VALIDATION OF THE ROSYS® AND HAMILTON ROBOTS USING FTA® PAPER TO ANALYZE OFFENDER LIQUID BLOOD SAMPLES

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The Illinois State Police began validation studies on the use of the Rosys® Anthos Gene Machine and Hamilton Microlab® 2200 for analyzing database samples in March 1999. The Rosys® spots thawed, mixed blood onto FTA®-bottomed microtiter plates. After washing and drying the FTA® paper, a 1 mm disk of paper is punched from each well. The punched disk falls into water in wells of an amplification plate designed for use in the GeneAmp PCR system 9600. Amplification master mix is added, the plate is covered with foil tape, and the samples are amplified for the specified number of cycles. An amplification plate and a 310 plate are placed on the Hamilton. The Hamilton adds an aliquot of Formamide/Rox (24:1) and an aliquot of amplified product to the 310 plate. This plate is sealed and placed on the ABI™ PRISM® 310 Genetic Analyzer for analysis.

The first validation study conducted demonstrated that a half reaction volume (25 ul) of AmpF/STR™ Profiler Plus™ and COfiler™ kits produced results consistent with those from full (50 ul) reaction volumes. This reduced reagent cost for the large number of offender samples to be analyzed. The second validation experiment was directed at selecting the appropriate cycle number for amplification. Twenty-six produced desirable results and twenty-eight produced many samples with off scale data. The cycle number was increased to twenty-seven (Profiler Plus™) and twenty-eight (COfiler™) once stored offender samples were analyzed. This was due to a much weaker signal displayed by the stored offender blood samples. The difference in cycle number is due to COfiler™ consistently displaying weaker RFU than Profiler Plus™. Contamination was addressed in the next validation study. Data from the contamination study illustrated that when a column of samples was punched followed by two columns of blanks, the first column of blanks showed considerable carryover contamination from the previous row of samples. The second column of blanks showed little or no carryover. As a result of the study, a blank column was inserted between each sample column. Contamination has not been an issue with this type of layout. Rosys® is investigating methods to eliminate carryover contamination. Amplified DNA from FTA® paper is not quantified and displays a wide range of sample to sample variation. One, two and three second injections were investigated and proved to be effective on samples with high RFU and/or pull up. Samples with allelic drop out can be injected for ten seconds, as previously validated for casework. The last series of validation studies addressed the amount of blood the Rosys® spots on the FTA® plate and if the robot could be used to mix the liquid blood samples. The Department of Public Safety, Phoenix, Arizona suggested spotting 5 ul of blood and 20 ul of distilled de-ionized (ddi) water on the FTA® plate. This mixture ensures uniform coverage of the FTA® disk. The Illinois State Police is currently spotting 5 ul of blood and 20 ul of ddi water on the FTA® plate. Frozen samples need to be mixed before being spotted on the FTA® plate. The Rosys® can mix the sample by aspirating and dispensing a certain volume of sample. During the validation studies the same samples were mixed on the Rosys® several different times. It became apparent that the Rosys® was diluting the samples during the mixing steps. After spotting blood on two consecutive plates, the blood on the second plate would appear much lighter in color. Hand Mixing and quick spinning proved to provide successful results.

Initial use of the Hamilton produced unacceptably high variation between samples. The aspirating and dispensing speeds were adjusted to alleviate this problem.

A Procedure manual, Bench Manual and Policy Manual were written for analyzing offender samples using the Rosys® Anthos and Hamilton Microlab® 2200. The Illinois State Police began analyzing offender samples July 6, 1999. Our projected number of samples using four 310 Genetic Analyzers is 1400 per month.