Optimization and Validation of the Biomek NX for the Automation of Forensic Sample Processing

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Multiple steps involved in forensic casework sample processing have been automated. For the past eight years, the Virginia Department of Forensic Science (VDFS) has utilized the Biomek® 2000 robot for automated DNA extraction (using the DNA IQ™ System), quantitation setup, normalization, STR setup and finally transfer of the DNA samples to tubes for long-term storage. While this platform has performed reliably, VDFS is currently validating the Biomek® NX robot as a replacement. The Biomek® NX is a liquid handling robot that relies on liquid displacement for pipetting fluids; it is equipped with a single Span-8 tool with independently controlled probes capable of dispensing different volumes simultaneously. The use of this versatile pipetting tool is expected to decrease the amount of time required for these automated steps.

VDFS previously observed that with the switch to all deep-well extraction, PCR inhibition suddenly became a problem for low template (undiluted for amplification) samples. Through testing, we determined that the likely source was droplets of the DNA IQ™ Lysis buffer adhering to the sides of the deepwell plate which later sporadically became introduced into DNA extracts. VDFS has demonstrated that utilizing an additional deepwell plate in the process prior to the elution step reduces the inhibition rate from approximately 1/8 undiluted (for amplification) samples to 1/80 or less and this modification has been implemented in the Biomek® 2000 and NX methods for DNA extraction.

Customized methods used for DNA extraction of forensic casework samples as well as the normalization and STR amp setup have been developed by Promega Corporation for use on the Biomek® NX platform. Both the DNA extraction and normalization/STR setup methods are outfitted with slick user interfaces that customize each run to the specific plate of samples. Moreover, it is easier to accommodate the different volumes and buffers used in the process. Our initial sensitivity testing of the Biomek® NX extraction method showed sub par results with respect to current protocols in use with the Biomek® 2000 platform. This was remedied by increasing the amount of the time the plate incubated on the heater during the elution step. Tests of method sensitivity included a binding and elution assay which involved placing a small quantity of purified DNA into wells, executing the extraction protocol, then assessing the quantity of DNA retrieved. Extraction of DNA from a bloodstain dilution series was performed and these results were comparable to those generated previously using the Biomek® 2000 and by organic extraction of the same series. Additionally, we are assessing the method for susceptibility to contamination and those ongoing tests suggest there is a very low risk of sample contamination.

Low template, degraded, problem substrates and aged samples can all pose challenges for successful DNA extraction using both manual and robotic approaches. These challenges can sometimes be more pronounced for solid phase extraction methods used by the automated platforms. One possible explanation for any disparity may be that the commonly employed stain extraction buffer (used with organic extraction) contains a 1% concentration of SDS, while proteinase K containing digest buffers used with the DNA

IQ™ system use Sarkosyl since a 1% SDS solution can cause the Guanidinium salt to precipitate out of solution. Preliminary data demonstrate that SDS can replace the Sarkosyl used for DNA IQ extraction if either the concentration is modified or other variables are altered and this may enhance DNA recovery from problem substrates and/or aged, low template and otherwise problematic samples.

Tests of the normalization/STR setup method involve assessing the method for susceptibility to contamination and accuracy of the normalization process, both of which appear to mimic the results obtained using the Biomek® 2000 platform; those data demonstrated a very low susceptibility to contamination and a STR typing quality comparable to manual amplification. In addition, studies are on-going that compare the average peak height obtained from samples normalized and setup for STR typing by the Biomek® NX method compared to the average peak height from the same samples manually prepared for STR amplification.

When evaluated in its entirety, the data suggest that the Biomek® NX can be successfully validated and implemented for automated casework sample processing.