Supplemental Validation of the Y-Screen Protocol for the Rapid Processing of Sexual Assault Kits

BACKGROUND

Since the original Y-screen Protocol was validated in 2017 and the supplemental validation in 2018, additional considerations regarding sampling size have arisen. The purpose of using a larger cutting size is to increase the potential for obtaining enough DNA for further analysis. In practice, it has been observed that taking additional cuttings for DNA has resulted in lower yields when compared to the initial cuttings. Additionally, taking larger cuttings up front for non-differential sample types eliminates the need for taking additional cuttings for DNA processing, and therefore preserves more of the evidence. This improved methodology could also allow for Y-screen processing of low-yield samples, like fingernail scrapings or skin swabs, that could be depleted with multiple sampling attempts [1].

OBJECTIVE

This supplemental validation will evaluate whether increasing the substrate size will negatively impact the original Y-screen Protocol.

PROCEDURE

The current ISPFS Y-screen Protocol uses up to four cuttings of ~1/8 swab per sample for a total of ~1/2 swab per tube. However, this study will use four cuttings of ~1/4 swab per sample for a total of 1 swab per tube. The SwabSolution™ and Y-screen quantitation protocols will otherwise remain unchanged.

For this study, a mixture of 6 semen and 8 non-semen sample types will be used. The 6 semen samples were from the original validation and have previous Y-screen results. A cutting of ~1/4 of each swab from each semen sample was taken and underwent Y-screen lysis and quantitation in duplicate. The results obtained from this quantitation were compared to the original data for these previously tested samples. The 8 non-semen sample types were comprised of four samples. Two of these samples (the high friction skin contact) were initially sampled for a total of ~1/2 or ~1/3 swab per tube (per the current ISPFS Y-screen Protocol), by either taking ~1/8 of four swabs or ~1/6 of two swabs, and then additionally sampled for a total of ~1 swab per additional tube, by either taking ~1/2 of two swabs or ~1/4 of four swabs. The remaining two of the non-semen samples (the saliva to skin lighter contact and saliva to skin heavier contact) were initially sampled for a total of ~1/3 swab per tube (per the current ISPFS Y-screen Protocol), by taking ~1/6 of two swabs, and then additionally sampled for a total of ~1 swab per additional tube, by taking ~1/2 of two swabs. Each of these also underwent Y-screen lysis and quantitation in duplicate. The results obtained from the two variations of these sample types were compared to one another.

Additionally, the current lysis volume was assessed to determine if it is sufficient to adequately saturate the larger substrate amount and lyse the increased amount of DNA present. The latter was assessed through evaluation of quantitation values.

MATERIALS

All materials needed for this study were already commonplace in the ISPFS Casework Unit as part of the current DNA processes. Refer to the Biology/DNA Casework analytical methods for the detection of male DNA on sexual assault kit evidence for the materials needed for this analysis.

RESULTS

Summary tables compiling the data for each of the sample categories are below, including non-semen sample types that were sampled as a ~1/2 total swab within each tube and ~1 swab within each tube, and semen sample types that were sampled as a ~1/2 total swab within each tube and ~1 swab within each tube. The autosomal DNA quantitation values, male DNA quantitation values, and the ratio of autosomal to male DNA are provided for each individual sample, as well as those averages of the duplicate sample types.

It was determined that a larger lysis volume for all Y-screen samples was not needed at this time.

Half Swab – Non-Differentials						
Sample	**Auto (ng/ul)**	**Y (ng/ul)**	**Ratio**	**Auto (ng/ul) Average**	**Y (ng/ul) Average**	**Ratio Average**
HFS_1	0.002	0.001	2.60	0.003	0.001	2.75
HFS_1	0.004	0.001	2.90			
HFS_2	0.009	0.002	4.60	0.0095	0.0025	4.25
HFS_2	0.010	0.003	3.90			
SS_K	0.023	0.013	1.70	0.021	0.0105	2.05
SS_K	0.019	0.008	2.40			
SS_L	0.025	0.011	2.30	0.033	0.015	2.20
SS_L	0.041	0.019	2.10			

Table 1: Non-differential (non-semen) samples' (with a half swab total) autosomal DNA, male DNA, and auto/Y ratio quantitation values. (HFS- high friction skin contact, SS_K- saliva to skin lighter contact, SS_L- saliva to skin heavier contact).

Whole Swab – Non-Differentials						
Sample	**Auto (ng/ul)**	**Y (ng/ul)**	**Ratio**	**Auto (ng/ul) Average**	**Y (ng/ul) Average**	**Ratio Average**
HFS_1	0.032	0.011	2.90	0.028	0.0125	2.30
HFS_1	0.024	0.014	1.70			
HFS_2	0.009	0.001	7.40	0.0105	0.001	8.75
HFS_2	0.012	0.001	10.10			
SS_K	0.006	0.002	2.60	0.009	0.004	2.30
SS_K	0.012	0.006	2.00			
SS_L	0.046	0.027	1.70	0.0565	0.0335	1.69
SS_L	0.067	0.040	1.68			

Table 2: Non-differential (non-semen) samples' (with a whole swab total) autosomal DNA, male DNA, and auto/Y ratio quantitation values. (HFS- high friction skin contact, SS_K- saliva to skin lighter contact, SS_L- saliva to skin heavier contact).

Half Swab – Differentials			
Sample	**Auto (ng/ul)**	**Y (ng/ul)**	**Ratio**
D36_2Days	27.400	0.068	404.30
D13_3.5Days	8.960	0.005	1971.60
D23_1Day	37.400	0.134	278.50
D2_1Day	111.00	0.010	11018.0
D37_6Days	112.00	0.0002	527851.2
D17_5Days	25.200	0.002	12313.10

Table 3: Original validation differential (semen) samples (with a half swab total) and the original autosomal DNA, male DNA, and auto/Y ratio quantitation values. (Differential samples are post-coital samples at various intervals dictated in the previous validation).

Whole Swab – Differentials						
Sample	**Auto (ng/ul)**	**Y (ng/ul)**	**Ratio**	**Auto (ng/ul) Average**	**Y (ng/ul) Average**	**Ratio Average**
D36_2Days	19.60	0.104	188.30	20.15	0.097	209.15
D36_2Days	20.70	0.090	230.00			
D13_3.5Days	9.450	0.020	475.50	9.54	0.020	474.70
D13_3.5Days	9.630	0.020	473.90			
D23_1Day	14.20	0.119	119.20	16.1	0.13	123.7
D23_1Day	18.0	0.141	128.20			
D2_1Day	71.60	0.006	12602.5	72.65	0.007	10984.8
D2_1Day	73.70	0.008	9367.10			
D37_6Days	113.0	0.003	38299.9	107.0	0.0016	262410.4
D37_6Days	101.0	0.0002	486520.9			
D17_5Days	32.70	0.003	12267.0	38.6	0.002	11105.15
D17_5Days	44.5	0.001	9943.30			

Table 4: Original validation differential (semen) samples' (with a whole swab total) autosomal DNA, male DNA, and auto/Y ratio quantitation values. (Differential samples are post-coital samples at various intervals dictated in the previous validation).

CONCLUSIONS

In general, increasing the substrate size of the samples undergoing lysis did not negatively impact the original Y-screen Protocol. There was an overall increase in the quantitation values of most sample types. Several samples demonstrated more than a doubling of their quantitation values when comparing the half swab and whole swab cuttings of each sample. This can be seen with the high friction skin contact (HFS_1) and saliva to skin heavier contact (SS_L) as viewed in Table 1 and Table 2. The increase in quantitation value of these samples, even of initially low-level DNA samples, exemplifies that there is potential for obtaining sufficient DNA for further analysis with this improved methodology.

There was a lack of increase in some sample types, such as the non-semen samples high friction skin contact (HFS_2) and the saliva to skin lighter contact (SS_K) when comparing their quantitation values from Table 1 and Table 2. While there is a lack of increase in general for these two sample types, this can be due to a variation in their sample collection. Although the half swab and whole swab cuttings from both samples were cut from the same sets of swabs, they could not be cut from the exact same location.

During the sample collection, there could have been a lack of complete uniform collection around all sides of each swab leading to the variation in quantitation values. This demonstrates that taking subsequent cuttings, such as taking initial cuttings for Y-screen and additional cuttings for DNA analysis, can result in lower yields during the second sampling.

Several semen samples had an increase in quantitation value when the sample included the equivalent of a whole swab of cuttings versus the equivalent of a half swab of cuttings (Table 3 and Table 4). However, there were some semen samples that displayed a lack of increase in quantitation value, such as samples D23_1Day and D2_1Day. This can also be due to the lack of complete uniform collection leading to a variation in what was sampled. Additionally, the semen samples were older and had been frozen in long-term storage between validation analyses. The age of these samples could explain why there wasn't an increase in all the quantitation values as expected, because DNA is known to breakdown over time. While these semen sample types helped further demonstrate the benefit of eliminating the need for subsequent cuttings that cause variation, this sampling method will not be necessary for this sample type and will only be used for non-semen (non-differential) sample types.

Overall, utilizing a larger cutting size for non-semen (non-differential) sample types provides the potential for obtaining higher quantitation values. By implementing this improved methodology in the Y-screen process it will provide enough DNA for further analysis of those samples as needed without the need for additional cuttings, allow for an up-front processing of low-yield samples, and preserve more of the evidence.

REFERENCES

[1] Idaho State Police Forensic Services (ISPFS). Y-screen Protocol Supplemental Validation Summary. 2019.

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