is phenol chloroform method. Many new methods of DNA extraction have been tried. The Chelex method is one among then. To know the efficacy of the Chelex method, it was compared with that of phenol chloroform method. Of the two methods studied the Chelex method proved to be more easy to handle and lesser time consuming in addition to yields higher amount of DNA, and is proved by quantification with ultraviolet (UV) spectrometer as shown in Figure 2.

Discussion

Forensic odontology is a branch of forensics which analyses stains and organic liquids from the oral cavity or its contents,

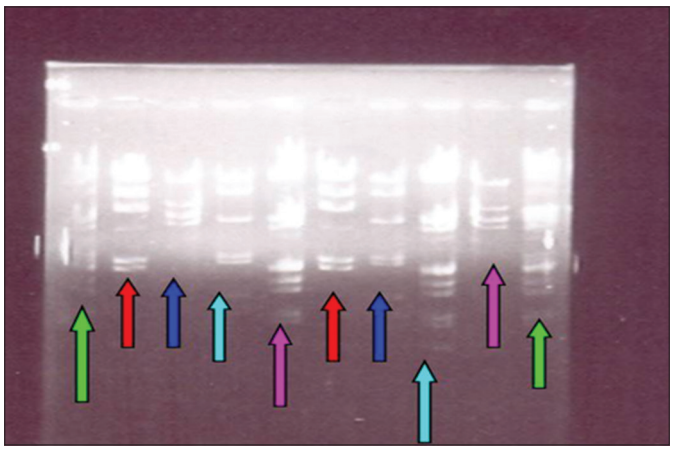

Figure 2: Comparison of deoxyribonucleic acid yield from manual and kit method.

Table 2: DNA obtained from an individual from various source produce the uniform banding pattern.

Parameter	Blood					Fresh saliva					Saliva at −20°C					Saliva stored at 37°C for 24 h				
Well number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
DNA of patient	A	B	C	D	E	C	B	A	D	E	C	D	A	B	E	E	D	C	B	A

DNA: Deoxyribonucleic acid

Table 3: DNA obtained from individual blood and BQ produce uniform banding pattern.

Well number	1	2	3	4	5	6	7	8	9	10
DNA banding pattern	A	B	C	D	E	B	C	D	E	A

DNA: Deoxyribonucleic acid, BQ: Beetal quid

bite mark comparison, investigation of trauma, and oral injuries such as personal injury cases, and dental malpractice. The fundamental requirement of a criminal investigation is that the victim and aggressor should be positively identified. Forensic dentistry aids in the forensic process by comparing the deceased's dentition with that of previous dental records or by facilitating to shape the profile of an individual in terms of age at the time of death, sex, and phylogeny to aid in identification.[11,12]

Saliva has been a potential source of identification and is usually found in bite marks, cigarette butts, BQ, postage stamps, envelopes, and other objects. The first phase of the study intended to isolate DNA from saliva (under different conditions), by phenol-chloroform method and Chelex method and compare the yield with that of blood. The second objective was to find out efficacy of these methods in extraction of DNA from traces of saliva, i.e. from buccal swab, and from BQ and which could be used for forensic application.[8]

The presence of residues is considerably important as biological evidences, but forensic analysis of such evidences has been hindered by failures in the extraction of human DNA. Consequently, it is indispensable in forensic science to establish a reliable method for extracting DNA from samples collected at the crime site. The most important objective was whether individuals can be identified from the samples of different source and to ascertain the applicability of the restriction digestion in forensics.[13,14]

Blood was taken as a control; saliva was divided into three parameters, i.e., from fresh saliva, from saliva stored at −20° for 24 h from saliva stored at room temperature for 24 h were obtained. Identification of individual has been done with the restriction enzyme EcoRI. The isolated DNA was digested using the restriction enzyme EcoRI (G|AATTC). The digested DNA was run on 1% agarose gel electrophoresis, and the bands produced in each individuals DNA were scored and is proved that identification of individual can also be done by DNA fingerprinting or profiling.

Agarose gel electrophoresis separates DNA fragments, according to their size. The most important objective was whether individuals can be identified from samples of different source and to ascertain the applicability of the restriction digestion in forensics.[16]

DNA fingerprinting is a technique that is used to represent like and unlike DNA that is present in different individuals. Nucleotide sequences which show significant variation from one individual to another are taken into consideration.[17] The most important objective of the study was to ascertain whether individuals can be identified from samples of different source and to ascertain the applicability of the restriction digestion in forensics, and the last objective was to compare the DNA yield from manual and kit method.

To prove that DNA could be extracted from traces of saliva, buccal swab, and BQ was used. DNA could be extracted from the buccal swab, BQ, and quantification was done with UV spectrometer. The comparison of DNA isolated from all the samples collected from all the individual using two different procedures has been done, and comparison of the yield of different sources showed the kit method to be more effective.

The use of biological evidences such as saliva, buccal swab, and BQ are compromised due to the quandary in the extraction of human DNA. The present study had proved to establish a reliable method for extracting DNA from samples collected from different sources of saliva and from traces of salivary stains, which was comparable to blood in proving identification. Samples collected from different sources of saliva and from traces of salivary stains can also be assessed by DNA fingerprinting or profiling, which is based on the fact that DNA is unique to every individual.

Conclusion

Residues like saliva, buccal swab and betel quid are important biological evidences relating to the suspects, the forensic analysis of evidence has been hindered by failures in extraction of human DNA. The study which is conducted in individuals had proved to establish a reliable method for extracting DNA from samples collected from different sources of saliva and from traces of salivary stains comprisable with the result of blood.

Identification of individual from samples collected from different sources of saliva and from traces of salivary stains can also be done by DNA fingerprinting or profiling which is based on the fact that although the DNA is common to all human beings differ from individual to individual as is evident from the banding pattern obtained in the present study.

References

1. Sweet D, Lorente M, Valenzuela A, Lorente JA, Alvarez JC. Increasing DNA extraction yield from saliva stains with a modified Chelex method. Forensic Sci Int 1996;83(3):167-77.
2. Sovinskia SM, Baird LS, Budowle B, Caruso JF, Cheemad DP, Duncanb GT, *et al*. Development of a deoxyribonucleic acid (DNA) restriction fragment length polymorphism (RFLP) database for Punjabis in East Punjab, India. Forensic Sci Int 1996;79(3):187-98.
3. Castella V, Dimo-Simonin N, Brandt-Casadevall C, Mangin P. Forensic evaluation of the QIAshredder/ QIAamp DNA extraction procedure. Forensic Sci Int 2006;156(1):70-3.
4. Gill P, Werrett DJ. Exclusion of a man charged with murder by DNA fingerprinting. Forensic Sci Int 1987;35(2-3):145-8.
5. Eriksen B, Bertelsen A, Svensmark O. Statistical analysis of the measurement errors in the determination of fragment length in DNA-RFLP analysis. Forensic Sci Int 1992;52(2):181-91.
6. Barbaro A, Staiti N, Cormaci P, Saravo L. DNA profiling by different extraction methods. Int Congr Ser 2004;1261:562-4.
7. Proff C, Schmitt C, Schneider PM, Foerster G, Rothschild MA. Experiments on the DNAcontamination risk via latent fingerprint brushes. Int Congr Ser 2006;1288:601-3.
8. Chiou FS, Pai CY, Hsu YP, Tsai CW, Yang CH. Extraction of human DNA from chewed residues of beetal quid using PVP/CTAB method. J Forensic Sci 2001;46(5):5.
9. Pizzamiglio M, Mameli A, My D, Garofano L. Forensic identification of a murderer by LCN DNA collected from the inside of the victim's car. Int Congr Ser 2004;1261:437-9.
10. Piccinini A, Betti F, Capra M, Cattaneo C. The identification of the victims of the Linate air crash by DNA analysis. Int Congr Ser 2004;1261:39-41.
11. Mályusz V, Schwark T, Simeoni E, Ritz-Timme S, Von Wurmb-Schwark N. Enzyrim: A new additive to increase the DNA yield from different materials such as teeth, blood or saliva. Int Congr Ser 2006;1288:562-4.
12. Wurmb-Schwark N, Mályusz V, Fremdt H, Koch C, Schwark T, Oehmichen M, *et al*. Fast and simple DNA extraction from saliva or sperm cells obtained from the skin or isolated from swabs. Int Congr Ser 2006;1288:565-7.
13. Krawczak M, Böhm I, Nürnberg P, Hampe J, Hundrieser J, Pöche H, *et al*. Paternity testing with oligonucleotide multilocus probe (CAC) 5/(GTG) 5: A multicenter study. Forensic Sci Int 1993;59(2):101-17.
14. Kemkes-Grottenthaler A. The reliability of forensic osteology – A case in point. Case study. Forensic Sci Int 2001;117(1-2):65-72.
15. Price C, Lingner J, Bickle TA, Firman K, Glover SW. Basis for changes in DNA recognition by the EcoR124 and EcoR124/3 type I DNA restriction and modification enzymes. J Mol Biol 1989;205(1):115-25.
16. Walker AH, Najarian D, White DL, Jaffe JM, Kanetsky PA, Rebbeck TR. Collection of genomic DNA by buccal swabs for polymerase chain reaction-based biomarker assays. Environ Health Perpect 1999;107(7):517-20.