A82 High - Throughput Chelex® - 100 Extraction of the Bode Buccal DNA Collector

Christopher Johnson, BS, Colin R. Steven, MS, Suzanne M. Barritt, MS, Theodore D. Anderson, MFS, Suni M. Edson, MS, and Louis N. Finelli, MD, Armed Forces DNA Identifcation Laboratory, 1413 Research Boulevard, Rockville, MD 20850*

After attending this presentation, attendees will understand a new high - throughput technique for processing buccal swabs.

This presentation will impact the forensic community by providing an alternative high - throughput technique for the processing of buccal swabs. This technique does not use robotics, but maintains the minization of human interaction and therefore possible errors.

The primary mission of the Armed Forces DNA Identification Laboratory (AFDIL) is to aid in the identification of missing service members from current and previous military conflicts. To this end, AFDIL works with the Armed Forces Medical Examiner System (AFMES) on current military cases and the Joint POW/MIA Accounting Command's Central Identification Laboratory (JPAC-CIL) for past military conflicts. On average AFDIL receives over one thousand reference samples per year that must be processed accurately and quickly. This presentation will discuss work primarily done by the Mitochondrial DNA (mtDNA) and Laboratory Automation and Biometrics (LABS) Sections in the efforts to process reference samples presumed to be associated with remains recovered by JPAC-CIL.

Bode Buccal DNA Collectors (The Bode Technology Group, Lorton, VA) offer a simple, non-invasive DNA sampling platform, suitable for the collection of reference DNA samples. While it is possible to extract these buccal swabs manually, a 96-well format was desired for a more efficient and time saving technique. Initially, AFDIL used a 96-well format involving DNA IQ™ system (Promega, Madison, WI) coupled with the Biomek® 2000 robotic platform (Beckman-Coulter, Fullerton, CA). Recently, the Biomek® underwent an upgrade to both the heating and the shaker elements, requiring a performance check to confirm that extraction efficiency was not affected by the changes. During this check, it was observed that both blood stain cards and swab extractions failed to produce DNA of suitable quality and quantity for use in nuclear DNA analysis. As extensive additional validation work would be required to return the Biomek® to casework, it was decided to investigate a more rapid and easier alternative for high-throughput extraction of Bode buccal collectors.

AFDIL currently uses an extraction protocol using Chelex®-100 resin (Bio-Rad, Hercules, CA) for manual extraction of DNA from multiple sample types, including oral cotton swabs, Bode buccal swabs, whole blood, bloodstain cards, soft tissue and fresh bone. Incorporating Chelex into a 96-well format offers a rapid, streamlined, and inexpensive solution. Using this system, 90 Bode buccal swabs can be extracted at once, the other six wells being held for extraction and amplification controls. The Bode swabs are punched into a 96-well plate using a Wallac DBS Puncher (PerkinElmer Life and Analytical Sciences, Boston, MA) and submerged in a 5% Chelex®-100 resin solution. Once the plate is sealed, it is placed in a thermal cycler and subjected to a 12.5 hour program, at the completion of which the samples are ready to be amplified for either mitochondrial or nuclear DNA. Multiple plates can be punched and prepared in a single day, the rate limiting steps being how quickly the plates can be prepared and how many thermal cyclers are available for use.

This protocol has proven to be highly efficient and cost effective. Not only does it minimize human interaction and therefore possible errors, it is markedly less expensive than the previously used procedure. Extracting one plate of 90 Bode swab samples with Chelex is approximately 21 times less expensive than the same extraction performed using the DNA IQ™ system on the Biomek®. To date, 1260 mtDNA and approximately 500 nucDNA reference samples have been successfully processed with this technique.

The views expressed herein are those of the authors and not necessarily those of the Armed Forces Institute of Pathology, the U.S. Army Surgeon General, nor the U.S. Department of Defense.

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 ** Presenting Author*