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DNA EXTRACTION FROM POST-MORTEM HUMAN TEETH SAMPLES FOR STR PROFILING: MANUAL VS AUTOMATED TECHNIQUE

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ABSTRACT

Aim and Objective: This study aims to compare the efficiency of two DNA extraction methods- manual Qiagen and EZ1 automated platform – in obtaining high quality DNA from post-mortem human teeth samples for STR (Short Tandem Repeat) profiling.

Introduction: Teeth are considered one of the most durable biological materials and serve as an excellent source of DNA particularly in challenging forensic scenarios like mass disasters and advanced decomposition.

Materials and Methods: Two Post-mortem human teeth samples were randomly selected from the workflow of **RFSL Chanakyapuri, New Delhi.** Only human premolar teeth were included in the study to ensure uniformity in sample type for both extraction techniques. STR amplification was performed using GlobalFiler and PowerPlex 21 kits, and genotyping was analyzed via the 3500 Genetic Analyzer and GeneMapper 1.4 IDX software. Real-Time PCR (RT-PCR) results were utilized to measure DNA amplification efficiency.

Results: The comparative analysis indicated variation in DNA yield, integrity and STR profiling success between the two methods. RT-PCR results were associated with both STR amplification and DNA quality.

Conclusion: Manual DNA isolation methods were observed to yield better quality DNA as compared to automated protocols. This observation supports the use of manual extraction methods, especially in circumstances where DNA quantity is limited or the sample quality is compromised. A minimum threshold of DNA was essential for successful PCR amplification.

Keywords: DNA yield, RT-PCR, Qiagen, EZ1 automation, Short tandem repeat profiling, Postmortem human dental sample, forensic identification.

1. INTRODUCTION

Forensic biology plays a vital role in human identification through DNA testing, whereas forensic science uses scientific ideas to support legal investigations[1]. Teeth are uniquely structured organs containing both hard and soft tissues, each contributing specific functions. The four main components of teeth are enamel, dentin, cementum, and pulp. The pulp, cementum, and dentin are all potential sources of DNA. Since the pulp is cellular and well-vascularized, it produces the most nuclear DNA. Cementum, which surrounds the tooth root, is another valuable DNA source due to the presence of cementocytes, particularly in aged or degraded teeth. Dentin may contain mitochondrial DNA, although its cellularity is limited. Enamel, in contrast, is acellular and lacks DNA, rendering it irrelevant in forensic applications[2,3,4].

DNA profiling of dental tissues has become increasingly important in forensic investigations, especially in cases involving decomposed, incinerated, or skeletal remains. However, effective DNA recovery can be influenced by several variables, including environmental exposure, microbial contamination, postmortem changes, and the condition of the tooth[5].

There are two types of tooth-based DNA extraction techniques: destructive and non-destructive. Destructive approaches, such as fracturing the tooth, provide high DNA yields but result in irreversible damage to the specimen. Non-destructive methods, To eliminate external debris, the sample were sequentially washed with 70% ethanol and distilled water. A sterile scalpel was used to meticulously remove any remaining surface debris from the external surface of the roots. Manual DNA isolation methods produced higher-quality DNA than automated protocols[6].

This finding supports the use of manual extraction methods, particularly when the DNA amount is low or the sample quality is impaired. A minimal amount of DNA was required for effective PCR amplification. Despite these advantages, there has been little research comparing the effectiveness of these approaches in extracting DNA from deteriorated or postmortem teeth. The present study has systematically evaluated the efficiency of manual DNA extraction techniques, with a specific focus on the Qiagen protocol, for obtaining viable DNA from human premolar teeth. Real-Time PCR for quantification and the GlobalFiler™ kit and Power-plex21 for STR analysis[7,8,9].

By comparative analysis indicated variation in DNA yielding and STR profiling success between the two methods. Real-Time PCR (RT-PCR) results were utilized to measure DNA amplification efficiency[10,11].

1.2 Methodological Approach To DNA Extraction: Comparative Evaluation And Selection

Post-mortem tooth samples will be randomly selected from the workflow of RFSL. The study will involve the extraction of DNA from the roots of human premolar teeth to ensure a consistent and manageable sample size for both manual and automated extraction techniques. A total of two teeth, with 1–2 roots per extraction, will be chosen for analysis. Teeth exhibiting severe degradation or evidence of dental treatments that could affect DNA yield will be excluded from the study. To reduce contamination, the samples will be thoroughly cleaned. Sterile gauze will be used to dry the tooth roots under aseptic conditions after they have been thoroughly cleaned with sterile saline solution. Following cleaning, the dentine portion of the tooth roots will be carefully fractured to expose the inner pulp region and prepared for subsequent DNA extraction. The dentine portion of the tooth roots will be carefully processed using both manual and automated DNA extraction methods. The study will utilize two extraction protocols: the Qiagen manual DNA isolation method) and the DNeasy automated protocol (EZ1 platform) In the Qiagen manual extraction method, the dentine will be fractured to access the pulp, ground into a fine powder, and subjected to the Qiagen manual DNA isolation protocol. This method allows for efficient DNA recovery from the inner pulp while minimizing external contamination. For the automated extraction method, a small perforation will be made by sectioning the dentine to access the pulp, followed by DNA extraction using the DNeasy automated protocol on the EZ1 platform. This approach reduces sample handling and improves consistency across extractions. Each method will be assessed for its effectiveness in DNA yield, purity, and integrity, ensuring a comprehensive comparison of manual and automated techniques for forensic applications. In contrast, automated DNA extraction methods, such as the EZ1 Advanced XL (Qiagen), utilize magnetic bead-based technology in a closed, standardized system to minimize human intervention and cross-contamination. Automated methods provide high-throughput processing and reproducibility, making them ideal for forensic laboratories handling large case volumes. However, studies indicate that automated systems may not be as effective in recovering DNA from aged, degraded, or calcified samples such as teeth, as their yield is often lower compared to manual methods. Given these factors, the selection of an appropriate extraction method depends on the specific forensic sample type, level of DNA degradation, and laboratory requirements.

2. MATERIALS AND METHODS

This research was carried out following a structured six-step approach to ensure systematic and accurate DNA analysis as shown in **Fig. 2.1** The steps included sample collection, DNA extraction, quantification of DNA, amplification of target sequences, STR (Short Tandem Repeat) genotyping, and data interpretation**.**

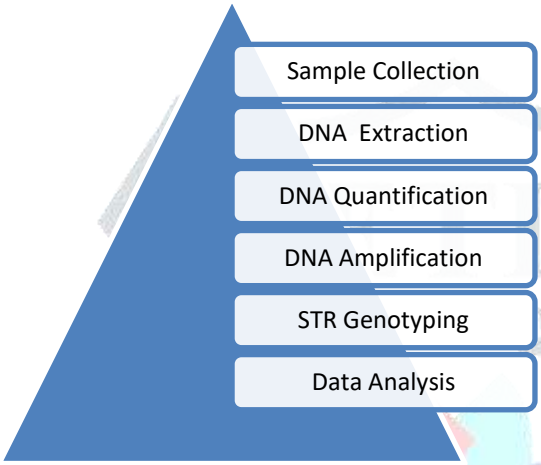


Fig 2.1: Sequential methodological steps employed in the present study

2.1 Materials

Both automated and manual DNA extraction methods were used in this investigation. Two commercial DNA extraction kits were used: the EZ1 DNA Investigator Kit (Qiagen, Hilden, Germany; Cat. No. 56504) and the QIAamp DNA Investigator Kit. DNA quantification was performed using Scientific's QuantifilerTM Trio DNA Quantification Kit. DNA Quantification Kit was utilized. Two commercial kits were used for STR amplification: the GlobalFilerTM PCR Amplification Kit (Thermo Fisher, USA) and the PowerPlex® 21 System (Promega). A ThermoMixer® F1.5 and Centrifuge 5424 R (Eppendorf AG, Germany), a Spinix Vortex Mixer (Tarsons, India), and standard laboratory refrigerators (4°C to -20°C) made up the equipment. DNA was extracted using the EZ1 Advanced XL automated instrument or the QIAamp spin-column method.

Quantification was done on the Applied Biosystems 7500 Real-Time PCR System with HID Software v1.2, while STR genotyping was performed using the (Thermofisher Scientific Applied Biosystem) 3500 Genetic Analyzer and GeneMapper™ IDX v1.4 software.

Pipetting was carried out using Transferpette® S micropipettes (BrandTech Scientific, Germany) with BIO-CERT® sterile filter tips (BRAND, Germany). DNA standards, low-bind microcentrifuge tubes, adhesive films, optical-grade 96-well plates, and 8-strip tubes were used in the PCR setup.

2.2 METHODS

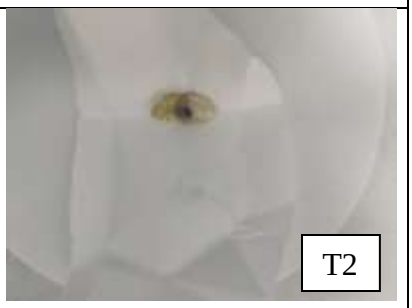
2.2.1 Sample Collection

Samples of post-mortem teeth were chosen at random from **RFSL Chanakyapuri New Delhi's** workflow. Only human premolar teeth were included in the study to ensure uniformity in sample type for both extraction techniques. Each analysis involved two teeth, typically containing one or two roots per sample. Teeth with extensive decay, structural damage, or previous dental treatments that could jeopardize DNA integrity were rejected. The selected samples represented a variety of physical states in order to evaluate technique performance under various scenarios. Pulp offers the highest DNA yield among tooth structures. In cases where pulp was degraded, cementum and dentin served as secondary sources, while enamel was unsuitable due to lack of nucleated cells. The pulp's rich cellular composition and vascularity make it the most reliable structure for forensic DNA extraction. Each sample was assigned a unique code using a standardized format, beginning with the letter "T" (representing tooth) followed by a assigned code are listed in **Table 2.1**

Sample 1: A dirty yet intact tooth, where pulp remained fully enclosed and uncontaminated internally, despite surface debris.

Sample 2: A broken premolar with exposed internal structures and external contamination.

Table 2.1 Assignment of Sample code are listed in below:

S. no	Sample Description	Assigned Code	SAMPLE PICTURE
1.	Tooth Sample 1	T1	
2.	Tooth Sample 2	T2	

2.2.1.1 Sample preparation

For Qiagen manual extraction: To reduce contamination, the samples will be thoroughly cleaned. Sterile gauze will be used to dry the tooth roots under aseptic conditions after they have been thoroughly cleaned with sterile saline solution. Following cleaning, the dentine portion of the tooth roots was carefully subjected to mechanical fracture using a sterile hammer. This process was performed with meticulous care to prevent damage to the pulp tissue to expose the inner pulp region and prepared for subsequent DNA extraction.

EZ1 automated extraction method: For EZ1 automated extraction method: To eliminate external debris, the sample were sequentially washed with 70% ethanol and distilled water. A sterile scalpel was used to meticulously remove any remaining surface debris from the external surface of the roots. The samples were then immersed in tubes containing 0.5 M EDTA solution (PH 8.0) and incubated at room temperature for 24 hours, with intermittent mixing to encourage decalcification. Following incubation, the softened tissue from the apical third of the roots was gently separated using a scalpel to collect decalcified material without requiring extensive force for further processing.

2.2.1.2 DNA EXTRACTION PROTOCOL

After retrieving the pulp tissue from the tooth, DNA extraction was carried out using two distinct methods: manual extraction and automated extraction.

1. Manual Extraction (Qiagen QIAamp DNA Investigator Kit)
2. Automated Extraction (EZ1 Advanced XL using EZ1 DNA Investigator Kit)

2.2.1.2.1 Manual DNA Extraction from Teeth Using Qiagen Protocol

The manual DNA extraction from tooth samples was carried out using the Qiagen QIAamp DNA Mini Kit, following a silica membrane-based protocol optimized for forensic analysis. So Pulverized samples (extracted pulp) were transferred to 1.5 mL microcentrifuge tubes.

1. The sample was treated with 500 µL of AT L buffer and 20 µL of Proteinase K (PK).
2. It was incubated at 56°C for at least one and half hour to ensure complete cell lysis and protein digestion.
3. The samples were then incubated at 56°C for one hour at 500 rpm in thermomixer to facilitate lysis and digestion of cellular material adhered to the fibers.
4. 400 µL each of AL buffer were added sequentially to enhance lysis and binding conditions.
5. The mixture was incubated again at 56°C for 15 minutes at 400 rpm in thermomixer to improve lysis efficiency.
6. Then, 400 µL of 100% ethanol was added to the lysate, mixed thoroughly, and transferred to a QIAamp spin column placed in a collection tube.
7. The column was centrifuged at 8000 rpm for 1 minute to bind the DNA to the silica membrane.

8. The flow-through was discarded, and the spin column was placed in a new collection tube.

9. The column was washed with 500 µL of AW1 buffer and centrifuged at 8000 rpm for 1 minute.

10. The collection tube was replaced, and a second wash was performed with 700 µL of AW2 buffer, followed by centrifugation at 8,000 rpm for 1 minute.

11. An additional wash using 700 µL of 100% ethanol was performed, centrifuged at 8000 rpm for 1 minute at 28°C.

12. To remove residual ethanol and allow membrane drying, the column was opened and left at room temperature for approximately 15 minutes.

13. For elution, 30 µL of ATE buffer was added directly to the center of the membrane.

14. The column was incubated for 15 minutes at room temperature, then centrifuged at 14,000 rpm for 1 minute to elute the DNA into a fresh microcentrifuge tube.

15. The eluted DNA was measured and stored at -20°C for further use in forensic applications such STR profiling or real-time PCR quantification.

16. This manual method, while time-consuming, allowed effective recovery of high-quality DNA suitable for forensic identification purposes.

2.2.1.2.1.2 Automated Extraction (EZ1 Advanced XL using EZ1 DNA Investigator Kit)

For EZ1 automated extraction method: Following incubation, the softened tissue from the apical third of the roots was gently separated using a scalpel to collect decalcified material without requiring extensive force for further processing.

1. Approximately 20–30 mg of pulp tissue was aseptically transferred into a sterile 1.5 mL microcentrifuge tube (Eppendorf).

2. 300 µL of ATL buffer and 20 µL of Proteinase K (from the Qiagen DNA extraction kit) were added to facilitate tissue lysis.

3. The mixture was briefly vortexed and incubated at 56°C for at least two hours to ensure complete digestion of the tissue.

4. After incubation, 140 µL of the lysate was carefully transferred to the sample tube designated for the EZ1 instrument.

5. The EZ1 Advanced automated system was prepared by loading sample tubes, reagent cartridges, and elution tubes into the appropriate slots following the manufacturer's protocol.

6. The instrument was programmed to run the DNA Tissue protocol, which typically required approximately 16 minutes to complete.

7. The pure DNA was then automatically eluted into a new tube and, depending on the process settings, typically had a final volume of 50 to 200 µL.

8. The isolated DNA was quantified and stored at -20°C until further downstream applications such as STR profiling or qPCR quantification.

9. It was especially beneficial when sample preservation was critical, and minimal sample handling was preferred.

3. DNA QUANTIFICATION:

DNA extracted from postmortem tooth samples was quantified using the Quantifiler Trio DNA Quantification Kit (Thermo Fisher Scientific) on a 7500 Real-Time PCR System (Applied Biosystems). This quantitative PCR (qPCR) system enabled assessment of DNA concentration (ng/µL) and degradation index (DI), calculated as the ratio of short to long autosomal target amplification, indicating DNA integrity.

3.1 AMPLIFICATION AND ANALYSIS

STR profiling was performed on DNA samples extracted using two methods using the globalfiler kit and the powerplex21 PCR amplification kit (Applied BioSystems). The isolated DNA was either diluted or concentrated to achieve a working concentration of 1 ng/ µL for each reaction.

The PCR amplification protocol included an initial denaturation at 95°C for 11 minutes, followed by 29 amplification cycles consisting of denaturation at 94 °C for 30 seconds, annealing at 59°C for 1 minutes, extension at 74°C for 1 minutes and a final extension at 60°C for 30 minutes.

PCR reaction for PowerPlex® 21 were carried out in 25 µL total volume, comprising 2.5 µL Master Mix, 2.5 µL Primer Mix, 1µL DNA template, and nuclease-free water.

For GlobalFiler™, reactions were prepared according to manufacturer recommendations using half-reaction volumes. The kit targets 21 autosomal STRs, Amelogenin, Y-Indel, and DYS391, including core CODIS and ESS loci, and SE33. PCR amplification included 29 cycles for 1 ng input and 30 cycles for ≤500 pg of DNA, with thermal cycling parameters similar to the PowerPlex® 21 protocol.

The PCR amplicon obtained from the globalfiler amplification kit were then subjected to DNA profiling was carried out using the GeneMapper™ IDX v1.4 software along with the Genetic Analyzer (8 capillaries) from Applied Biosystems. Peak detection threshold was set at 175 RFU, and allele assignment was carried out by comparison with the GlobalFiler™ allelic ladder and the LIZ® 600 internal size standard. Stutter artifacts and off-ladder peaks were manually reviewed and filtered in accordance with the manufacturer's guidelines.

Genotyping was performed using GeneMapper ID-X v1.4 software (Applied Biosystems), with allele calls assigned based on allelic ladders provided by the manufacturer. Only alleles showing peak heights of 100 RFU or greater were considered in the analysis.

4. RESULT AND DISCUSSION

The quantity and efficiency of a standard Qiagen manual isolation method, automated method, Manual DNA Extraction from Teeth Using Qiagen Protocol and Automated Extraction (EZ1 Advanced XL using EZ1 DNA Investigator Kit) were compared to obtain human DNA and short tandem repeats (STRs) Profiles from two teeth samples. DNA samples were quantified by quantitative PCR, and STR profiles were obtained using the Globalfiler and Power-plex 21 amplification kit.

The PCR amplification protocol included an initial denaturation at 95°C for 11 minutes, followed by 29 amplification cycles consisting of denaturation at 94 °C for 30 seconds, annealing at 59°C for 1 minutes, extension at 74°C for 1 minutes and a final extension at 60°C for 30 minutes.

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For GlobalFiler™, reactions were prepared according to manufacturer recommendations using half-reaction volumes. The kit focuses on 21 autosomal STRs, such as Amelogenin, Y-Indel, and DYS391, as well as core CODIS and ESS loci and SE33. PCR amplification included 29 cycles for 1 ng input and 30 cycles for ≤500 pg of DNA, with thermal cycling parameters similar to the PowerPlex® 21 protocol.

DNA profiling was performed on the PCR amplicon from the globalfiler amplification kit using the GeneMapper™ IDX v1.4 software and the Genetic Analyzer (8 capillaries) from Applied Biosystems. Peak detection threshold was set at 175 RFU, and allele assignment was carried out by comparison with the GlobalFiler™ allelic ladder and the LIZ® 600 internal size standard. Stutter artifacts and off-ladder peaks were manually reviewed and filtered in accordance with the manufacturer's guidelines.

Genotyping was performed using GeneMapper ID-X v1.4 software (Applied Biosystems), with allele calls assigned based on allelic ladders provided by the manufacturer. The analysis included only alleles with peak heights of 100 RFU or above.

Premolar teeth's forensic-quality DNA could be successfully extracted using the manual Qiagen extraction method, especially when the samples were well-preserved. The following findings were reached: DNA yield and quality were higher in intact samples, with DI values near to one. Degraded samples exhibited poor amplification and high DI values, which indicated fragmentation. Forensic analysis relied on comprehensive and trustworthy STR profiles obtained from high -integrity sample.

A comprehensive look of the DNA extraction results acquired from premolar tooth samples by the manual Qiagen method. The purpose was to determine DNA quality and quantity from tooth tissue for forensic profiling, especially in cases when DNA deterioration could occur. DNA quantity, degradation index (DI), internal PCR control (IPC) flags, PCR inhibition, and the quality of the Short Tandem Repeat (STR) profile were all taken into account. **Tables 4.1 and 4.2** illustrate the results, which provide insight into the efficacy of the manual method under various sample conditions. **Sample 1** produced roughly equal amounts of small and large DNA fragments, with a DI close to 1.0, indicating little to no degradation In contrast, **Sample 2** showed a

marked difference between small and large fragments, resulting in a DI above 1.0. This indicates moderate degradation, likely due to environmental or post-mortem factors affecting DNA stability.

Sample 1 exhibited no IPC flag or signs of inhibition, indicating ideal conditions for STR amplification. On the other hand, **Sample 2** exhibited IPC flagging and PCR inhibition, indicating the presence of co-extracted inhibitors or degradation byproducts that impede amplification.

4.1 Sample Analysis

4.1.1 STR Profiling and Electropherogram Assessment

Utilizing electropherogram peak intensities and pattern completeness, the STR profiles produced from the two samples were examined. These factors help determine whether the DNA is suitable for forensic identification as shown in **Table 4.3 & 4.4**

Observations:

Sample 1: A complete STR profile was observed. All loci were successfully amplified with consistent peak heights and minimal noise. As illustrated in **Figure 4.3,** this profile is ideal for forensic comparison and individual identification.

Sample 2: The STR profile was incomplete. Some loci had allele dropouts, lower peak heights, and unbalanced peak ratios.

As seen in **Fig 4.4,** increased fragment size was associated with decreased peak height,indicating DNA degradation.

Table 4.1: DNA Quantification and Degradation Index

Sample	Teeth	Quantification(ng/ µL)			Degradation index	Degraded profile
		Small	Large	Y		
1.	Premolar	0.452	0.863	1.771	0.52	NO
2.	Premolar	0.56	0.061	1.01	9.180	YES

Table 4.2: PCR Inhibition and IPC Status

Sample	IPC Flag	PCR Inhibition	Amplification Status
1.	NO	NO	Successful
2.	YES	YES	Partially Inhibited

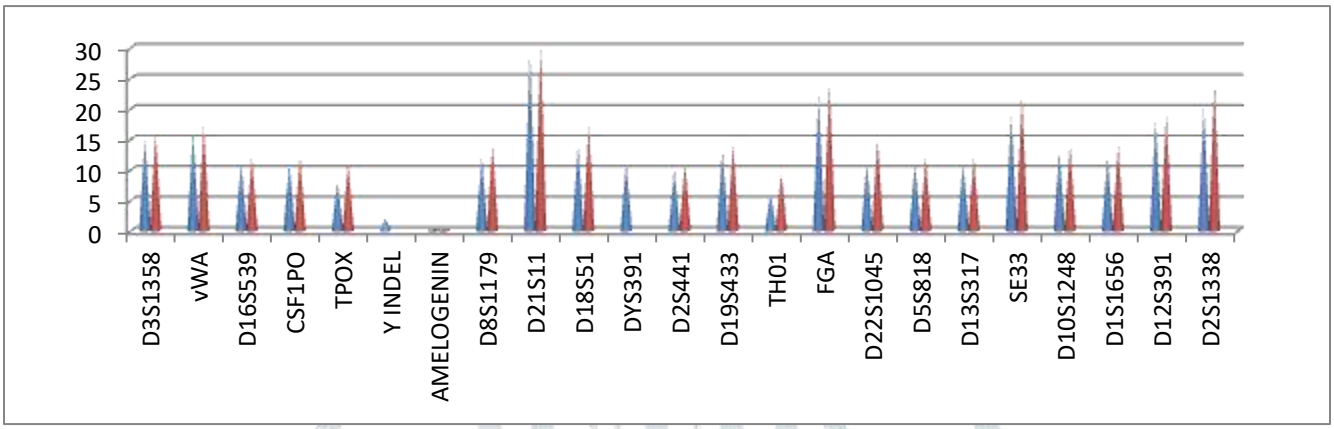

Fig 4.3 Sample 1 (Tooth Sample Manual extraction global filer kit)

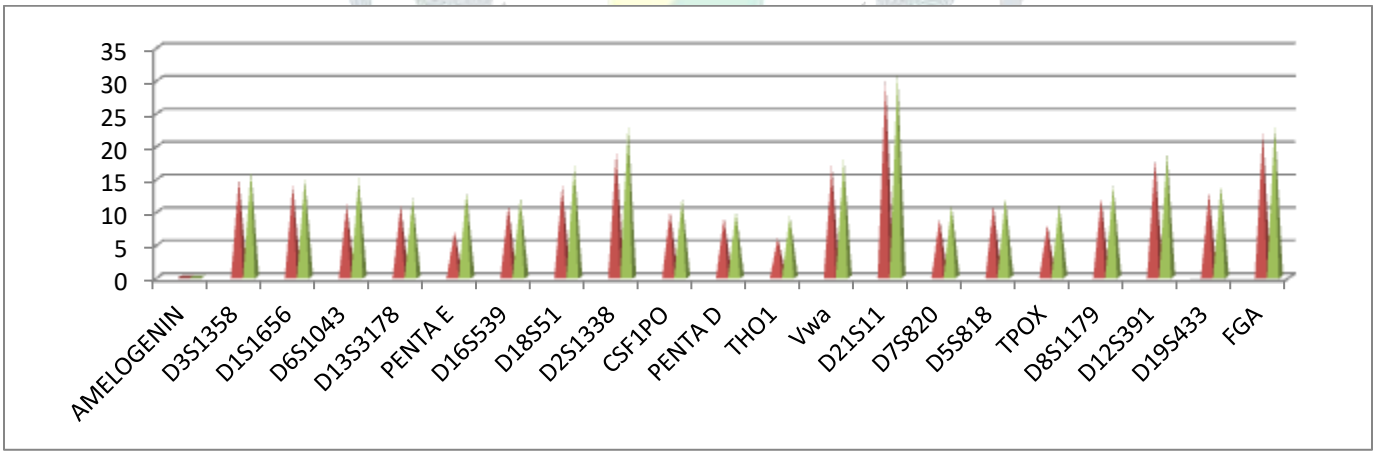

Fig 4.4: Sample 2 (Tooth Sample automated extraction power plex 21 kit)

4.2 Comparative Analysis within Manual Method

Even within the same extraction method, results varied significantly depending on the condition of the tooth. The principle behind the effectiveness of DNA amplification was assessed using cycle threshold (Ct) values obtained during real-time PCR analysis. Ct values are widely accepted as an indirect measure of DNA concentration in the sample, with lower Ct values reflecting higher initial DNA quantities and higher Ct values suggesting limited or degraded DNA content When the Ct values fell between 24 and 28, it was thought that the DNA concentration was enough for effective amplification. However, if the Ct values exceeded 30–35 or were absent, it indicated either substantial DNA degradation or insufficient template quantity. In such cases, re-extraction of DNA was deemed necessary to achieve effective amplification. Manual DNA isolation methods were shown to produce higher-quality DNA than automated protocols. The manually processed samples generally exhibited higher DNA recovery and integrity, as demonstrated in comparative graphical analysis. This observation supports the use of manual extraction methods, especially in cases where DNA quantity is minimal or the sample quality is compromised. Successful PCR amplification required a minimum amount of DNA. The samples were classified as follows according to the amplification response and Ct value readings:

1. Sufficient DNA Yield

Samples producing Ct values within the acceptable range and showing clear amplification curves were considered to contain high-quality DNA. These samples did not require any additional processing and were appropriate for subsequent molecular applications.

2. Insufficient or Degraded DNA

Samples that did not give a Ct value or took more than 35 cycles to detect were considered degraded DNA or non- human, or DNA in very low quantities. Such samples either showed no amplification or could not proceed beyond the denaturation step in the PCR process. These circumstances demanded more DNA extraction or purification before amplification could be attempted again.

4.3 Conclusion

After through and extensive work on the above two DNA isolation methods, our results indicate that the Qiagen manual DNA isolation method is the most suitable and reliable method of DNA isolation for determining DNA amount and quality, followed by (Qiagen QIAamp DNA Investigator Kit) and EZ1 Advanced XL using the EZ1 DNA Investigator Kit procedure[12,13,14,15].The present study has systematically evaluated the efficiency of manual DNA extraction techniques, with a specific focus on the Qiagen protocol, for obtaining viable DNA from human premolar teeth. The investigation highlights that the structural preservation of dental tissue plays a pivotal role in the quality and quantity of DNA recovered. Well-preserved tooth samples consistently yielded high molecular weight DNA, leading to complete and reliable Short Tandem Repeat (STR) profiles[16,17].In conclusion, the findings of this research reinforce the applicability of manual extraction protocols, especially the Qiagen method, in forensic odontology[18,19,20].

5. Source of funding

None

6. Conflict of Interest

None

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