AUTOMATED DIFFERENTIAL EXTRACTIONS USING THE QIAGEN QIACUBE

Darby Stienmetz, B.S., Brittany Baguley, PhD., Lisa Smyth-Roam, PhD.

Washoe County Sheriff's Office Forensic Science Division, 911 Parr Boulevard, Reno, NV

The thousands of untested sexual assault kits being found across the country are developing into a massive tidal wave that is beginning to flood crime laboratories with an overwhelming amount of samples that must be processed. The already backlogged laboratories not only have to process their current cases but now have to take on the untested kits that have started to flow in. Currently, a manual differential extraction takes criminalists at the Washoe County Sherriff's Office Forensic Science Division (WCSO-FSD) approximately five hours to process approximately six samples. Automating the labor intensive manual differential process will eliminate human error, make the process more efficient, and allow analysts to take on a hands-off approach. Some laboratories have automated this process by using the Qiagen QIAcube to separate the male and female DNA and then purified these samples using the Qiagen EZ1 instrument. The WCSO-FSD does not have an EZ1 instrument; therefore, we have investigated automating the entire differential extraction process using just the QIAcube. The QIAcubes in our laboratory are currently used for straight extractions, i.e. to process blood, touch, and saliva samples. By combining the QIAcube automated wash protocol with our currently used QIAcube purification protocol, the majority of this process will be automated using equipment that criminalists in our laboratory are already familiar with.

The internal validation of the QIAcube differential extraction process included studies that addressed sensitivity, precision, reproducibility, contamination, and mock casework samples. For comparison to the QIAcube results, the sensitivity, precision, and reproducibility studies were also performed by an experienced criminalist and both the yield and separation effectiveness were measured. The sensitivity study utilized three sets of a semen dilution series, with and without female epithelial cells. In the precision and reproducibility study, thirty five replicates were processed. The contamination study consisted of male-female mixture samples and blank samples in a checkerboard pattern on the QIAcube. The mock casework samples were comprised of a variety of physiological fluids with and without semen, added to several different types of substrates, along with previously analyzed proficiency test samples. Quantitation and amplification of the samples from the manual and automated processes were performed using the Plexor HY system and PowerPlex 16 HS system, respectively. The samples were analyzed using an Applied Biosystems 3130.