



### F35 DNA Collection From Used Toothbrushes as a Means to Decedent Identification

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After attending this presentation, attendees will understand a refinement of existing methods to recover and extract DNA from used toothbrushes in order to provide a recommended protocol for laboratories to use when provided toothbrushes as known DNA reference samples.

This presentation will impact the forensic science community by describing a simple and effective method to collect the DNA from the used toothbrush while preserving the remainder of the brush for future testing.

This presentation will show that there is no significant difference in the quantity and quality of DNA recovered from a toothbrush that has been used for only one month vs. three months. Additionally, it will be shown that any PCR inhibitors present in the DNA samples will not significantly affect the usefulness of the DNA sample.

A method using aviation snips can be used to remove the distal end of the toothbrush head to leave sufficient area for further analysis intact

and attached to the toothbrush handle. Testing randomly used toothbrushes collected outside of the controlled study will yield similar amounts and quality of DNA as the test controlled brushes.

Fifty-two adult subjects who are not biologically related to each other were recruited as volunteers. The number of subjects will add significance to the results of this study since previous related studies generally used smaller groups of subjects. A used toothbrush was provided by each subject along with a small bloodstain control. Samples were numbered in such a fashion that they could not be attributed to any individual person in this study.

The subjects were divided into three groups and given new toothbrushes: 20 subjects used their toothbrushes in the normal way for four weeks; 20 subjects used their toothbrushes in the normal way for 12 weeks; and 12 subjects surrendered their current toothbrush for DNA testing. Two new, unused toothbrushes were used as negative controls.

**DNA Recovery:** It was assumed that sufficient DNA would be present on the head of a toothbrush to provide opportunity to complete many DNA analyses. Thus, a technique to recover DNA from a representative sample of the head of the subjects' toothbrushes was sought.

The distal end of the toothbrush head was removed to leave sufficient area for further analysis intact and attached to the toothbrush handle. The sample for use in this study was removed from the head using aviation snips, which are inexpensive and commonly available in hardware stores. The aviation snips were used for each sample and the snips were cleaned and decontaminated between each sample according to established laboratory guidelines and protocols.

**DNA Extraction:** All experimental samples (52) and controls (2) were extracted using the organic, phenol-chloroform extraction method to ensure consistency and avoid introducing variables.

All toothbrush DNA samples were quantified, amplified, and analyzed at ten STR loci to obtain a full DNA profile. The DNA profile obtained from each toothbrush was compared against the known reference bloodstain DNA profile from the user of the toothbrush.

**External Validation:** The toothbrush supplied by each of 12 subjects after normal use for random periods of time were analyzed in the same way to determine if these "normal" toothbrushes contain DNA of similar quality and quantity as the experimental samples, and whether it is possible to determine which toothbrush was used by a subject based on the DNA profile.

The results of this study confirm earlier conclusions that a used toothbrush is a good, reliable source of antemortem DNA from a putative decedent. The use of aviation snips to remove a small portion of the toothbrush head provides an easy, inexpensive method of obtaining a DNA sample while preserving the remainder of the sample brush for possible future sampling. This method is recommended as a standardized technique for use in forensic DNA laboratories.

**Used Toothbrush, DNA, Aviation Snips**