## Y27 The Evaluation of DNA Extraction Methods for Chewing Gum Samples

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**Learning Overview:** After attending this presentation, attendees will better understand three different DNA extraction methods, including the PrepFiler® BTA™ method, Phenol-Chloroform-Isoamyl Alcohol (PCIA) purification, and a combined protocol. A comparison of the extraction yields, degree of DNA degradation, sample purity, and the advantages and disadvantages of each method will be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by ultimately enabling analysts to make informed decisions regarding the selection of the most appropriate DNA extraction method for chewing gum samples.

Chewing gum samples are potential sources of DNA in forensic casework. Historically, several different methods have been used to extract DNA from chewing gum samples, however, few systematic studies have been conducted to show whether one method is superior to others. In this work, two commonly used methods were tested, including the Applied Biosystems® PrepFiler® BTA™ kit and the PCIA purification method. The PrepFiler® BTA™ kit is often employed in crime laboratories because it is efficient and designed for problematic sample matrices, including bones, teeth, and adhesives. The PCIA method, however, is not as commonly used today because it is labor intensive and requires the use of toxic organic solvents. Per research, no study has been conducted to show that the PrepFiler® BTA™ method results in higher DNA yields than PCIA purification for chewing gum samples. In addition, an optimized protocol was also tested, which included sample lysis using the PrepFiler® BTA™ buffer followed by DNA purification with PCIA and PrepFiler® BTA™ magnetic beads.

It was initially hypothesized that the optimized protocol would be superior because: (1) it would lead to a high yield given that peer-reviewed literature suggested that the use of PCIA results in complete digestion of the chewing gum sample, leading to the release of all DNA present within the gum; and (2) that the extract would be free of inhibitors and other contaminants since multiple purification steps were included. Each method was tested independently by extracting DNA from forensically relevant chewing gum samples. To create these mock samples, gum was chewed for a period of five minutes by a male donor and was then buried outdoors in soil for one week. During this time, environmental humidity and temperature were monitored. DNA was then extracted from the samples, using one of the three protocols described above. The extracts were then assessed for yield, degree of degradation, and presence of inhibitors using quantitative Polymerase Chain Reaction (qPCR) and the Applied Biosystems® Quantifiler® Trio kit. Results were then compared to determine which method, if any, was most efficient at producing large quantities of high-purity, high-molecular weight DNA. DNA yields obtained from qPCR were normalized, then statistically compared using Analysis of Variance (ANOVA). This analysis showed that PCIA purification alone produced statistically significantly higher yields of DNA than the other two extraction protocols tested.

After these results were obtained, the experiment was conducted again, using more controlled variables, including flavor of the gum, extraction incubation times, environmental exposure times, and time intervals between sample chewing and burial. The extraction methods described above were also used for this additional set of samples.

Based on the results obtained from both rounds of experiments, it was determined that the most effective method for DNA extraction from a forensically relevant chewing gum sample was the PCIA extraction method, rather than the PrepFiler® BTA™ or custom extraction methods also tested.

**PrepFiler® BTA™, DNA Extraction, Chewing Gum**



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