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Review Article

Comparative study of extraction and analysis of DNA from different temperatures using phenol chloroform method

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ABSTRACT

The extraction of DNA from biological samples is a fundamental process in forensic investigations, providing invaluable information for the identification and profiling of individuals involved in criminal activities. Saliva is a commonly encountered biological fluid at crime scenes and can serve as a rich source of DNA evidence. This article focuses on the extraction of DNA from saliva samples and explores the methods and techniques employed in forensic investigations to optimize DNA recovery and maximize profiling success. Saliva samples were collected in saline in a swab tube and half of the samples were kept at 4^{o}C and half at Room Temperature (RT) to understand the temperature variance. After that, DNA extraction was done using the phenol-chloroform method. The quality and quantity of the extracted DNA are assessed using established techniques such as Gel electrophoresis and qPCR amplification. The results of the experiments reveal the efficiency and reliability of the DNA extraction method for saliva samples. DNA was successfully extracted from the set conditions. Although the DNA bands obtained from 4oC were more prominent compared to the samples kept at RT. The identification of optimal DNA extraction methods for saliva samples can contribute to the development of standardized protocols and guidelines for forensic DNA analysis. In conclusion, this article sheds light on the extraction of DNA from saliva samples for its use in forensic investigation. The outcomes of this study have the potential to improve the efficiency and effectiveness of forensic investigations. Further research in this field is warranted to explore emerging technologies and refine existing methodologies for DNA extraction from saliva samples in forensic contexts.

1. Introduction

Saliva is a fluid found in the mouth cavity. It is made up of an intricate blend of substances from the oropharynx, gastrointestinal reflux, deposits of food, and organic and inorganic components from the salivary glands.[1,2]

One of the most intricate, adaptable, and vital body fluids, saliva satisfies a wide variety of physiological requirements. Saliva is crucial to the function of the digestive tract, and stomach cell protection. Saliva is important for speech function, antimicrobial activity, ionic balance regulation, tissue lubrication, and mastication.[3–5] Analysis done on saliva has proved to be resourceful for the identification of different types of salivary diseases. It is also helpful in the diagnosis of disease conditions. It is a straightforward, non-intrusive method, also simple to store, and more cost-effective than blood collecting. Lately, the use of salivary analysis has shown growth in its use in laboratories, dentistry, and medical practices with the sum of new protocols and instrumentations,

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and equipment. Numerous researchers have focused on the importance of saliva in diagnostic for both oral and systemic disorders with the hope of expanding its use as a potential supplemental test.[6–10] In forensics, the ability to detect the DNA of a human in saliva has been helpful. Saliva can be discovered in a variety of places at a crime scene, including bite marks on tools or victims of violent crimes, cigars, stamps, envelopes, and other items.[11] Saliva is an important bodily fluid for identifying physiological and pathological conditions in humans. Saliva helps with many laboratory investigations, including the identification of infectious diseases, malignant neoplasms, pharmaceutical and illegal drug nursing, hormonal analysis, autoimmune diseases, and supporting forensic drugs. The benefits of employing saliva in test center diagnostics include its accessibility, ease of collection, non-invasive nature, and low-cost, straightforward storage. The fact that saliva's biological marker concentrations are lower than those of plasma and that there are still no reference values makes it difficult to use saliva. This scenario is getting improved thanks to the advancement of superior technology (nanotechnology). Saliva has typically been employed as a popular source of research material for oral squamous cell carcinoma[12,13] Saliva has recently come to be recognized as a useful indicator of the body's health status.[14] Additionally genotyping, saliva has been utilized for hepatitis A screening,[15] hepatitis A typing,[16,17] and DNA screening.[18] The isolation of cells from saliva is a less invasive way that epidemiological research for the creation of DNA gene banks is constantly being increased.[19,20]

2. Materials and Methods

2.1. Requirement

Reagents: Solution B, 20% SDS, Proteinase K, Phenol-chloroform-Isoamyl alcohol mixture (25:24:1), 5M Sodium Acetate (pH 5.2), Chilled Isopropanol (IPA), 70% ethanol, Nuclease free water, Eppendorf tubes 2 ml, Micropipette, Agarose 1% agarose, 10X TAE buffer, Loading dye - 0.25% bromophenol blue, 0.4M Tris, 48.5g per L, Ethidium bromide (10mg/ml).

2.2. Equipment

Agarose Gel Electrophoresis, Real-Time Polymerase Chain Reaction

2.3. Standard preparation

1. Sol B: Dissolved in 40 ml of 1 M Tris HCl (pH 8), 15 ml of 1 M NaCl, 10 ml of 0.5 M EDTA was autoclaved the content and cooled down. Added 5 ml of 20% SDS solution to this mixture and mixed it well.
2. 20% SDS: Dissolved In 100 ml, 20 g of SDS solution.
3. Proteinase K: Dissolved In 1 ml of autoclaved distilled water, 10 mg of Proteinase K.
4. Phenol – Chloroform Isoamyl Alcohol Mixture (PCI):: Mixed 25 ml phenol, 24 ml chloroform, and 1 ml isoamyl alcohol.
5. 5M Sodium Acetate: Dissolved In 100 ml distilled water (pH- 5.2), 41 g of sodium acetate.
6. Isopropanol: Kept the IPA at -200C before use.
7. 70% Ethanol: Dissolved In 30 ml distilled water, 70 ml absolute ethanol.

2.4. Sample collection

30 saliva samples were collected from 15 person, Two from each volunteer in a swab collecting tube filled with 1.5 ml saline. At 40C, a sample from each volunteer was kept, and another sample was kept at Room Temperature (RT). So, in this way, 15 samples were kept at 40C and 15 at RT incubate for five days and start processing the sample for DNA extraction every alternate day.

2.5. Sample pretreatment

Samples were processed every alternate day for DNA extraction

2.6. Procedure

2.6.1. DNA extraction from saliva samples

1. The saline containing saliva should be transferred into the Eppendorf tube and centrifuged at 10000 RPM for 10 mins.
2. 1 ml Solution B, 50 μl 20% SDS, and 5 μl Proteinase K were added, and the supernatant was discarded.
3. The above mixture was mixed well and incubated for half an hour at RT and half an hour at 560C.
4. After incubation, 250 μl of Sodium acetate and 500 μl of PCI solution were added to the contents, mixed well by inverting.
5. Centrifuged the mixture at 12,000 rpm for 15 minutes.
6. Three layers were formed. Carefully, the upper layer that contained DNA was transferred into a fresh tube.
7. 500 μl of chilled IPA was added to precipitate the DNA. The Mixture was incubated at -200C.
8. The Mixture was centrifuged at 10000 RPM for 10 min. The Supernatant was discarded.
9. The Pellet was washed with 500ul 70% ethanol by centrifuging the content at 10000 RPM for 5 mins.
10. The Supernatant was discarded and the pellet was air-dried.
11. After air dry, the DNA pellets were dissolved in 15μl of Nuclease free water.
12. The dissolved DNA was visualize under UV in agarose gel electrophoresis.

3. Agarose Gel Electrophoresis

1. Diluted 10X TAE buffer to 1X for gel formation. (Mixed 3 ml TAE with 27 ml d/w to make it 1X). Mixed 300 mg agarose with 30 ml 1X TAE buffer in a flask.
2. Heated the flask and dissolved the agarose. Heat-protecting gloves were used when heating the agarose.
3. The agarose when dissolved, but not in a boil. Once fully melted and allowed to cool without setting and the edges of a gel-casting tray were sealed with tape.
4. 0.5 μl of ethidium bromide was added to the agarose solution at about 60oC and gently mixed, then a fine comb was inserted into the casting tray.
5. Poured the agarose to a depth of about 1 cm and allowed it to solidify and removed the tape and 10μl of a 500μl DNA sample were taken with 2.5 μl loading dye as added.
6. A marker was created using 1μl of 1kb ladder (in a refrigerator), 9μl water, and 2.5 μl loading dye.
7. The tank was filled with 1X TAE buffer to adjust above the gel bed, and the gel was placed in the tank, ensuring that the gel was submerged.
8. The sample wells were filled with samples, and the gel was run at 80V for about an hour until the front dye reached the bottom of the gel.
9. The electrodes were bubbling indicating that the circuit was complete.
10. After the electrophoresis, the gel was photographed under UV transillumination.

3.1. Preparation of master mix for q-Polymerase chain reaction

The Reaction mixture is prepared by adding all the components in one tube except the sample and dividing the content equally in all wells and then adding the sample in the last. The Reaction mixture was prepared for a total volume of 10μl.

Table 1: Reaction mixture for q-PCR

S /No.	Content	Volume (μl)
1	2X qPCR Mix	5
2	Fw Primer (n65)	0.5
3	Rv Primer (n65)	0.5
4	RNAse free water	3.8
5	Sample (DNA)	0.2

3.2. Running the q-polymerase chain reaction

The 2-step q-PCR was operated in which the first step was hot start which was carried out at 950C for 5 min, then the amplification step which was divided into two stages first being denaturation which was carried at 950C for 10 sec, annealing of primer and extension at 600C for 20 sec. The amplification cycle was carried out for 45 cycles leading to the last stage of PCR which was melting.

Table 2: Steps in q-PCR

S/ No.	Content	Temp (^{o} C)	Duration
1	Pre-denaturation	95	3 min
2	Denaturation	95	10 sec
3	Annealing & Extension	60	20 sec
4	Melting	72	3 min

4. Results and Discussions

From the results obtained after gel electrophoresis, it can be clearly seen that the saliva samples which were kept at 4oC yielded more DNA compared to RT. So, it can be inferred that colder temperatures can be used to store the samples for more time duration.

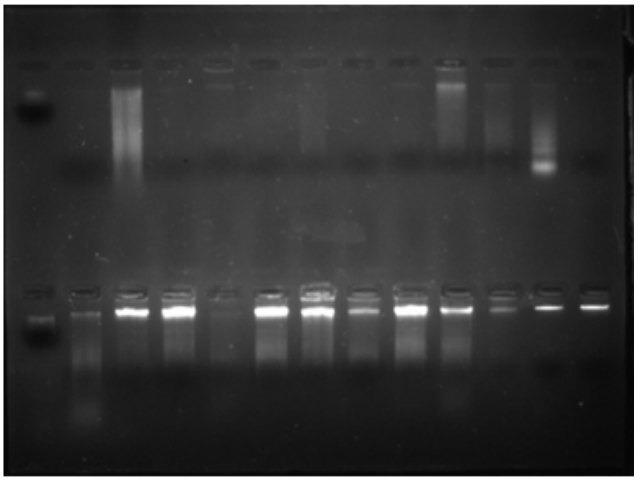

Fig. 1: DNA bands obtained from saliva under

qPCR analysis: The fold change depicted in Table 3 corresponds to the level of expression of the genomic DNA. Higher the fold change higher is the level of gene expression and thus higher was the genome in the initial sample.

Table 3: CT value and fold change of IFN-G

Well	CT value(Mean)	Fold Change(Mean)
B1-B12	20.33	78.5

The CT Value and Fold Change of samples were 20.33 and 78.5.

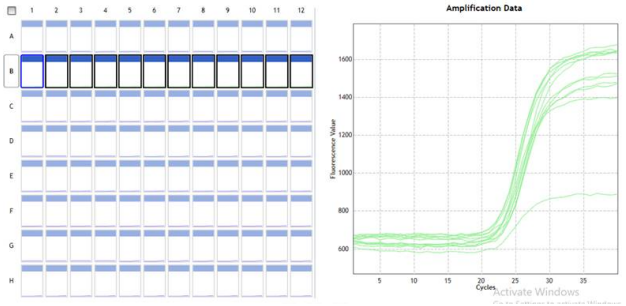

Fig. 2: Raw data plot of qPCR

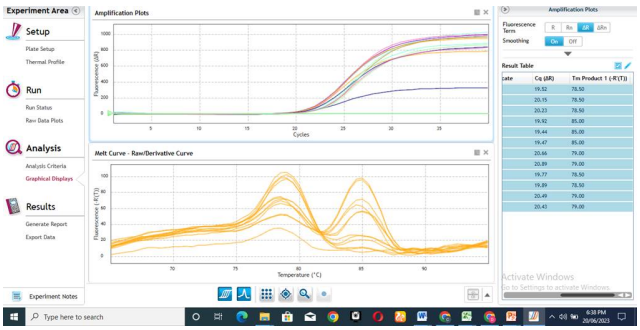

Fig. 3: Melt curve of qPCR

5. Conclusion

The present study explored the extraction of DNA from the saliva samples at two different temperature conditions using the phenol-chloroform method. The results obtained through in study indicate that DNA can be successfully retrieved from both conditions, however, DNA obtained from the saliva kept at 40C gave more prominent results compared to the samples kept at RT and this protocol thus can be used for further forensic investigations. Also, qPCR analysis has proved that the DNA extracted is functional and can be used for future forensics studies.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. Dodds MW, Jonson DA, Yeh CK. Health benefits of saliva: a review. *J Dent*. 2005;33(3):223–56.
2. Douglas CR. Tratado de fisiologia aplicada a‘ sau´de. Sa~o Paulo: Robe Editorial. and others, editor; 2002. Available from: https://www.amazon.com.br/Tratado-Fisiologia-Aplicada-%C3%80-Sa%C3%BAde/dp/8573630256.
3. Mandel ID. The role of saliva in maintaining oral homeostasis. *J Am Dent Assoc*. 1989;119(2):298–304.
4. Sreebny L. Saliva: its role in health and disease. *Int Dent J*. 1992;42:291–304.
5. Denny PC, Denny PA, Klauser DK, Hong SH, Navazesh M, Tabak LA. Agerelated changes in mucins from human whole saliva. *J Dent Res*. 1991;70(10):1320–7.
6. Chiappin S, Antonelli G, Gatti R. Saliva specimen: A new laboratory tool for diagnostic and basic investigation. *Clinica Chimica Acta*. 2007;383(1-2):30–40.
7. Lima DP, Diniz DG, Moimaz S. Saliva: reflection of the body. *Int J Infect Dis*. 2010;14(3):184–8.
8. Moura S, Medeiros AMC, Costa F, Moraes PH, Filho O. Valor diagno´stico da saliva em doenças orais e sistemicas: uma revisa~o de literatura. *Pesq Bras Odontoped Clin Integr*. 2007;7(2):187–94.
9. Medina ML, Merino LA, Gorodner JO. Utilidad de la saliva como fluido diagno´stico. *Bol Inst Med Reg*. 2004;p. 78–88.
10. Gilks CF, Balbirnie E. Testing sputum samples for HIV antibodies using a rapid, saliva-card test system. *Ann Trop Med Parasitol*. 1997;91(1):115–22.
11. Silveira EM. Odontologia legal: a importancia do DNA para as perı´cias e peritos. *Sau´de E´tica Justiça*. 2006;11(1-2):12–20.
12. Nagler RM. Saliva as a tool for oral cancer diagnosis and prognosis. *Oral Oncol*. 2009;45(12):1006–16.
13. Liao PH, Chang YC, Huang MF. Mutation of p53 gene codon 63 in saliva as a molecular marker for oral squamous cell carcinomas. *Oral Oncology*. 2000;36(3):272–8.
14. Lima DP, Diniz DG, Moimaz S. Saliva: reflection of the body. *Int J Infect Dis*. 2010;14(3):184–8.
15. Amado LA, Villar LM, De Paula V. Comparison between serum and saliva for the detection of hepatitis A virus RNA. *J Virological Methods*. 2008;148(1-2):74–80.
16. Xie X, Zhang X, Yu B. Rapid extraction of genomic DNA from saliva for HLA typing on microarray based on magnetic nanobeads. *J Magnetism Magneti*. 2004;280(2-3):164–72.
17. Terasaki P, Chia D, Sugich L. Saliva as DNA source for HLA typing. *Human Immunology*. 1998;59:597–605.
18. Yamamoto AY, Mussi-Pinhata MM, Marin LJ. Is saliva as reliable as urine for detection of cytomegalovirus DNA for neonatal screening of congenital CMV infection. *Journal of Clinical Virology*. 2006;36:228–258.
19. Koni AC, Scott RA, Wang G, Bailey ME, Peplies J, Bammann K, et al. DNA yield and quality of saliva samples and suitability for large-scale epidemiological studies in children. *International journal of obesity*. 2005;35(1):113–118.
20. Turner S, Ayres J, Macfarlane T, Mehta A, Mehta G, Palmer C, et al. 2010.

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