H112 Comparison of QIAcube® Differential to Manual Differential Extraction When Purified Using the QIAamp® DNA Blood Mini Kit

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The goal of this presentation is to provide a comparison of an automated differential wash protocol utilizing the QIAcube® and the QIAamp® DNA Blood Mini Kit protocol and a manual differential extraction procedure. These preemptive studies were needed to complete an internal validation at the Washoe County Sheriff's Office to ensure reliability of these systems and techniques for casework. Prior to placing a new method into service in a crime laboratory setting, accredited laboratories must perform internal validations according to Standard 8 of the Federal Bureau of Investigation (FBI) Quality Assurance Standards to verify that developmentally validated methods work reliably and robustly.

This presentation will impact the forensic science community by introducing studies performed to internally validate the QIAcube® automated differential wash protocol prior to purification using the QIAamp® DNA Blood Mini Kit protocol on the QIAcube®, as well as educating laboratory staff interested in exploring automated differential extraction techniques. Furthermore, the combination of the QIAcube® differential wash protocol with the QIAamp® DNA Blood Mini Kit protocol on the QIAcube® will highlight difficulties encountered when compared to other purification protocols more commonly coupled with the QIAcube® differential wash protocol.

In a 2008-2012 survey performed by the United States Department of Justice, an average of 237,868 victims reported being sexually assaulted each year, which calculates to an occurrence approximately every two minutes. Although only about half of all sexual assaults are reported, a great deal of time and effort goes into processing evidence from these cases due to the potential of samples containing female-male mixtures on which differential extractions must be performed. In forensic casework, a differential extraction is a method that incorporates the combination of phase separation with differential centrifugation to isolate sperm cells from other cell types in order to generate two distinct profiles of the victim and the assailant. Traditionally, differential extractions have been performed manually, requiring an analyst to undergo repeated pipetting and multiple centrifugation steps. Due to the hands-on nature of the approach, the quality and consistency of the separations tend to be variable from analyst to analyst. A combination of the number of sexual assault cases reported along with the time required to analyze samples from these cases has caused backlogs to become commonplace among many crime laboratories across the country. Bringing automated differential extraction procedure online would benefit analysts by not only reducing the backlog of the laboratory but also by streamlining the workflow of a lengthy process.

This study focused on determining the utility of QIAGEN's® QIAcube® for differential extraction of samples and compared it to the manual method currently being used by the Washoe County Sheriff's Office. The QIAcube®, introduced in 2007, was originally designed to extract nucleic acids and proteins and, therefore, capable of centrifuging, vortexing, pipetting, and extracting a supernatant from a pelleted sample. This study evaluated the QIAcube's® abilities, using a custom protocol, to perform differential separations on up to 12 mock sexual assault samples at a time. Experiments included a buffer study comparing three potential buffers incorporated into the lysis mixtures; a sensitivity and reproducibility study based on a 1:3 semen dilution series, with and without female epithelial cells present; a mixture study utilizing mixed female epithelial cells and semen; a cross contamination study using mixed female blood and semen; as well as a matrix and mock evidence study consisting of a mixture of female epithelial cells and semen pipetted onto different substrates along with various proficiency test samples. All studies were performed by a graduate student using a combination of four QIAcubes®. For comparison, the sensitivity and reproducibility studies were also performed by an experienced analyst. There was no sign of cross-contamination between samples, even though the tubes remain open all at the same time in the instrument. Interestingly, the manual method consistently yielded DNA concentrations approximately twice as high as the QIAcube® for the sperm fraction. Extensive troubleshooting was performed to include the use of different reagents and temperatures as well as a variety of protocol variations. In conclusion, the Washoe County Sheriff's Office will not be utilizing the QIAcube® to perform differential extractions unless future modifications of the standard protocols result in higher male yields.

Differential Extraction, QIAcube®, Troubleshooting