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TECHNICAL NOTE

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A Minimally Destructive Technique for Sampling Dentin Powder for Mitochondrial DNA Testing*

ABSTRACT: The purpose of this paper is to present the horizontal sectioning technique used by odontologists at the Central Identification Laboratory to sample dentin for mtDNA analysis. From the perspective of DNA testing, anthropologists and odontologists at the Central Identification Laboratory work with ancient remains. In many instances, the lack of comprehensive antemortem records, the potential for fragmentation and commingling, and environmental exposure makes the use of traditional forensic identification techniques difficult or impossible. Teeth are highly resistant to environmental degradation and are an excellent source of mitochondrial DNA (mtDNA). This technique is simple, quick, and relatively conservative, allowing for preservation of the majority of the external portion of the tooth structure.

KEYWORDS: forensic science, forensic odontology, mitochondrial DNA, dentin sampling, JPAC, Central Identification Laboratory

The mission of the Joint POW/MIA Accounting Command (JPAC) is to conduct global investigation, recovery, and identification operations to achieve the fullest possible accounting of those missing as a result of service to our nation. The majority of the cases evaluated at JPAC's Central Identification Laboratory (CIL) are from past wars including World War II, the Korean War, and the Vietnam War. Forensic dental identification, development of biological profiles, material evidence analysis, and historical research are the procedures most commonly used to identify unknown remains at the CIL. Efforts to identify the remains of service members may be thwarted for a number of reasons including the lack of sufficient antemortem dental information. An option with some of these cases is to sample the teeth and osseous structures for mitochondrial DNA (mtDNA) analysis. During the past two years, approximately 75% of the CIL cases have included the use of mtDNA comparison to aid in the identification of remains.

Pulpal and dentinal tissues are potential sources of mtDNA. Although teeth are highly resistant to degradation from adverse environmental conditions, pulpal tissue is virtually never found under these circumstances. In these situations, an attempt can be made to sequence mtDNA from the dentin. Mitochondrial DNA is more readily recoverable than nuclear DNA with the additional advantage that genealogists are able to look farther out into the family tree to search for a suitable family reference sample (FRS).

There are several methods that can be used to sample tooth dentin for mtDNA analysis. These techniques include splitting, crushing, scraping, and filing of the teeth (1,2). Cryogenic grinding (3) is one of the more common methods used. A drawback of this technique is that it can result in the total destruction of the tooth sample. This can be a limiting factor when there are few dental remains to work with.

In 1993, Smith, et al. published a technical note that described a horizontal sectioning technique (4). This paper presents a refinement of that procedure. The described technique is a method of sampling dentin which does not result in the total destruction of the tooth sample, yet still provides sufficient material to obtain usable mtDNA sequences.

Methods and Materials

Prior to sampling, the tooth is photographed and radiographed to provide a permanent record. The tooth is weighed on a digital balance scale (Sartorius Model BP 1105, Elk Grove, Illinois) and the weight is recorded in grams. Fifteen-milliliter polypropylene reagent tubes (Sarstedt Inc., Newton, North Carolina) are labeled and weighed. These reagent tubes serve two functions. They are used to collect the final dentin powder sample and to mix reagents during the mtDNA analytical procedures conducted by the Armed Forces DNA Identification Laboratory (AFDIL) in Rockville, Maryland. One reagent tube is set aside as a control for every three tubes that will be used for collecting dentin samples. Chain of custody photographs are taken throughout the sampling process.

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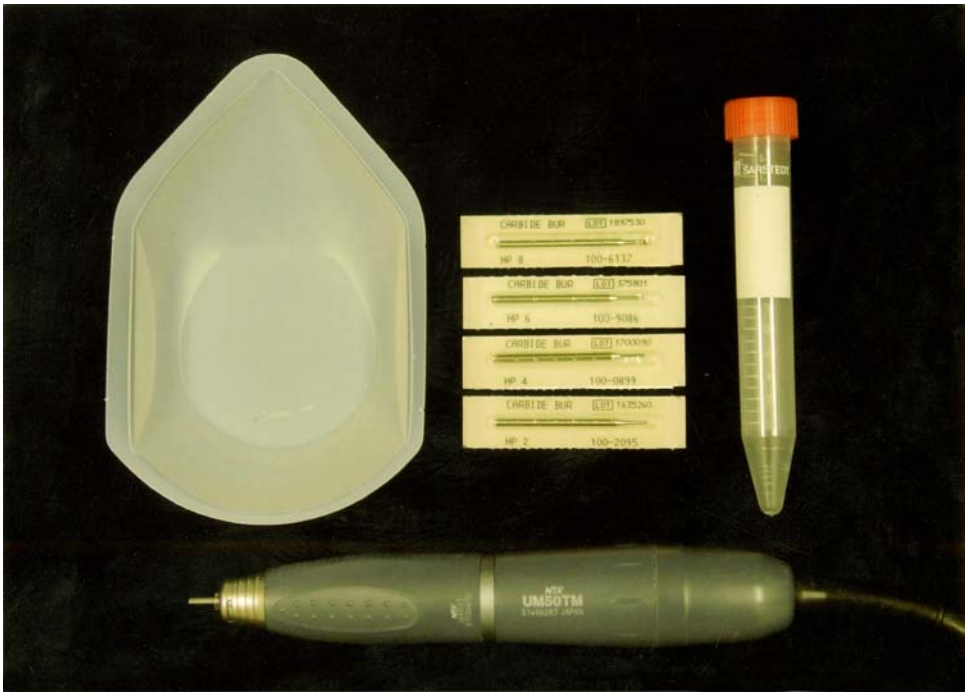

FIG. 1—*Armamentarium including a weigh boat, long-shanked round burs, 15 mL polypropylene reagent tubes, and laboratory handpiece.*

To minimize the chances of contaminating the dentin powder sample with the operator's DNA, all reagent tubes, weigh boats, dental handpieces (NSK America, Schaumburg Illinois), and sterile long-shanked dental round burs (Fig. 1) are run through a calibrated ultraviolet crosslinker (Spectronics Corporation, Westbury, New York). The crosslinker uses ultraviolet radiation to render extraneous DNA material non-amplifiable in the analytical process.

Dentin samples are harvested under an enclosed fume hood. The internal aspects of the fume hood and the adjacent work areas are wiped down three consecutive times with a diluted solution (1/5 bleach, 4/5 water) of 5.5% sodium hypochlorite. The work area under the hood is then exposed to an ultraviolet flood lamp (Cole-Parmer 9815 – Series Lamps, Vernon Hills, IL) for 10 min.

To further decrease the potential of contaminating the dentin sample with foreign DNA, the odontologist harvesting the sample wears personal protective equipment which includes surgical scrubs, sterile surgical gowns, surgical face masks, surgical head cover, two pairs of sterile gloves, and safety glasses.

Fractures, exposed dentin, and open root apices are covered with dental utility wax to minimize the uptake of sodium hypochlorite used during the preparation phase of the sampling process. The tooth is then placed in a clean specimen bottle containing undiluted 5.5% sodium hypochlorite. The bottle is placed in an ultrasonic cleaner (Sultan Chemicals, Inc., Englewood, New Jersey) for 6 min. The sodium hypochlorite denatures foreign DNA contaminates.

A sterile drape and sterile 4 × 4 gauze are placed in the fume hood adjacent to the UV flood lamp. The tooth is removed from the ultrasonic cleaner, placed on the gauze, and exposed to the UV light for a total of 10 min (5 min per side). Any wax on the tooth is removed, to allow for maximum exposure of the UV light on to the tooth surface. The fume hood door is kept closed until the operator begins the actual sampling procedure. Upon completion of the UV light cycle, the lamp is removed from the hood. Prior to starting the sampling procedure, the odontologist wipes the outer pair of surgical gloves with 4 × 4 gauze dampened with diluted 5.5% sodium hypochlorite.

The dentinal sampling is done over a weigh boat in order to collect as much dentin powder as possible. Initially, the tooth is circumferentially scored 1 mm below the cemento-enamel junction with a #2 long-shanked round bur leaving a 2–3 mm wide isthmus of intact tooth structure on the facial surface. The crown is then manually separated from the root (Fig. 2). A large round bur is then used to remove as much coronal and root dentin as possible. Differnt sized burs are placed down the canal systems to complete the removal of the radicular dentin (Fig. 3). The powdered dentin is transferred to the reagent tube by transforming the weigh boat into a funnel and vibrating or tapping the powder out (Fig. 4). If additional dentin powder is needed, slots or windows from the root structure may be prepared. The root is selected for additional dentin powder since it is the area with the highest potential yield of DNA (4).

After collecting the dentin powder, the reagent tube cap is secured and wiped with 4 × 4 gauze moistened with diluted sodium hypochlorite. The weight of the prepared tooth and the reagent tube are recorded. The dentin sample weight is obtained by subtracting the empty reagent tube weight from the reagent tube/sample weight and is recorded in grams. The dentin powder sample is now ready for analysis. The entire process is repeated with each additional tooth to be sampled.

Upon completion of the analytical procedures and publishing of the results, the tooth is waxed back together for return to the surviving family members. It is possible to reconstruct the tooth back to its presampled appearance (Fig. 5) by using the uncut isthmus of tooth structure to reapproximate the crown and root portions. It is important to note that this waxing procedure should be completed only after the analysis is finalized by the DNA laboratory. Wax could become an additional source of contamination, decreasing

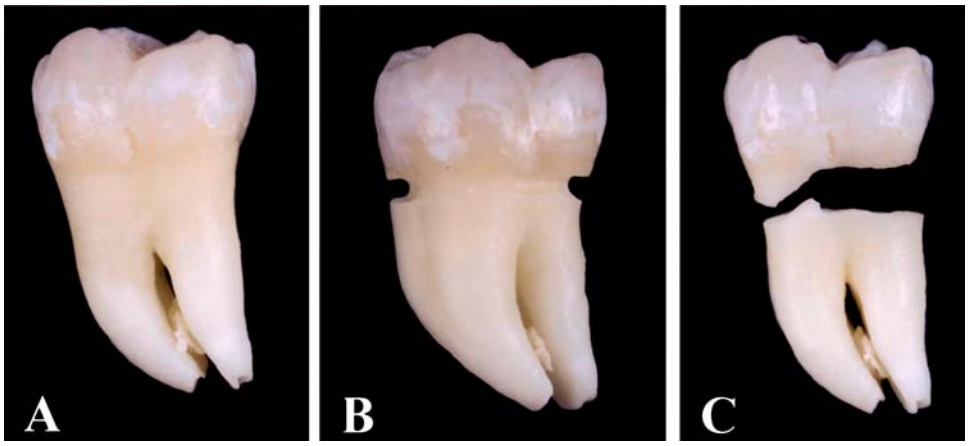

FIG. 2—*Various stages of tooth sectioning: (A) prior to sampling; (B) initial scoring of tooth; and (C) sectioned tooth.*

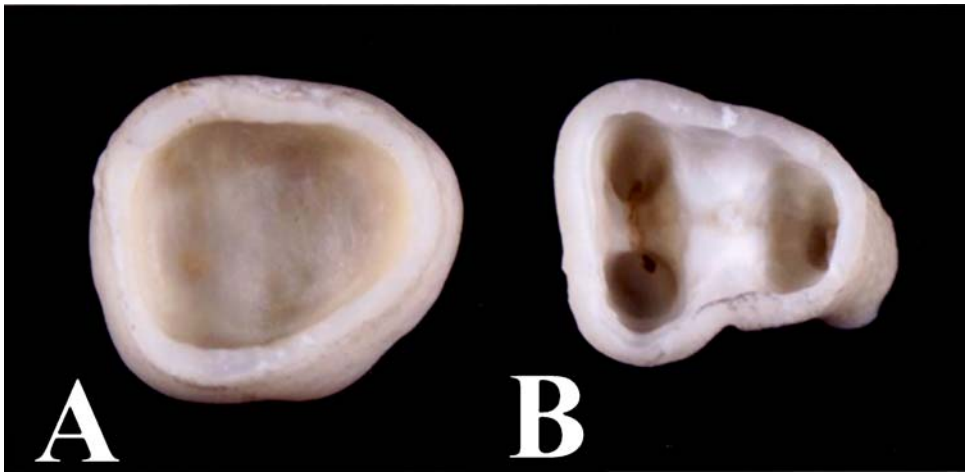

FIG. 3—*Internal view of sampled tooth with dentin removed: (A) crown portion and (B) root portion.*

the ability of the DNA laboratory to obtain a mtDNA sequence in the event that a second sample is required.

Case Study

In January 1968, a U.S. aircraft was lost on a combat mission in Laos. All nine individuals on board died in this incident. From 1996 through 2002, multiple joint U.S. and Lao People's Democratic Republic teams excavated the crash site. Material evidence recovered at the site correlated the crash with the January 1968 loss.

The recovered osseous remains were highly fragmented, commingled, and eroded after burial in an acidic soil environment. Individual osseous remains were segregated from the larger assemblage through mtDNA analysis.

The recovered dental remains consisted of maxillary and mandibular fragments with both associated and disassociated teeth. Dental radiographs were available for three individuals (2 sets of bitewings and a periapical radiograph). Narrative dental records were available for eight of the nine individuals. Due to the limited amount of antemortem information, mtDNA analysis would have to be used to help individually identify the lost U.S. service members.

Teeth were selected for sampling based on their potential to yield a mtDNA sequence. Historically, large, intact, and well-preserved teeth have been considered the best candidates for sampling. All five teeth sampled yielded mtDNA sequences.

All nine missing Americans were identified through various combinations of their biological profiles, mtDNA, and dental remains. Remains for two of the missing members consisted only of a single disarticulated tooth for each individual. These limited dental remains were associated with the lost individuals by mtDNA testing. It is important to note that if a more destructive technique for sampling dentin (e.g., the cryogenic technique) had been used, there would have been no remains available for turnover and subsequent burial by the surviving family members.

Discussion

This technique involves the horizontal sectioning of teeth to allow access for dentin sampling. The advantages of horizontally sectioning a tooth include its simplicity, ease of access, preservation of crown and root structure, and the ability to restore the tooth very close to its presampled state. If an additional DNA source is required, the root surface may be used for resampling and if a single tooth is the only biological material left for an individual, it can be retained for presentation to the surviving family members.

In order to detect potential contamination of the dentin sample with the operator's mtDNA, all laboratory personnel who handle

FIG. 4—*Transfer of dentin powder from weigh boat to reagent tube.*

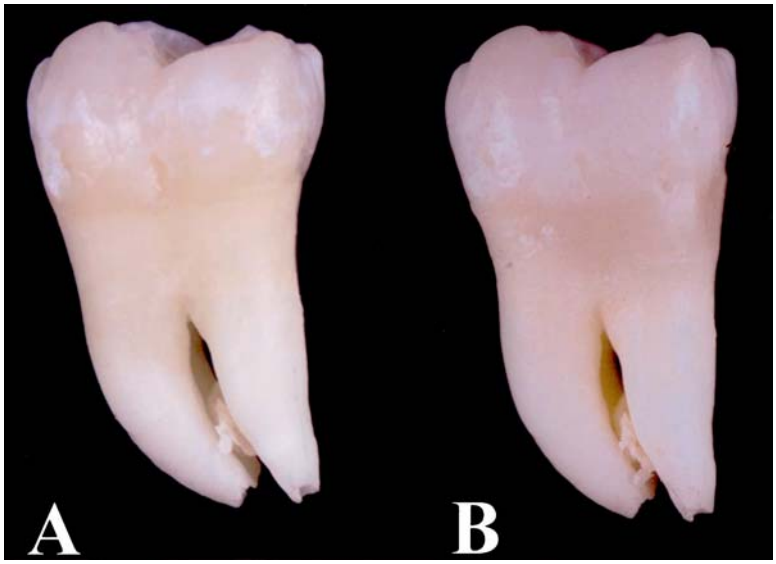

FIG. 5—*Facial views of tooth: (A) tooth prior to sampling and (B) tooth after sampling and waxed back together.*

remains are required to have a sample of their mtDNA on file at AFDIL.

Originally, full strength sodium hypochlorite (5.5%) was used to wipe down the fume hood for a number of cases. However, due to severe corrosion of the metal and degradation of the glass, a dilute solution is recommended.

At this time, the authors are in the process of evaluating the effects of several variables on the ability to obtain mtDNA sequences from dentin powder samples using the technique described. The variables being assessed include the tooth type, the amount of dentin powder obtained, environmental conditions where the dental remains were recovered, and restored versus unrestored teeth. Preliminary results

indicate that the teeth do not have to be in pristine condition to be successful in acquiring usable mtDNA sequences.

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