B4   Optimization and Validation of the ForensicGEM™ Rapid Extraction Method for High-Throughput Processing of Cotton Buccal Swabs

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After attending this presentation, attendees will understand the benefits of using forensicGEM™ as an optimal extraction method for the High-Throughput (HTP) processing of reference samples.

This presentation will impact the forensic science community by demonstrating an HTP, rapid extraction method that decreases the time and cost of extraction. By removing the need for lengthy extraction protocols, this method will assist in the elimination of reference backlogs in forensic laboratories.

Most extraction methods are time consuming and have a high risk for contamination due to numerous steps involved in the purification of the DNA. The Armed Forces DNA Identification Laboratory (AFDIL) currently uses DNA IQ™ for HTP processing of cotton buccal swabs, which is expensive and time consuming. The forensicGEM™ method uses a proteinase from the thermophilic *Bacillus* sp. EA1 and robust buffers to perform a single-step extraction in approximately ten minutes without the need for purification. ForensicGEM™ chemistry is rapid, compatible with downstream processing methods, adaptable to tubes or plates, amenable to automation, and inexpensive. The ability to do a single-step extraction without purification will facilitate the rapid low-cost HTP processing of cotton buccal swabs.

For use at the AFDIL, the forensicGEM™ Storage Card (Saliva) kit was optimized and validated for HTP processing of cotton buccal swabs. The optimization experiments examined three steps in the procedure: (1) cell elution; (2) eluate input volume; and, (3) extraction buffer volume. First, the five-minute elution step, which is performed in a ThermoMixer® C to dislodge the buccal cells from the swabs, was evaluated at two different speeds: 900rpm and 2,000rpm. These two conditions were each tested on 90 buccal samples to assess DNA yield and the impact of the swab position on the 96-well plate. Agitating samples at 2,000rpm corresponded to a 79% success rate (full profiles) when typed with AmpF*l*STR Yfiler®, whereas agitating swabs at 900rpm demonstrated only a 46% success rate. Plate location of the buccal swab appeared to make no difference in the success of profiles. Based on these results, 2,000rpm was determined to be the optimal elution condition.

Next, the volumes of the eluate input and extraction buffer were also investigated to further improve DNA yield. Cotton buccal swabs were collected from a total of 15 sources (in duplicate) and were extracted using five extraction conditions, testing a range of 40μL-100μL of eluate with a range of 10μL-25μL of buffer. The extracts were evaluated with several commercially available Short Tandem Repeat (STR) kits as well as the Control Region (CR) of the mitochondrial DNA (mtDNA) genome. The extraction condition that used 70μL of eluate input with 10μL of forensicGEM™ buffer outperformed the other conditions, generating at least a 90% first pass success rate with CR amplification and all STR kits. The STR profiles were of high quality (e.g., optimal peak heights, low intracolor imbalance) and required minimal reprocessing. This performance mimicked current processing methods and was therefore selected as the optimized protocol. Further testing is currently being performed to validate the optimized forensicGEM™ method for sensitivity, repeatability, and reproducibility according to the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines using quantitative Polymerase Chain Reaction (PCR), STR typing, and mtDNA analysis. Implementation of this optimized HTP extraction will allow for increased throughput capabilities by reducing both costs and processing time by approximately 65%.

The opinions or assertions presented herein are the private views of the authors and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the United States Army Medical Research and Materiel Command, or the Armed Forces Medical Examiner System.

Rapid Extraction, DNA Databasing, ForensicGEM™

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