An evaluation of DNA yield, DNA quality and bite registration from a dental impression wafer

Mark A. Ellis, DDS, MSD; Fengyu Song, DDS, MS, PhD; Edwin T. Parks, DMD, MS; George J. Eckert, MAS; Jeffrey A. Dean, DDS, MSD; L. Jack Windsor, PhD

ABSTRACT

Background. The authors determined the amount and quality of the DNA captured by a bite impression wafer and analyzed any inaccuracies in the impression wafer.

Methods. The authors made bite registrations for subjects aged 7 to 12 years by using a dental impression wafer (Toothprints, Kerr, Orange, Calif.), obtained an oral rinse sample, took cheek cells by using buccal swabs and made an alginate impression to pour a stone model. They extracted and quantified the DNA from the dental impression wafer, mouthwash and buccal swabs by using the Quant-iT PicoGreen (Invitrogen, Carlsbad, Calif.) assay and a real-time polymerase chain reaction (RT-PCR) assay. They compared the stone models and imprints from the wafer.

Results. The average amounts of DNA determined by using Quant-iT PicoGreen from the buccal swab, mouthwash and dental impression wafer samples were 113.61, 509.57 and 1.03 micrograms, respectively. The average amounts of DNA determined by using RT-PCR from the buccal swab, mouthwash and dental impression wafer samples were 11.5240, 22.2540 and 0.0279 µg, respectively. The bite registrations and stone models had an average of 14 percent of mismatches.

Conclusion. The dental impression wafers captured DNA but not in high quantities. They did not produce an accurate representation of the dentition.

Clinical Implications. The dental impression wafers captured enough DNA to permit amplification. The accuracy of the bite registration was not sufficient for identification purposes. Therefore, dental impression wafers may be useful only as a reservoir for DNA.

Key Words. Real-time polymerase chain reaction; bite registration; mouthwash; buccal swab; dental impression wafer.

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Law enforcement agencies regularly ask dentists for assistance in identifying unknown living and deceased children. Traditionally, they have used radiographs and patient files. However, with the decrease in dental caries owing to rigorous preventive programs, many children do not have distinguishable radiographs or any type of dental impressions. Delattre and Stimson[1] asked dentists at two different component dental society meetings to self-assess their patient records. They found that only 56 percent of these dentists thought that their patients' files would be useful in identifying missing or abducted children.

Forensic dentists use DNA analyses to identify recovered children. Significant quantities of DNA can be recovered from saliva and teeth,[2-6] but although DNA analysis is a powerful and accurate tool for identifying humans, the methods for recovering DNA from teeth have not been efficient or cost-effective. In a study by Sivagami and colleagues,[7] however, ultrasonication of tooth samples yielded enough DNA to use in polymerase chain reaction (PCR) analysis to be able to determine the

Dr. Ellis is a pediatric dentist, Department of Pediatric Dentistry, Indiana University School of Dentistry, Indianapolis.
Dr. Song is an assistant professor, Department of Oral Biology, Indiana University School of Dentistry, Indianapolis.
Dr. Parks is a professor, Department of Oral Pathology, Medicine, and Radiology, Indiana University School of Dentistry, Indianapolis.
Mr. Eckert is a biostatistician, Division of Biostatistics, Indiana University School of Medicine, Indianapolis.
Dr. Dean is a professor, Department of Oral Facial Development, Indiana University School of Dentistry, Indianapolis.
Dr. Windsor is an associate professor, Department of Oral Biology, Indiana University School of Dentistry, 1121 W. Michigan St., Indianapolis, Ind. 46202, e-mail "ljwindso@iupui.edu". Address reprint requests to Dr. Windsor.

sex of the study subjects appropriately. The authors concluded that DNA could be obtained by using this method from any tooth, regardless of the age of the patient. A domestic violence case in which a 16-year-old girl was bitten and placed in a river for 5.5 hours revealed that saliva from the bite mark on her body still had enough DNA for PCR analysis and, thus, played an important role in identifying the suspect.[2] This is why swabs of saliva in bite mark investigations should be obtained even though the amount of DNA available initially might seem minimal.[8]

Epithelial cells of the oral mucosa slough off as they contact the teeth. Lijnen and Willems[9] used a double-swab technique for the buccal mucosa and obtained a high yield of DNA. King and colleagues[6] expanded on this technique by comparing the quality and quantity of DNA from 22 subjects obtained by using buccal swab and mouthwash samples. They found that PCR was 100 percent successful in quantifying the DNA isolated by both modalities, although the mouthwash samples yielded slightly more DNA. They also determined that there were no significant differences among repeated swabs of the same area. Walsh and colleagues[4] reported that whether the source of DNA is saliva, a buccal swab, blood or hair, the DNA banding patterns are indistinguishable among these four sources.

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PCR is the simplest method to use to produce multiple copies of DNA.[2,5-7,10,11] The strands of DNA are unwound and duplicated by a polymerase using each strand as a template. PCR has great sensitivity and applicability in analyzing DNA from limited biological material.[10,11] Gall and colleagues[10] and Dimo-Simonin and colleagues[11] reported that DNA could be amplified from cytological stained smears. PCR also is an important technique for amplifying DNA that may be old and partially degraded.[5]

The real-time polymerase chain reaction (RT-PCR) assay has the ability to monitor the progression of DNA quantification. Reactions are characterized at the point during cycling when amplification of a PCR product is first detected rather than by the amount of PCR product accumulated after a fixed number of cycles. RT-PCR assays are sensitive and require minimal attention. They also are cost-effective, fast and accurate.[12,13]

The Quant-iT PicoGreen (Invitrogen, Carlsbad, Calif.) assay is another method used to quantify DNA. It is a nonspecific method that relies strictly on the total amount of DNA present rather than the presence of a specific gene.

Toothprints (Kerr, Orange, Calif.) dental impression wafers are a commercial product that has been reported to be able to register patients' unique bite characteristics, as well as capture their DNA.[14] The developer, however, has stated that "no specific DNA tests have been done" to verify the amount or quality of the DNA present.[15] It also is unclear how long the DNA will be able to be extracted from the bite registrations stored in the plastic bags that are provided (this issue was beyond the scope of this study). The manufacturer recommends that Toothprints be used to make bite registrations when the children are 3, 8 and 13 years of age to correspond to the three main stages of dentition development: primary, mixed and adult.

There are no data that verify the product's ability to capture DNA or to provide accurate impressions for use in identifying people. Therefore, we conducted a study to test the ability of the dental impression wafer to capture DNA, to analyze the quantity and quality of that DNA and to analyze any inaccuracies in the impression technique. We used the Quant-iT PicoGreen assay to determine the total amount of DNA and the RT-PCR assay to determine the overall quality of the DNA. Establishing the validity of Toothprints as an effective tool may help with the genetic and dental matching processes that are used to identify recovered living and deceased children.[4]

SUBJECTS, MATERIALS AND METHODS

Subjects. We recruited 20 healthy patients (nine boys and 11 girls) with mixed dentition who ranged in age from 7 to 12 years from Riley Hospital for Children, Indianapolis. None of the subjects had systemic disease or oral pathological lesions. The Indiana University Institutional Review Board approved the study, and we

ABBREVIATION KEY. ABFO: American Board of Forensic Odontology. **DPI:** Dots per inch. **PCR:** Polymerase chain reaction. **RT-PCR:** Real-time polymerase chain reaction.