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VALIDATION OF FULLY AUTOMATED QPCR AND PCR METHODS FOR FORENSIC CASEWORK SAMPLES ON THE TECAN® FREEDOM EVO®

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In the last years, forensic labs have faced a dramatic increase in the number of crime scene samples submitted for DNA testing, a situation related to both the improved technological performance and the creation of national crime scene and offender DNA databanks. It has become obvious that automation is needed to face this increase. Automated systems have already been developed to feed convicted offender databanks in various countries. However, concerns are different in processing crime scene samples: contamination issues, variability of sample types, etc. Here we present the validation of fully automated, hands-off QPCR quantification and STR amplification methods for forensic casework samples on a Freedom Evo® workstation from TECAN®. This two-meter workstation is equipped with an extended deck, a liquid handler arm (LiHa) with eight fixed tips, a robotic manipulator arm (ROMA), as well as an integrated plate sealer, microplate centrifuge and code readers (sample tracking). Extracted DNA samples are stored in ABgene® micro-tubes equipped with septa plugs and a unique 2D code embedded under the tube. Automated preparation of sample dilution plates (10 µl volume), PCR plate assembly for DNA quantification (25 µl reaction volume) and STR amplification (400 pg template DNA, 10 µl reaction volume) is integrated to our in-house LIMS (www.DNAProfiles.ca), which generates worklists used by the TECAN® Gemini™ software. Versatile scripts minimize user intervention at the beginning of the session and a message is sent to a pager when plates are ready. Pipetting conditions were optimized to allow precision and reproducibility down to 0,5µl. Contamination issues were addressed by designing experiments using DNA concentrations much higher than typically used or encountered in forensic samples. A washing routine with 2% bleach was developed for fixed tips and no signs of contamination by carry-over were observed when pipetting DNA at concentrations up to 500 ng/µl, as demonstrated by ProfilerPlus® and Cofiler® amplification. Contamination by aerosols was also assessed for both sample dilution plate preparation and STR amplification plate assembly. In dilution plates, only residual contamination at the limit of detection threshold was observed when handling extremely high concentration DNA samples. The safety threshold under which no aerosol contamination is observed has been established to ~1 µg/µl of extracted DNA, and it was determined that no DNA above this concentration should be allowed on the deck. For ProfilerPlus® and Cofiler® amplification, contamination by aerosols during plate assembly was tested with wells containing 1 µg template DNA surrounded with blanks. All blanks tested negative. The validation also included systematic comparison between manual and robotic results for a number of casework samples. Similar results were obtained with the two methods for both QPCR quantification and STR profiles. Our workstation is operational for casework since the Fall of 2005. As of this Summer, automation of amplification has allowed us to eliminate QPCR quantification and STR amplification backlogs, and to cut our technical turnaround time by half.