	Intermountain Forensics	VAL #	EXT-V01 (supplemental 2.0)
Forensic DNA Technical Leader Approval		**Issue Date**	
		6/30/20	

EZ1 Advanced XL DNA Investigator Supplemental Studies

1. Summary and Conclusions

Combinations and Supernatant Collection
This study supplements the EX1 Advanced XL DNA investigator validation. Within these studies we attempt to determine the viability of 1) using the lyse and spin baskets to incorporate multiple substrate tubes into one final extract (tube combination study), 2) using supernatant collected after pelleting the cellular material in the initial spin down of the sample for serology testing (supernatant testing study) 3) determining if incubation time,15 or 30 minutes, affects serological testing results (incubation time study) 4) determining whether yield is better as a result of just incubation or incubation + shaking (incubation shaking study) and 5) determining if TE buffer can be utilized in lieu of universal buffer in RSID serological testing (buffer study).

Summary:
Tube combination Study:
A combination method prior to EZ1 extraction, which can include supernatant collection for bodily fluid testing, was performed to determine its suitability for use on casework samples using RSID Saliva, RSID Semen, and phenolphthalein for blood. One set of each cell type was placed into a tube and spin basket and incubated on the lab bench and the other was shaken on the themomixer at 600rpm without heat for 1 hour. Samples were centrifuged at max speed for 5 minutes to remove the substrate and pellet the cells. After removal of the supernatant, 10 samples were combined, resulting in 5 samples, ensuring a female saliva or blood sample was mixed with a semen sample. Like dilutions were combined (1:10 into 1:10). 6 Samples containing semen were separated using the QIAcube Connect and extracted on the EZ1 instrument. 2 Non-differential samples were extracted on the EZ1 instrument. All samples were quantified with Quantifiler Trio and Quantiplex Pro.

Supernatant Collection study:
Dilutions of 1:10, 1:100, 1:1,000, and 1:10,000 were made for semen, blood, and saliva. 50ul of each was added to swabs and each swab was cut into 2 tubes. 250ul of TE was added to each tube. The samples were incubated and spun down as per extraction protocol. The supernatant was removed and RSID Saliva, RSID Seminal Fluid and Phenolphthalein tests were run.

Buffer study:
The supernatant collection study was run with TE Buffer, an additional RSID saliva and RSID Seminal Fluid test using the Universal Buffer provided by RSID was performed to determine if any results were different using the manufacture provided buffer verses TE.

Incubation Shaking study:
One set of samples (from supernatant collection study) was incubated for 1 hour on the lab bench while a second set of samples was incubated while shaking in the thermomixer without heat and run with RSID Saliva and RSID Seminal Fluid.

Incubation time:
Three sets of samples (from supernatant collection study) were incubated for 15, 30 and 60minutes and run with RSID Saliva and RSID Seminal Fluid.

Conclusions:
Tube combination Study:
The combination and supernatant collection method tested has shown to be able to be successful at detecting both profiles used for the combination and obtain positive supernatant test results for semen, saliva and blood with an incubation time of 15 minutes in a thermomixer shaking at 600RPM. Combination results were not able to be visualized simply by the quantification Auto:Y Ratio so 4 epithelial their associated sperm fractions were amplified with Globalfiler to assess the electropherogram data.

Table 1: Non Differential tube combinations quant data and representative Egram:

Sem. Tube	Contains	Quaniplex Human (ng/ul)	Quantiplex Y (ng/ul)	Auto:Y Ratio
1:1,000	1:1,000 Saliva	0.00277	0.00172	1.60
1:10,000	1:10,000 Blood	0.000873	0.000616	1.42

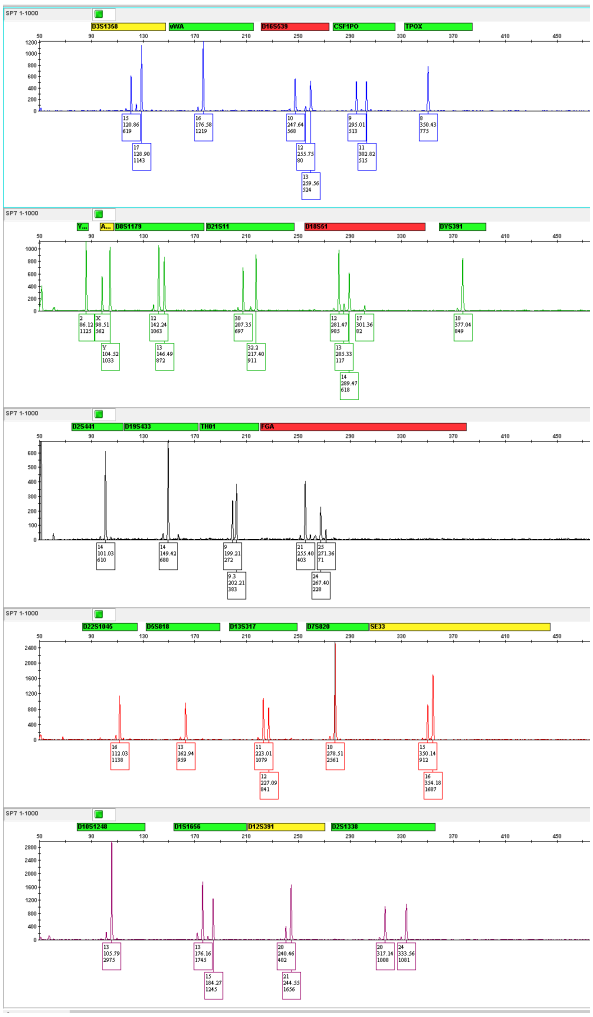
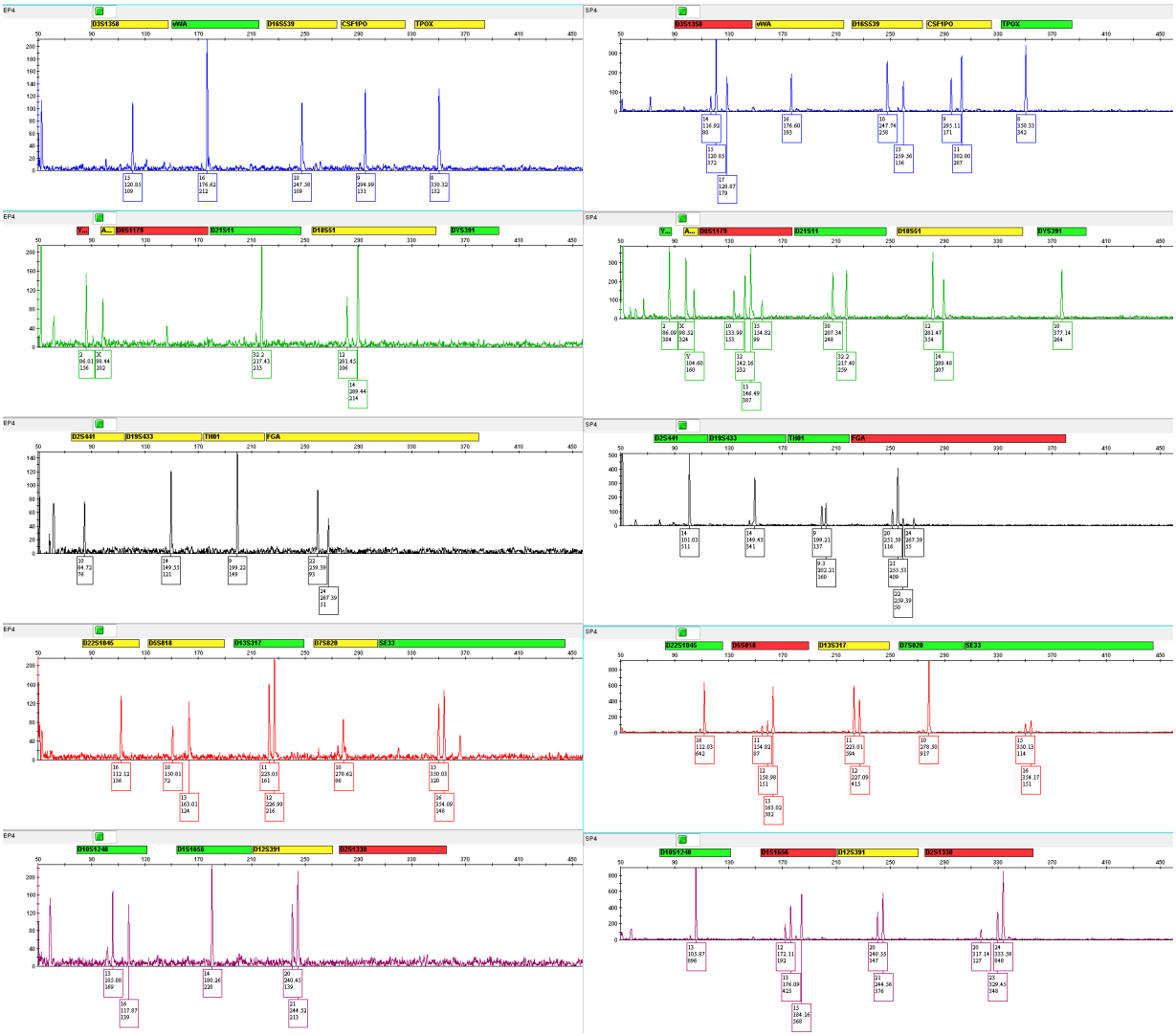


Table 2: Differential tube combinations quant data and representative Egrams:

Contains	Tube	Quaniplex Human (ng/ul)	Quantiplex Y (ng/ul)	Auto:Y Ratio
1:10 Semen S 1:10 Blood S	EP1	0.14733	0.10552	1.40
	SP1	0.228	0.151	1.51
1:100 Semen S 1:100 Saliva S	EP2	0.01811	0.01038	1.75
	SP2	0.02427	0.01482	1.64
1:1,000Semen S 1:1,000 Blood S	EP3	0.00082	0.00038	2.17
	SP3	0.00116	0.00031	3.75
1:10,000Semen S 1:10,000 Saliva S	EP4	0.00041	0.00033	1.26
	SP4	0.00167	0.00119	1.40
1:10 Semen 1:10 Blood	EP5	0.18815	0.13084	1.44

	SP5	0.19530	0.14658	1.33
1:100 Semen 1:100 Saliva	EP6	0.01102	0.00016	67.93
	SP6	0.00028	0	N/A



Tube combinations were not able to be confirmed based on the quantification Auto:Y ratios alone. An internet search revealed that the number of cells/ul in semen could be 5-27 times higher than in blood and saliva. The results obtained support this. 4 epithelial fractions and their associated sperm fractions were amplified with Globalfiler to observe any separation. A major male profile was observed in the epithelial fraction that is consistent with the semen donor and a minor profile was observed consistent with the female donor in the 1:10 and 1:100 dilutions. The 1:1,000 and 1:10,000 dilutions resulted in low RFU heights and a minor profile was not observed in both differential and non-differential samples. DNA concentrations appear to indicate that a higher amount of DNA can be obtained from lower level samples if shaken vs. incubation on the lab bench.

As per the results of this study, combining contents of substrate tubes is able to effectively concentrate DNA samples from multiple substrate tubes. Where multiple substrate tubes are obtained from one sample, the laboratory will utilize

this method to combine the substrates into one DNA extract (note: combining different items into one extract is against general laboratory processing policy)

Supernatant Collection study:
Positive results were obtained down to the 1:10 dilution of semen, 1:100 dilution for Saliva, and 1:100 dilution for blood (See table).

As per the results of this study, utilizing supernatant for serological testing with RSID Saliva and RSID Seminal Fluid is an effective way to characterize results without further testing and/or destruction of sample. This process will be utilized as standard practice for sexual assault kits and samples requiring serological testing.

Buffer study:
No changes in results were observed between using TE and Universal Buffer on the dilutions made (See table 3).

As per the results of this study, supernatant serology testing will utilize TE buffer as this is more practical and in line with DNA extraction processing.

Incubation Shaking study:
No difference was observed between sample shaking and non-shaking, however, Product brochure indicates serology results were consistent down to a 1 minute incubation time while shaking (See table 3).

As per the results of this study, supernatant serology testing will utilize shaking during incubation as recommended from the manufacturer.

Incubation time:
No difference was observed between sample incubation times of 1 hour, 30 minutes, and 15 minutes. Product brochure indicates serology results were consistent down to a 1 minute incubation time while shaking (See table 3).

As per the results of this study, supernatant serology testing will utilize a 15 minute incubation time to reduce processing time while still maintaining highest quality results.

Table 3: Serogical Results – Supernatant testing

Body Fluid	Dilution	1 Hour Incubation		30 Minute Incubation	15 Minute Incubation
		Result with TE	Result with Universal Buffer	Result with TE	Result with TE
Semen	1:10	Positive			
	1:10Shaken	Positive	Positive	Positive	Positive
	1:100	Negative			
	1:100Shaken	Negative	Negative	Negative	Negative
	1:1,000	Negative			
	1:1,000Shaken	Negative	Negative	Negative	Negative
	1:10,000	Negative			
	1:10,000Shaken	Negative	Negative	Negative	Negative
Saliva	1:10	Positive			
	1:10Shaken	Positive	Positive	Positive	Positive
	1:100	Positive			
	1:100Shaken	Positive	Positive	Positive	Positive
	1:1,000	Negative			
	1:1,000Shaken	Negative	Negative	Negative	Negative
	1:10,000	Negative			
	1:10,000Shaken	Negative	Negative	Negative	Negative
Blood	1:10	Positive			
	1:10Shaken	Positive	Positive	Positive	Positive
	1:100	Positive			
	1:100Shaken	Positive	Positive	Positive	Positive
	1:1,000	Negative			
	1:1,000Shaken	Negative	Negative	Negative	Negative
	1:10,000	Negative			
	1:10,000Shaken	Negative	Negative	Negative	Negative

2. Validation Personnel

The following individuals were integral to the validation and have gained competency as a result of this validation:
Alyssa McElreath
Derek Cutler
Daniel Hellwig

3. Validation Components

Benchmark Thermomixer #1 (SN: A057-02839)
Benchmark Thermomixer #2 (SN: A058-03087)
RSID Saliva
RSID Semen
Phenolphthalein Presumptive Blood Test Kit (Medtech Forensics)
Costar Spin-X Insert Columns