Method Modification of EZ1 Advanced XL Instrument

July 2026

BACKGROUND

Idaho State Police Forensic Services (ISPFS) previously validated the use of the Qiagen BioRobot EZ1 and EZ1 Advanced XL instruments for automated DNA purification. This method modification would be to make adjustments to the current Biology/DNA Casework Analytical Methods for EZ1 Advanced XL extractions.

OBJECTIVE

The goal of this method modification would be three-fold:

- Implement the use of Lyse & Spin Baskets and the addition of dithiothreitol (DTT), along with proteinase K (ProK), for large volume extractions and reference samples that are not processed using direct amplification
- Eliminate the PBS soak during differential extractions and update the lysis step of the F2 fraction
- Evaluate the use of carrier RNA in all extraction types

Reagent blanks will be run with each extraction set and all reagent blanks will be evaluated for any potential contamination.

METHOD UPDATES

Implementation of Lyse & Spin Baskets

Two blood stain cards and three buccal swabs will be collected. Two ~5mm^2 cuttings from the blood cards and two ~1/4 of the buccal swabs will be cut, one set will be labeled Ref_X and be placed a 2mL extraction tube and the other set will be labeled Ref_X_LS and will be placed into a Lyse & Spin Basket. The X will be updated to reflect the appropriate sample number (See Sample List – EZ1 Method Modification Spreadsheet). All of the samples will be extracted on the EZ1 Advanced XL using the current Biology/DNA Casework Analytical Methods. All samples will be quantified and the quantitation results compared. Pending the quantitation values of this study, the remainder of the validation will be performed using Lyse & Spin Baskets where applicable.

Lyse & Spin Basket Samples

Where the current Biology/DNA Casework Analytical Methods state to use a spin basket the Lyse & Spin Basket will be utilized instead. The Lyse & Spin Basket will be loaded into the centrifuge and run on high speed (>10,000 rpm) for 5 minutes. The

top portion of the tube can be discarded, and the bottom portion of the tube can be loaded directly onto the EZ1 Advanced XL for purification.

Use of Carrier RNA in Large Volume and Trace Protocol Extractions

Ten low level / trace samples will be collected and cut in ~½ into separate Lyse & Spin baskets. Both sets of samples will be extracted on the EZ1 Advanced XL, 5 samples will be extracted using the large volume protocol and 5 will be extracted using the trace protocol. The second set of samples will be extracted the same way, with the addition of 1µL of carrier RNA (cRNA) to each lysate. The cRNA from the DNA Investigator kit will be reconstituted and utilized. All samples will be quantified and the quantitation results compared.

Addition of DTT to all Non-Differential Extractions

Ten samples will be collected from the environment (i.e. steering wheel, can, clothing, etc.) to mimic casework samples, which will include varying concentrations of DNA and number of contributors. ~½ of each swab will be cut into a Lyse & Spin Basket and labeled LV_X_DTT and the other ~½ of the swab will be cut into a new Lyse & Spin Basket and labeled LV_X_ProK. The X will be updated to reflect the appropriate sample number. The digest master mix for the DTT samples will be 480µL of SEB, 10µL of ProK and 10µL of DTT. The ProK samples will use the standard master mix of 490 µL of SEB and 10 µL of ProK. All of these samples will be extracted on the EZ1 Advanced XL using the current Biology/DNA Casework Analytical Methods. All samples will be quantified and the quantitation results compared. All samples will also be amplified to determine profile quality.

Elimination of PBS Soak and F2 Lysis Update in Differential Extractions

Ten buccal swabs will be collected and spotted with 50µL of varying concentrations of semen (Table 1). ~½ of each swab will be cut into separate 1.5mL tubes. One set of tubes will be labeled Diff_X_PBS and the other set will be labeled Diff_X. The X will be updated to reflect the appropriate sample number. The samples with PBS in the name will be extracted using the current Biology/DNA Casework Analytical Methods; while the second set of samples will be processed using the differential sample preparation listed below. Both sets of samples will be extracted using the EZ1 Advanced XL instrument using the trace protocol. All samples will be quantified and the quantitation results compared. All samples will also be amplified to determine profile quality.

Table 1: Semen Dilutions

Sample Number	Dilution Factor	Semen (µL)	TE (µL)
1 and 2	Neat	50	0
3 and 4	1:10	5	45
5 and 6	1:15	5	70
7 and 8	1:25	4	96
9 and 10	1:32	4	124

Differential Sample Preparation:

1. Add 10µL ProK and 190µL SEB.
2. Incubate at 56°C for 1 – 2 hours, do not exceed 2 hours, in a thermomixer shaking at 900rpm or a heat block.
3. Centrifuge briefly to get liquid off lid. Remove the substrate using piggyback/spin basket centrifugation on high speed (>10,000 rpm) for 5 minutes. Discard substrate.
4. Remove all but ~10-50µL of the supernatant, taking care not to disrupt the cell pellet in the bottom of the tube. Transfer this supernatant (Fraction 1, epithelial cell fraction) to the new, labeled sample tube. Proceed to loading this sample onto the EZ1 Advanced XL instrument and run using the Trace protocol.
5. Wash the sperm pellet as follows: resuspend the pellet in 500µL of PBS by vortexing briefly. Spin in a microcentrifuge for 5 minutes at maximum speed (>10,000 rpm). Remove all but about ~10-50µL of the supernatant and discard it. Repeat this step 3 more times, using sterile deionized water for the last wash.
6. Add 160µL of SEB, 10µL ProK and 40µL DTT and mix thoroughly (a total of 210µL to each sample).
7. Incubate at 70°C for 10min in a thermomixer shaking at 900rpm or a heat block.
8. This sample (Fraction 2, sperm fraction) is now ready to be processed on the EZ1 Advanced XL using the Trace Protocol.

RESULTS

Implementation of Lyse & Spin Baskets

Five reference samples were extracted and the quantitation values compared. The quantitation values for the samples extracted in the Lyse & Spin baskets were not significantly different than the quantitation values obtained for the samples extracted in the 1.5mL tubes currently used (Tables 2 and 3).

Table 2: Comparison between 1.5mL tubes and Lyse & Spin tubes – Auto quant

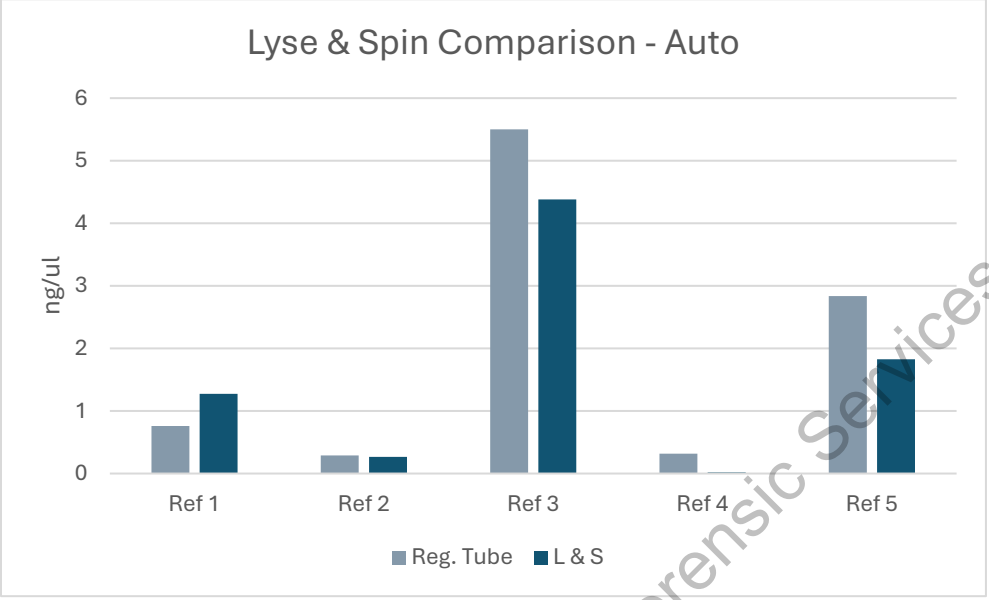
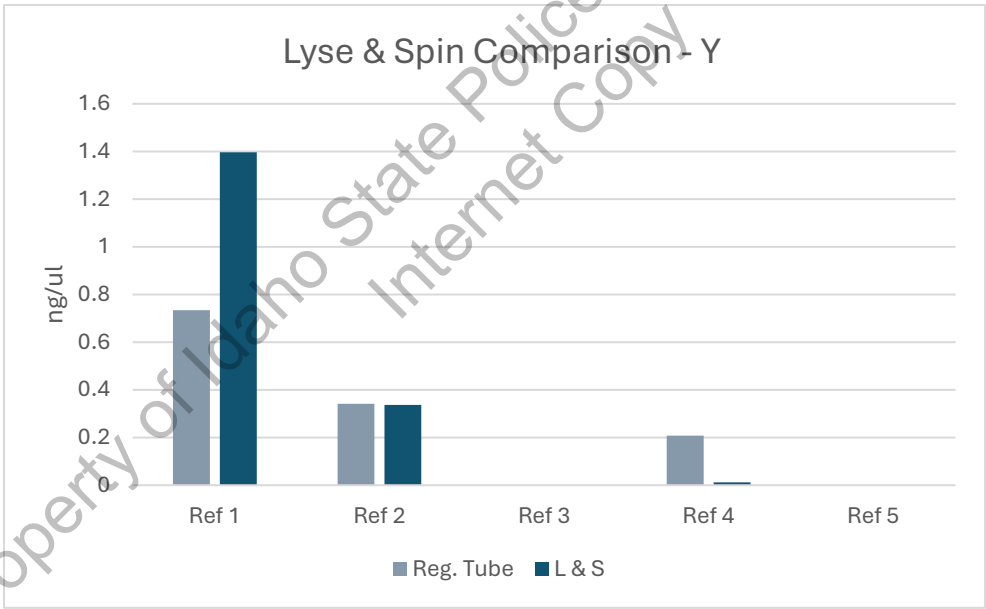


Table 3: Comparison between 1.5mL tubes and Lyse & Spin tubes – Y quant



The Lyse & Spin baskets are suitable for extraction and will be used for the addition of DTT to all non-differential extractions and the EZ2™ Connect Fx Instrument Validation.

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_____ 11/26/2025

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Use of Carrier RNA in Large Volume and Trace Protocol Extractions

Ten non-probative samples were processed using the large volume and the trace extraction protocols. To one set of samples, cRNA was added prior to loading the extracts onto the instrument. The quantitation values for the samples extracted with the cRNA were not significantly different than the quantitation values obtained for the samples extracted without the addition of cRNA (Tables 4 and 5). Sample 10 had a significantly higher level of DNA than the other non-probative samples; this was expected as this sample is a cigarette butt. Sample NP10 had auto/Y quantitation values of 0.3044/0.2837 (without cRNA) and 0.2792/0.2851 (with cRNA). These values are not accurately represented in the tables below.

Table 4: Evaluation of cRNA – Auto quant

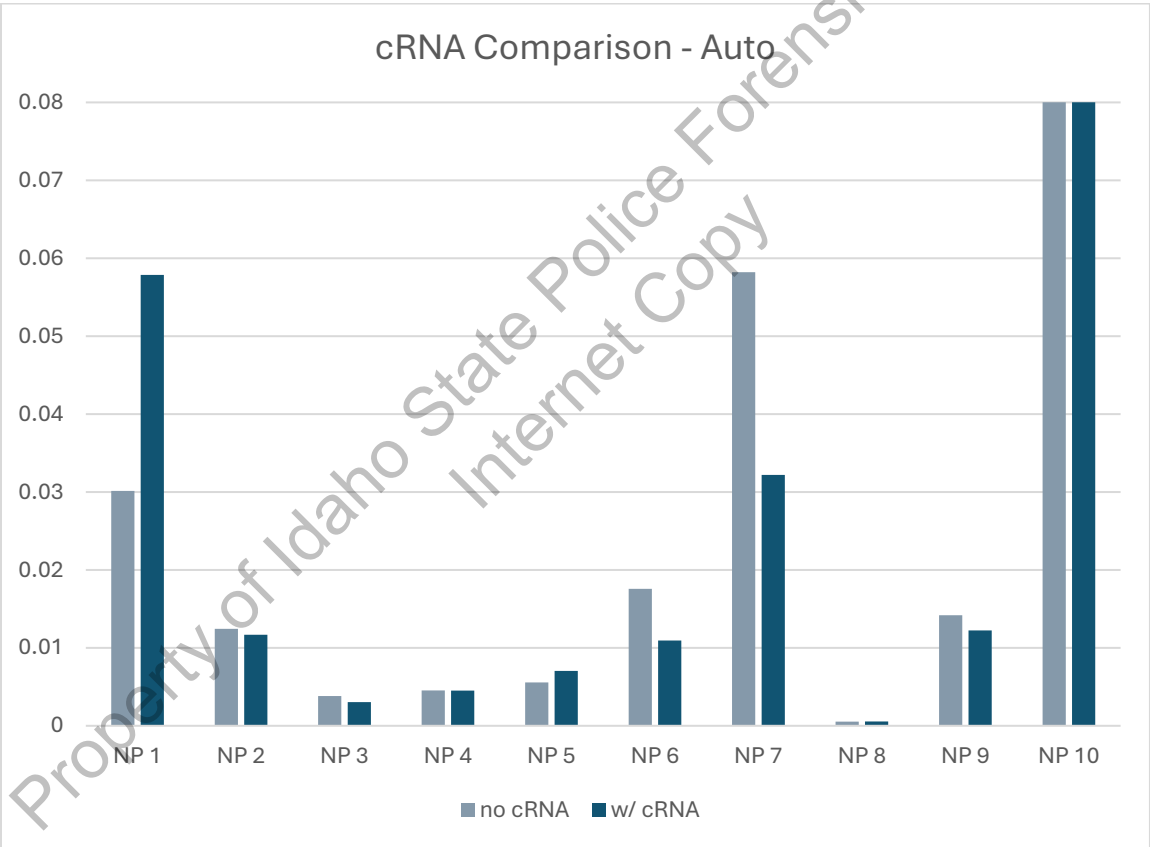
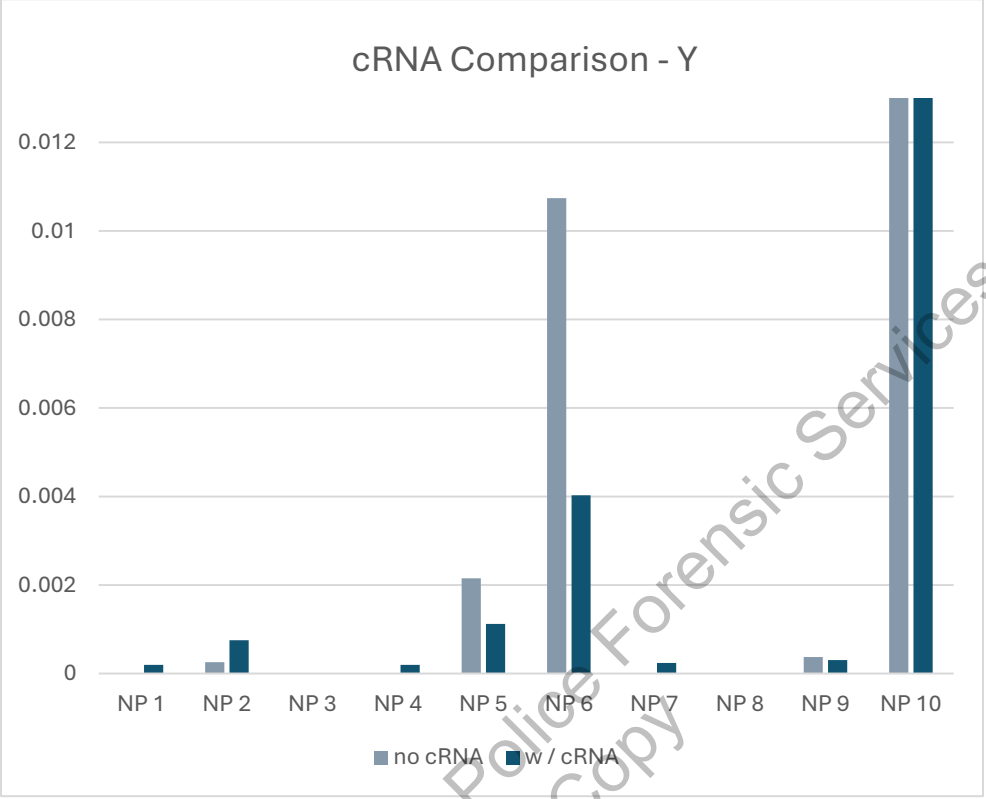


Table 5: Evaluation of cRNA – Y quant



The quantitation results of these samples do not suggest an increased yield is obtained with the addition of cRNA to all samples. Based on this information, cRNA will not be incorporated into the remainder of the EZ1 modification or the EZ2™ Connect Fx Instrument Validation.

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11/26/2025
Date

Addition of DTT to all Non-Differential Extractions

Ten non-probative samples were processed using the large volume extraction protocol. One set of samples had DTT and ProK added to the master mix and the second set of samples had only ProK added to the master mix. The quantitation values for the samples extracted with the DTT and ProK were not significantly different than the quantitation values obtained for the samples extracted with the addition of only ProK (Tables 6 and 7). The quantitation values for Samples LV7 and LV10 were significantly higher than the other

samples, and the quantitation values are not accurately represented in the Y Quant table below. Sample LV7 had a male quantitation value of 0.1492ng/µL and Sample LV10 had a male quantitation value of 1.585ng/µL.

Table 6: Evaluation of DTT/ProK vs ProK – Auto quant

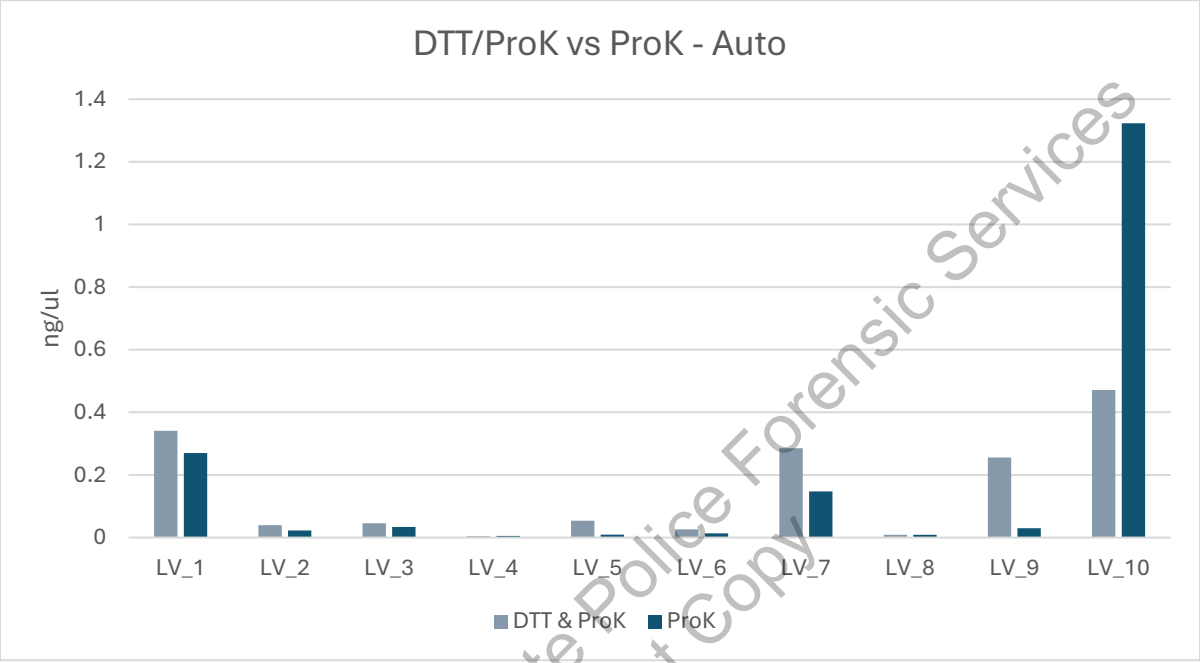
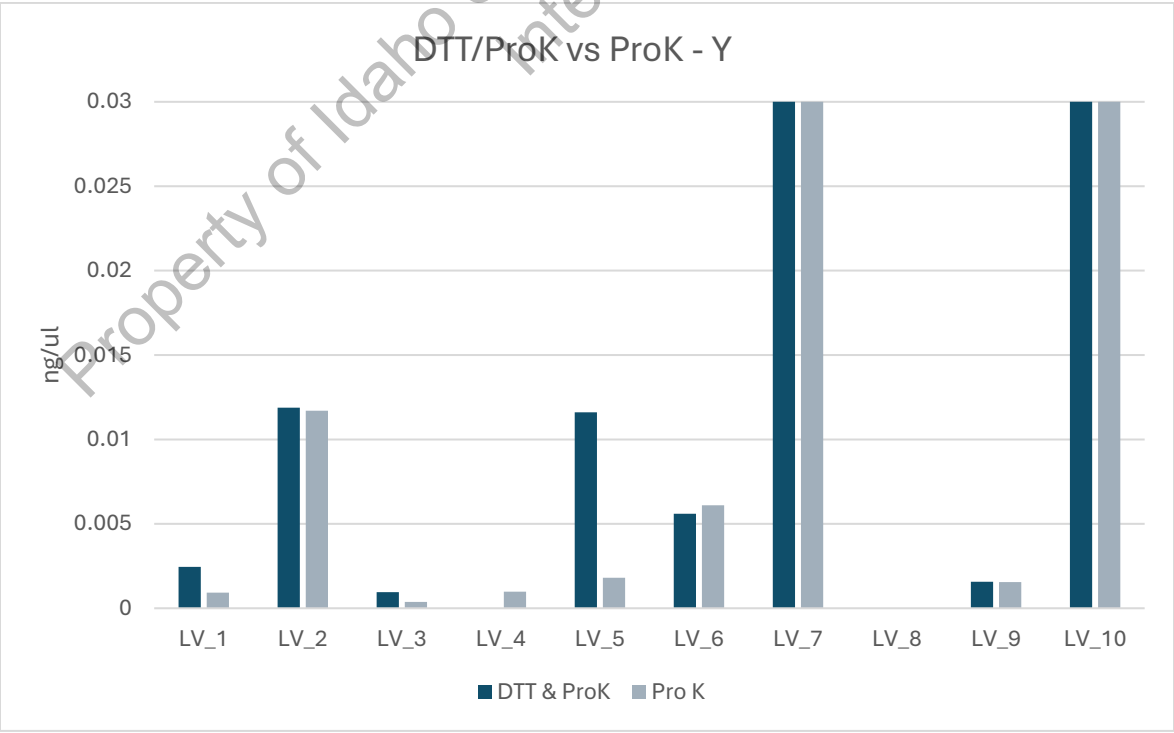


Table 7: Evaluation of DTT/ProK vs ProK – Y quant

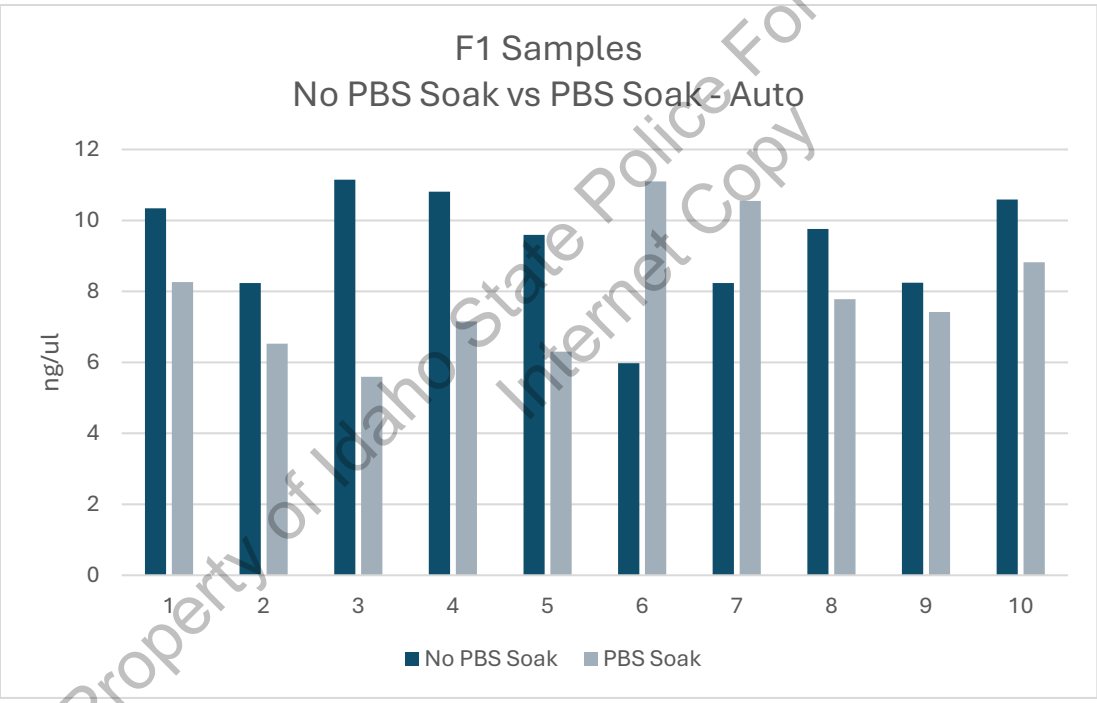


DNA profiles were developed from these samples and compared. The addition of DTT during the extraction process did not have any negative effects on the DNA profiles developed. Similar number of alleles and profile quality were developed from the samples extracted using just ProK and the samples that were extracted using both ProK and DTT.

Elimination of PBS Soak and F2 Lysis Update in Differential Extractions

Ten buccal swabs spotted with varying concentrations of semen samples were processed using the trace extraction protocol. One set of samples had a PBS overnight soak performed and the second set of samples did not have a PBS overnight soak. The quantitation values for the samples extracted with the PBS soak were not significantly different than the quantitation values obtained for the samples extracted with the PBS soak (Tables 8 and 9).

Table 8: Evaluation of PBS Soak vs No PBS Soak – Auto quant



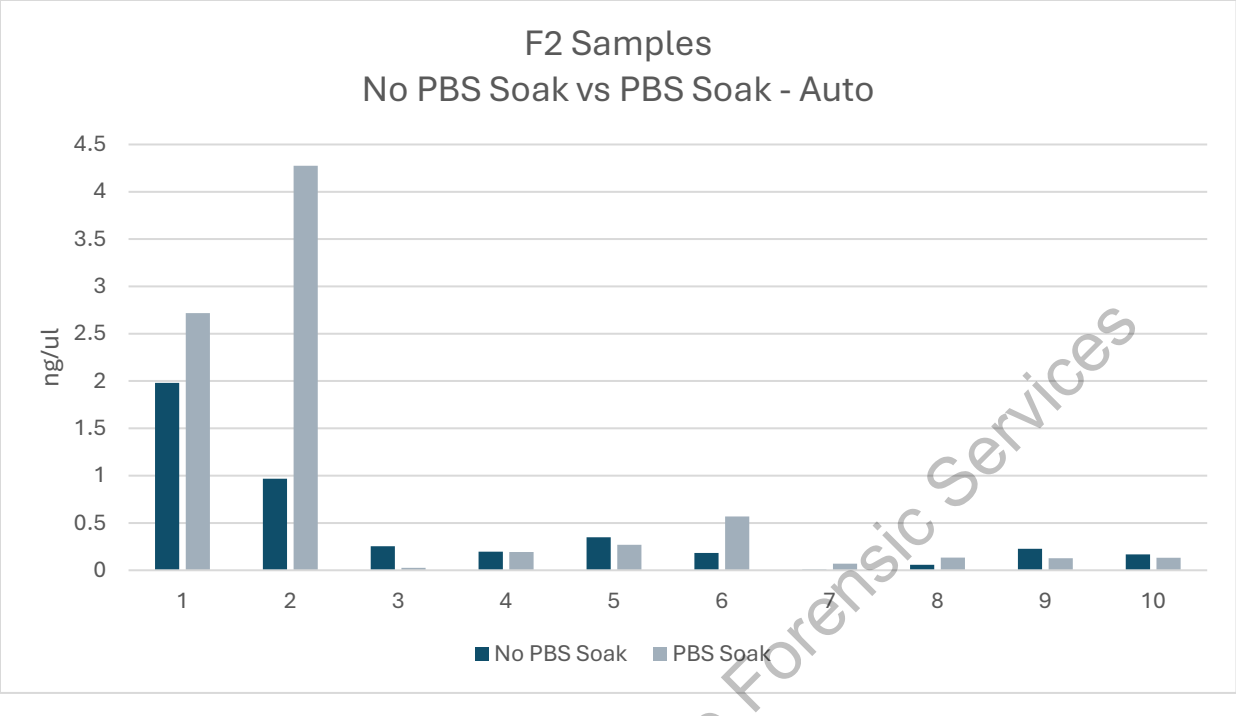
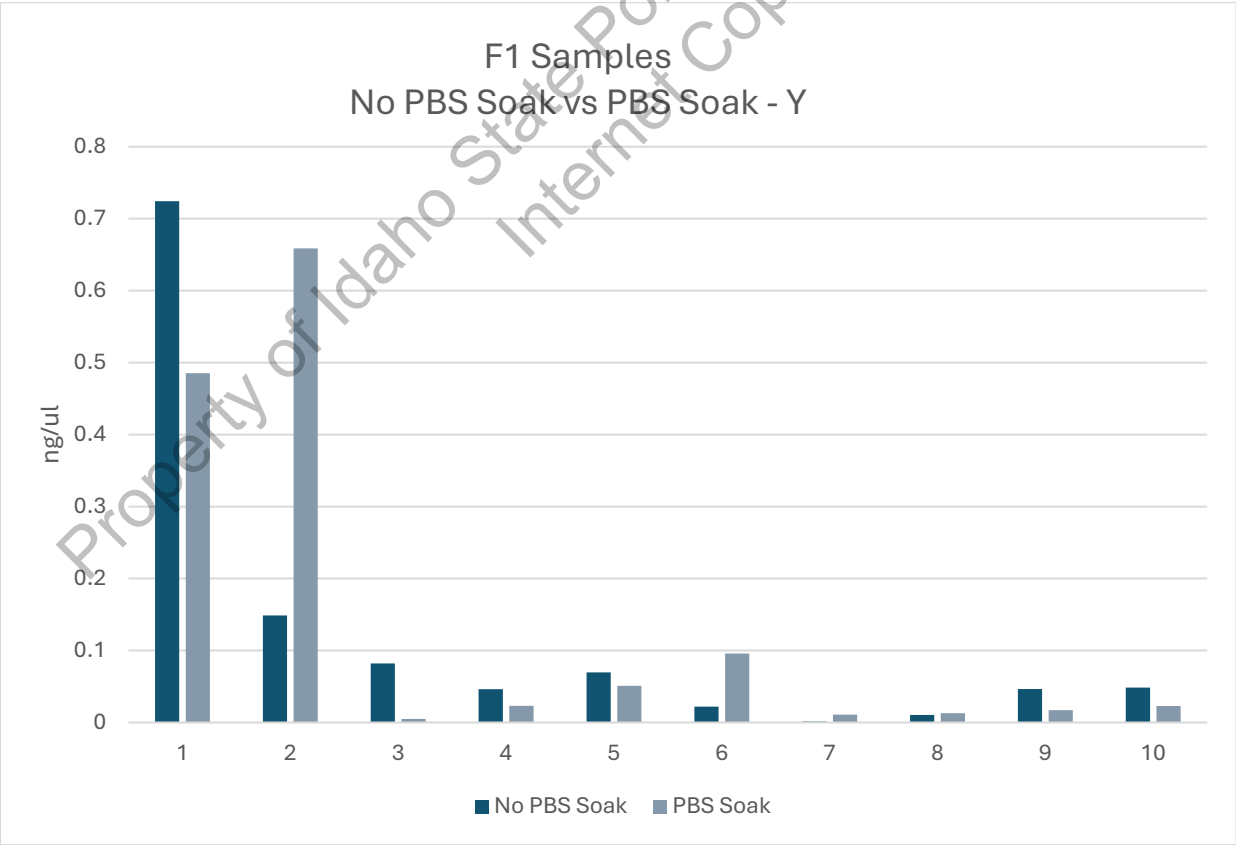
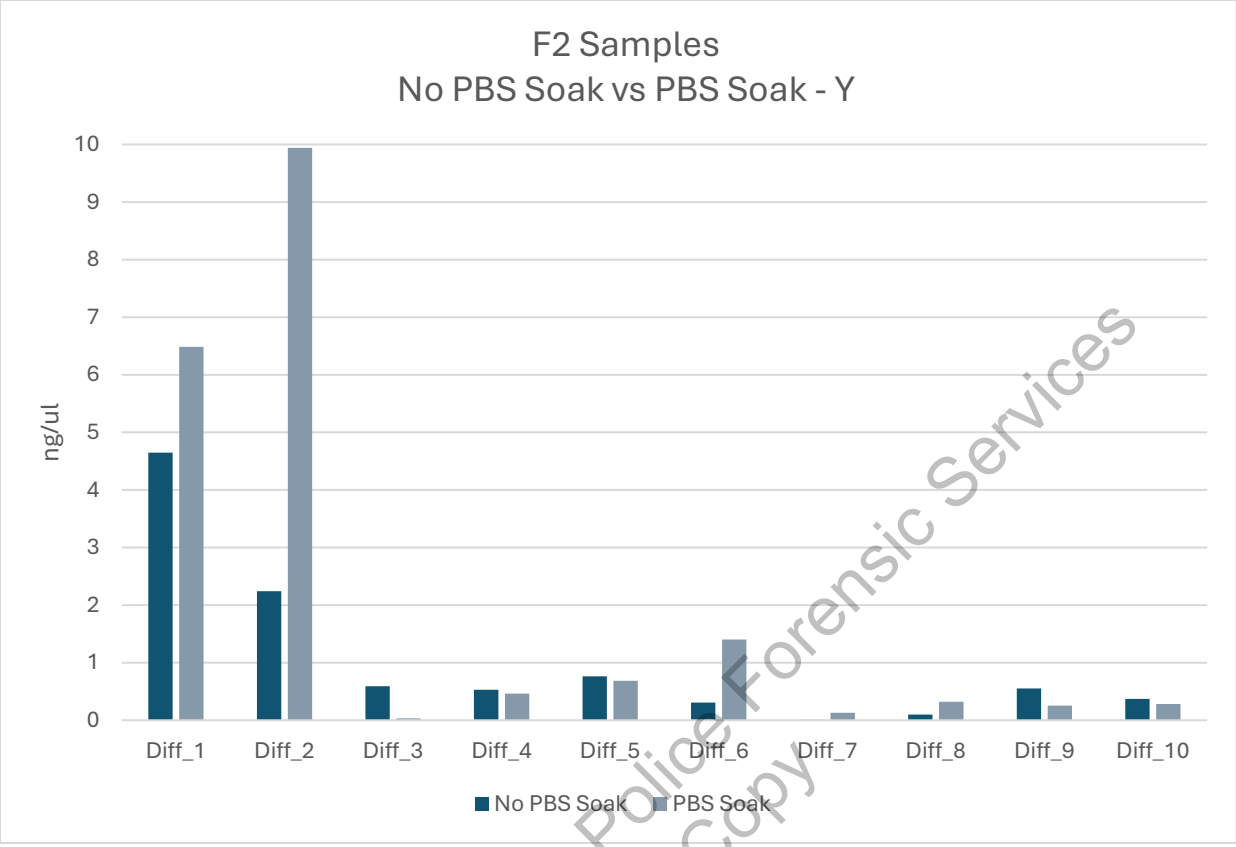


Table 8: Evaluation of PBS Soak vs No PBS Soak – Auto quant





DNA profiles were developed from these samples and compared. The removal of the PBS soak during the extraction process did not have any negative effects on the DNA profiles developed. Similar number of alleles and profile quality were developed from the samples extracted using the PBS soak and the samples that were extracted without the PBS soak.

RECOMMENDATIONS

It is recommended that non-differential samples be cut into Lyse & Spin baskets (approved above) and have DTT added during extraction. The addition of cRNA is not recommended based on these results (approved above). The PBS soak in differential extractions can be removed to streamline the differential extraction procedure and the master mix ratios and volume can be changed for Fraction 2 of differential samples.

REFERENCES

1. Biology/DNA Casework Analytical Methods, Revision17.
2. QAS Forensic DNA Testing Laboratories, 07/01/2025.
3. EZ1&2™ DNA Investigator® Kit Handbook, April 2025.
4. Excel document, Data Analysis – EZ1 Modification

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