A129 Evaluation of Six Methods to Extract DNA From Chewing Gum Simulated Forensic Samples

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After attending this presentation, attendees will learn the best methods for extracting DNA from chewing gum of the six methods evaluated. This presentation will also include results with a previously unpublished extraction method.

This presentation will impact the forensic science community by providing systematic data that can be used by the analyst in selecting a method for extracting DNA from chewing gum especially when there is limited sample.

DNA evidence recovered from a crime scene is known to be a valuable tool in criminal investigations and a number of methods to extract DNA from a wide variety of substrates have been employed. However, there is very little information on the use of chewing gum as a source of DNA in the literature though this type of evidence is known to be encountered occasionally at crime scenes. Bond and Hammond[1] have noted that, though chewing gum is rarely encountered at crime scenes, it is a very rich source of DNA. They also point out the difficulty with which this type of evidence may be attributed to a suspect due to the location of recovery, which may be outside of the immediate crime scene and/or in a communal area. The condition of the chewing gum sample has also been shown to have little effect on the ability to obtain a DNA profile.[1,2] Thacker et al.[2] were able to obtain full profiles from chewing gum following incubation at room temperature or in a humidity chamber or after 30 hours of exposure to sunlight. Of the extraction methods they used, Chelex®100 (Sigma) was found to be the most effective for recovering DNA from degraded samples. This presentation presents results of six DNA extraction methods on chewed chewing gum samples. A chewing gum sample from a crime scene can be divided into a small number of samples (e.g., approximately three replicates). Samples not tested initially are often saved for retesting or use by the defense. The method employed should be known to have produced useful results in the past. In this study, the methods that would yield the most DNA with the greatest efficiency and with consideration to cost were evaluated.

The research described in this presentation includes both the detailed systematic methods constructed in this study and answers to the question posed by concluding the results of each simulated forensic sample including non-destructive methods agarose gel (1%) electrophoresis, UV- Vis spectroscopy (260/280 nm ratio), and real-time PCR. The six methods employed were Chelex-100 (Sigma), Phenol-Chloroform- Isoamyl Alcohol (PCIA), DNA IQ™ (Promega), Silica G gel (Merck), Silicycle (TAAcONa, Silicycle Inc.), and EDTA dialysis (Spectra-Por). All extraction methods were validated using buccal cells. The simulated forensic samples were obtained in triplicate for each extraction method to demonstrate reproducibility. Six pieces of Trident Watermelon Twist with xylitol were chewed for thirty minutes each on separate days. Each chewed piece of gum was divided into three approximately equal pieces that were weighed in tarred spin baskets (Fitzco) or 1.5-mL microcentrifuge tubes prior to extraction. Extracted samples were stored at -20ºC. Real-time PCR was conducted using undiluted DNA extracts, iQ SYBR Green Supermix, and forward and reverse TPOX primers[3] on a BioRad iQ5 instrument. Negative controls were prepared using nuclease- free water in lieu of extracted DNA and a K562 DNA standard (Promega) was used for the positive control. The melting temperature of the expected 64-bp amplicon was calculated to be 78ºC by both the basic and nearest neighbor methods using Northwestern University's online biotools software.[4] DNA was successfully extracted from buccal cells using all six extraction types. Preliminary results of real-time PCR of the chewing gum extraction products indicated that the PCIA method yielded the highest quantity of DNA, the DNA IQ system yielded the second highest DNA quantity, dialysis the third, and Chelex the fourth. The K562 DNA amplified while the negative control did not amplify.

References:

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4. Horsman KM, Hickey JA, Cotton RW, Landers JP, Maddox LO. Development of a human-specific real-time PCR assay for the simultaneous quantitation of total genomic and male DNA. J Forensic Sci 2006;51:758-65.

DNA Extraction, Chewing Gum, Real-Time PCR

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