M48 BioRobot Forensic Validation-Progress to Date

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This paper will describe progress made applying automated extraction techniques to casework-type samples pairing QIAGEN's M48 BioRobot with their own MagAttract DNA Mini M48 extraction kit as well as Promega's Differex reagents.

Our current technique of choice for extracting nuclear DNA from cells is the Chelex-based extraction. The M48 is a fully enclosed extraction robot using magnetic beads to pull DNA out of solution and move it through a series of wash steps, all the while minimizing human contact with the sample. The instrument utilizes individual screw cap tubes and includes UV decontamination and a drip tray to further prevent cross transfer between wells. Therefore, this new automated technique promises not only an increase in casework throughput but also a reduction in human interaction with samples, decreasing the overall risk of contamination.

Labs have previously relied on the time-consuming differential Chelex extraction for epithelial-sperm cell mixtures. Using Promega's Differex reagents in conjunction with the QIAGEN M48 BioRobot decreased the time spent handling samples by 2-3 hours. The Differex system of reagents applies the principles of both differential lysis and phase separation to resolve epithelial DNA from sperm cell DNA. After a 1-2 hour set-up period consisting of preparation, incubation, and wash steps, the samples are put onto the robot for purification.

Forensic validation involved many different sample types such as blood, tissue, cigarette butts, saliva, semen, and epithelial cells. Samples were incubated for 30 minutes using QIAGEN's MagAttract DNA Mini Kit reagents and Proteinase K followed by purification with the included series of wash buffers on the robotic platform. Using Promega's Differex reagents, differential type samples were incubated for 1 hour with the yellow, aqueous Digestion Buffer, phase separated using the organic Separation Solution and washed with sterile water up to two times. Sperm fractions and swab remains fractions were further incubated with DTT and Proteinase K to ensure lysis of the sperm head. At this point the resultant three fractions- sperm fraction, epithelial cell fraction, and swab remains fractions- were put directly onto the Biorobot and purified, removing contaminants, PCR inhibitors and any remnants of the separation solution and yellow-dyed buffer. All DNA samples were quantified, amplified in the Cofiler system, and run on ABI 3100s.

By utilizing the Differex extraction system in lieu of the Chelex differential protocol, we reduced the number of man hours typically required for that particular sample type. Initial results show cleaner fractions and a higher amplification success rate offset by slightly lower yields than those observed with traditional Chelex extractions. The M48 extraction performs well for almost all sample types. Samples with lower expected DNA yields such as epithelial cell scrapings taken from clothing items showed better results with the Chelex approach. An inevitable loss of DNA for these samples types was observed due to the multiple wash steps involved, affecting our typing success. Overall the robotic extracts contained fewer inhibitors yielding better

amplification success rates than Chelex derived samples.