2/22/2026

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

DNA DATA BANK

VALIDATION OF THE BSD600 ASCENT A2 INSTRUMENT: PRECISION AND ACCURACY

Prepared September 2025

PURPOSE

The goal of this study was to assess the BSD600 Ascent A2 instrument for precision and accuracy while punching data bank samples. The instrument was tested to ensure it could consistently deliver the punch to the assigned plate well without issue over multiple days with multiple analysts operating the instrument.

MATERIALS AND METHODS

A set of 22 samples were punched a total of four times on four different days by two analysts using the BSD600 Ascent A2 instrument. The selected data bank samples consisted of blood cards, saliva Bode swabs, and saliva on FTA cards, all of which are currently punched with the current instrument. There were also two NIST traceable standards (blood cards) that were included in these studies. To mimic current practices in the data bank, two punches were taken from each blood sample and one punch was taken from each saliva sample. The onboard camera was used to visually check that the punch or punches fell into the correct well.

All samples, blanks, and controls were punched on the BSD600 Ascent A2 instrument into a plate with 5 µl PunchSolution™. The samples were then dried on a 70°C heat block for 20 minutes and then amplified using the PowerPlex® Fusion System following the data bank PS kit, half reaction protocol with 12.5 µl total volume, consisting of: 2.5 µl 5X master mix, 2.5 µl 5X primer pair mix, 2.5 µl 5X AmpSolution™ reagent, and 5 µl amplification grade water. All plates were amplified using a 9700 thermal cycler (ABI) following the 25 cycle thermocycling reaction for direct amplification and subsequently separated on the 3500*xl* Genetic Analyzer (ABI) for analysis. For consistency, 1 µl of PCR product was loaded onto the CE plate and all plates were injected at 12 and 24 seconds with the following settings: 1.2 kV injection voltage, 15 kV run voltage, 36 cm (length), and Data Collection Software v.3.0. Analysis was completed using the GeneMapper® ID-X v1.4 software (ABI), with 20% global and stutter cutoff filters. The limit of detection (LOD) currently utilized in the DNA data bank (blue 94, green 95, yellow 90 and red 95 RFUs) was also used for this validation. No deviations from approved data bank protocols for amplification, capillary electrophoresis or analysis were made during this validation.

RESULTS

Out of the 88 sample profiles, two partial profiles were obtained from two different samples on two different runs. All profiles analyzed were concordant with the expected results. There were no issues detected by either analyst with the instrument or the sample analysis throughout these four runs.

CONCLUSIONS

The partial profiles obtained are not indicative of inconsistent performance by the BSD600 Ascent A2 but rather reflect the variation in the amount of DNA present throughout the samples. For each run, a new spot on the card/swab must be punched for analysis, which can cause a slight variation in the amount of DNA present for the same sample throughout the four runs.

The BSD600 Ascent A2 instrument reliably and consistently punched the data bank samples into the correct corresponding plate wells. The various sample types being punched had no effect on the instrument's ability to successfully punch the sample into the intended well. Static electricity issues can be a known problem with some BSD instruments and these issues can cause samples to "jump" out of their assigned well or miss the designated well all together; however, no static issues were seen during any of the runs. The BSD600 Ascent A2 instrument accurately and without issue scanned the sample barcode, punched the sample, and delivered the sample punch to the correct and intended well on the sample plate.

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

DNA DATA BANK

VALIDATION OF THE BSD600 ASCENT A2 INSTRUMENT: CONTAMINATION

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PURPOSE

The goal of this study was to assess the contamination rate when using the BSD600 Ascent A2 instrument for punching DNA data bank samples.

MATERIALS AND METHODS

Checkerboard and zebra stripe plates were punched using the BSD600 Ascent A2 instrument. The checkerboard consisted of 46 blanks and 46 samples while the zebra stripe plate consisted of 44 blanks and 48 samples. All blanks and samples were analyzed for the presence of any extraneous alleles called that could be attributed to contamination from the direct amplification punching workflow on the instrument.

All samples and blanks were punched on the BSD600 Ascent A2 instrument into a plate with 5 µl PunchSolution™. A cleaning punch was taken in between the punching of each sample. The samples were then dried on a 70°C heat block for 20 minutes and then amplified using the PowerPlex® Fusion System following the data bank PS kit, half reaction protocol with 12.5 µl total volume, consisting of: 2.5 µl 5X master mix, 2.5 µl 5X primer pair mix, 2.5 µl 5X AmpSolution™ reagent, and 5 µl amplification grade water. All plates were amplified using a 9700 thermal cycler (ABI) following the 25 cycle thermocycling reaction for direct amplification and subsequently separated on the 3500*xl* Genetic Analyzer (ABI) for analysis. For consistency, 1 µl of PCR product was loaded onto the CE plate and all plates were injected at 12 and 24 seconds with the following settings: 1.2 kV injection voltage, 15 kV run voltage, 36 cm (length), and Data Collection Software v.3.0. Analysis was completed using the GeneMapper® ID-X v1.4 software (ABI), with 20% global and stutter cutoff filters. The limit of detection (LOD) currently utilized in the DNA data bank (blue 94, green 95, yellow 90 and red 95 RFUs) was also used for this validation. No deviations from approved data bank protocols for amplification, capillary electrophoresis or analysis were made during this validation.

RESULTS

For all blanks, the 24 second injection was pulled into the project and assessed for any contamination. There were no alleles present in the blanks above LOD for both the checkerboard and zebra stripe plates. All known samples produced profiles concordant with the

expected results from previous runs. One sample on the zebra stripe plate had an artifact present at D18S51, but no exogenous DNA was detected in any of the known types.

A total of 5 partial profiles were obtained out of the 94 samples run. These samples were still accessed for concordance with their previous Fusion run to ensure that no exogenous DNA was detected.

CONCLUSIONS

The checkerboard and zebra strip plates were processed with current data bank protocols to access contamination using the new instrument. Due to the longer injection time, the 24 second injection data is more sensitive than the 12 second data and was utilized to determine if any contamination was present. Contamination was not observed in any of the blanks or known samples punched on the BSD600 Ascent A2. The blanks did not have alleles called above the limit of detection and the known samples did not have any drop-in alleles or signs of exogenous DNA.

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

2/22/2026

DNA DATA BANK

VALIDATION OF THE BSD600 ASCENT A2 INSTRUMENT: DB POPULATABLE WORKSHEET

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PURPOSE

The goal of this study was to ensure that the new BSD600 Ascent A2 software was compatible with the currently used data bank populatable worksheet (211-F600). Since this study was only assessing the software with the populatable worksheet, the samples were not processed for downstream analysis and no DNA profiles were developed for this specific study.

MATERIALS AND METHODS

The same samples from a recently processed DB plate (plate 2024-123) that were punched with the current BSD/software system were processed using the new BSD instrument/software to ensure the barcodes can be scanned properly and the samples fall into the correct well. The first two columns of 8 samples from plate 2024-123 were processed on the BSD600 Ascent A2. The barcodes were scanned by the system and a sample was punched into the plate. The onboard camera was used to visually check that the punch fell into the correct well. The file was exported to the in-house populatable worksheet and it was checked to ensure that the correct barcodes were reflected in each proper well. The sample barcodes, well/plate position, and populatable worksheet were compared against plate 2024-123 for accuracy.

Additionally, a full plate was tested to ensure that the import batch and import BSD functions continued to work with the BSD output files from the new instrument. The batch used for plate 2024-123 was imported into the populatable worksheet, which auto-populated 90 data bank sample numbers into wells A01-B12, starting with column 1 and ending with column 12. The barcodes were scanned by the system and a sample was punched into the plate. The onboard camera was used to visually check that the punch fell into the correct well. To further test the populatable worksheet, two samples were intentionally switched on the BSD output file produced from the full plate functional test. The modified BSD output file was imported into the populatable worksheet to ensure the two samples switched would be detected by the worksheet.

RESULTS

All barcodes and well placements on the populatable worksheet were correct and concordant with plate 2024-123. When a full batch was imported into the worksheet and the BSD data was subsequently imported, no issues arose. When two samples were purposely

switched in the BSD output file and that data was imported into the worksheet with the batch already imported, the two switched sample numbers were red in the worksheet. The functionality of the worksheet converts these red sample numbers to strikethrough when printed.

CONCLUSIONS

The software from the new BSD600 Ascent A2 instrument reliably functioned without issue with the currently existing populatable worksheet. Barcodes scanned from the BSD software were accurately imported into the currently used worksheet. The BSD output files imported seamlessly into the worksheet when a DB LIMS batch was imported and the worksheet properly functioned to detect a discrepancy between the sample number expected in the well and the sample barcode scanned by the BSD. Since the BSD software could function with the current populatable worksheet utilized in the data bank, no edits to the populatable worksheet were required.

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

DNA DATA BANK

VALIDATION OF THE BSD600 ASCENT A2 INSTRUMENT: CONCORDANCE 2/22/2026

Prepared September 2025

PURPOSE

The goal of this study was to assess that the anticipated DNA profiles were obtained for all samples analyzed throughout the course of this validation.

MATERIALS AND METHODS

A total of 184 DNA profiles were generated throughout the seven validation runs. The profiles generated were compared to their previous Fusion run to assess concordance. A NIST SRM (SRM 2391d component E) and two NIST traceable blood cards were included and compared to the expected results for concordance. The NIST SRM was run twice, one sample with one punch and the other with two punches. Two punches were also taken from the NIST traceable blood samples.

All samples were punched on the BSD600 Ascent A2 instrument into a plate with 5 µl PunchSolution™. The samples were then dried on a 70°C heat block for 20 minutes and then amplified using the PowerPlex® Fusion System following the data bank PS kit, half reaction protocol with 12.5 µl total volume, consisting of: 2.5 µl 5X master mix, 2.5 µl 5X primer pair mix, 2.5 µl 5X AmpSolution™ reagent, and 5 µl amplification grade water. All plates were amplified using a 9700 thermal cycler (ABI) following the 25 cycle thermocycling reaction for direct amplification and subsequently separated on the 3500*xl* Genetic Analyzer (ABI) for analysis. For consistency, 1 µl of PCR product was loaded onto the CE plate and all plates were injected at 12 and 24 seconds with the following settings: 1.2 kV injection voltage, 15 kV run voltage, 36 cm (length), and Data Collection Software v.3.0. Analysis was completed using the GeneMapper® ID-X v1.4 software (ABI), with 20% global and stutter cutoff filters. The limit of detection (LOD) currently utilized in the DNA data bank (blue 94, green 95, yellow 90 and red 95 RFUs) was also used for this validation. No deviations from approved data bank protocols for amplification, capillary electrophoresis or analysis were made during this validation.

RESULTS

All DNA profiles/alleles detected during the validation process were concordant with the expected results. Seven profiles out of the 184 had drop out; however, these profiles were still accessed for concordance with the alleles present.

CONCLUSIONS

For each well, the instrument was able to scan a barcode, or receive a typed barcode from the analyst, position the plate accurately under the shoot and punch the sample into the corresponding well. Since the expected profiles were developed from all samples, the BSD600 Ascent A2 instrument was able to successfully punch all samples for downstream analysis without any issues. Issues with punching and delivering the sample to the designated well would have been detected while the analyst was using the instrument or during analysis. The partial profiles obtained are not indicative of inconsistent performance by the BSD600 Ascent A2 but rather reflect the variation in the amount of DNA present throughout the samples. Since currently approved and validated data bank direct amplification protocols were used for the punching and downstream analysis of all samples processed, no new sensitivity or stochastic studies were conducted for this validation.