Evaluation of Swab Solution™ Extraction Method in Single Sourced Mock Case Samples

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Abstract: An increasing number of samples for DNA processing induces crime laboratories to implement efficient workflows. At present, Swab Solution™ Kit extracts directly amplified using PowerPlex® Fusion 6C has proven to be an effective tool for routine analysis of blood and buccal swabs. With the aim of abbreviating workflow for casework samples, the capacity of Swab Solution™ kit in extracting DNA and its success in generating genetic profiles when amplified with PowerPlex® Fusion 6C was evaluated and compared with the performance of DNA IQ™ System, the routine procedure used by the PNP Crime Laboratory. Mock casework samples involving single profiles on different matrices (blood-stained item, fingernail clippings, and cigarette butt and hair samples) were prepared and extracted using the Swab Solution™ Kit and DNA IQ™ System. Samples from both group were simultaneously amplified with a reduced volume of PowerPlex® Fusion 6C followed by capillary electrophoresis using AB3500 Genetic Analyzer. Experimental results demonstrated that Swab Solution™ method has superior performance over DNA IQ™ System in generating DNA profiles from epithelial cell containing specimen such as cigarette butts, and facemasks. Single profile analysis of blood on different matrices processed with Swab Solution™ produced full and well balanced profiles similar to DNA IQ™ extracts. In addition, this experiment demonstrates that Swab Solution™- PowerPlex® Fusion 6C Method can be a powerful strategy in decreasing the examination cost and reducing laboratory processing time

Key Words: forensic; DNA extraction; direct amplification

1. Introduction

Increasing influx of casework samples prompts forensic laboratories to evaluate methods that will abbreviate workflow, increase laboratory throughput and promote efficient allocation of resources. Recent advancements in the field of DNA technology promote the use of direct amplification, a laboratory method that shortens processing time by eliminating purification and quantitation steps from the standard DNA Analysis procedure (Brito et. al, 2015; Gray et. al, 2014; Hall et. al, 2014). Direct amplification method which uses innovative STR multiplexes that are tolerant of amplification inhibitors were found to be effective on FTA® cards but not on non-FTA® treated substrates. For this type of samples, pre-treatment which involves the addition of lysing and deproteinating agent to make DNA accessible prior to amplification was recommended (McLaren et. al, 2012).

In the PNP Crime Laboratory DNA Analysis Branch, direct amplification using Powerplex Fusion™ 6C (Promega Corporation, Madison USA) were found to be successful on ordinary buccal swab samples extracted with Swab Solution™ Kit (Promega Corporation, Madison USA) as proven by validation studies and routine reference sample

analysis. Consistent and reproducible result obtained during sample processing shows that this two-step process is robust and tolerant to large amounts of DNA inputs.

Considering that casework samples are usually embedded into substrates that do not contain lysing nor deproteinating agent, the possibility of using of Swab Solution™ method in extracting DNA from evidentiary specimen were explored. A research was performed to test the efficacy of Swab Solution Kit on mock casework samples and compare it with the existing laboratory protocol involving magnetic bead extraction using DNA IQ. This study intends to evaluate the success of Swab Solution Kit extraction in tandem with the direct amplification STR multiplex currently used in the laboratory. If found feasible, Swab Solution™ Extraction-Powerplex® Fusion 6C Amplification (Promega Corporation, Madison USA) can be used as an alternative approach to the existing protocols implemented in the laboratory.

2. Materials and Methods

Single source mock casework samples were obtained from PNP DNA Analysis Branch Laboratory. To test the performance of Swab Solution™ versus DNA IQ™, two sets were prepared from the following mock casework evidence (cigarette butt, facemask, hair samples, nail clippings and bloodstained items).

2.1 DNA Extraction

2.1 .1 Swab Solution™ Extraction

100µL of Swab Solution™ was added into each hair sample tube while 200µL of Swab Solution™ was added into each tube of all other mock case samples. All tubes were placed into the Eppendorf thermomixer incubated at 70°C at 850 rpm. DNA containing supernatant was collected and transferred to a clean tube. Alternatively, substrate was simply removed from the tubes and was discarded.

2.1.2 DNA IQ™ Extraction

Lysis buffer were prepared by adding 6µL DTT for every 100µL DTT. 150uL of prepared Lysis Buffer was added into each sample tube. Sample was incubated at 70°C for 30 minutes. After brief centrifugation, supernatant was collected and transferred into a fresh 1.5 ml microtube. 7uL of the DNA IQ resin were added into the supernatant containing tube and incubated at room temperature for 5 minutes with 3 seconds at high speed for every minute of incubation. Then, tube is placed in a magnetic stand. All the solution was discarded without disturbing the pellet on the side of the tube. The sample was washed with 100µL lysis buffer followed by three times washing using a wash buffer. Sample tube was air dried for 5 minutes. Release of DNA was facilitated by adding 35µL elution buffer followed by incubation at 65°C for 5 minutes. Magnetic beads were finally separated from the solution by placing the tube in a magnetic stand. DNA bearing solution is transferred to a clean new tube.

2.2 DNA Quantification

Quantification was performed using ABI 7500 and Powerquant®System. Four standards ranging from 50ng/µL down to 0.0032 ng/µL were used. Using the PowerQuant™ kit components, a mastermix was created in a 1.5mL microcentrifuge, consisting of 5µL PowerQuant™ 2X Master Mix, 0.5µL PowerQuant™ 20X Primer/Probe/IPC Mix and 3.5 µL amplification grade water for each sample. Once created, the cocktail was vortexed and then centrifuged. 9µL of this mixture was dispensed into each reaction well containing 1 µL of sample, standard or control into appropriate wells. The standards were processed in duplicate while water is used for the negative control. The plate covered with an optical adhesive and completely sealed. Afterwhich, the plate was centrifuged briefly and then loaded onto the AB 7500 Real Time PCR System-HID using the HID Real Time PCR Analysis Software v1.2. The analyzed data was exported from the HID Real Time PCR Analysis Software v1.2 and processed using the Powerquant™ Analysis Tool.

2.2 DNA Amplification

Amplification was performed using AB Veriti thermal cycler and Powerplex Fusion® 6C Amplification System. Using the Powerplex Fusion® 6C kit components, a mastermix was created in a 1.5mL microcentrifuge, consisting of 2µl Powerplex Fusion 6C Master Mix, 2µl Powerplex Fusion® 6C Primer Mix, and 5 µL amplification grade water for

each sample. Once created, the cocktail was vortexed and then centrifuged. 9µL of this mixture was dispensed into each reaction tube containing 1 µL of sample, standard or control into appropriate tubes. Water is used for the negative control. After which, tubes are loaded onto the AB Veriti thermal cycler. Thermal cycling consisted of 96˚C initial hold for 1 minute, followed by 29 cycles of a denaturing step for 5 seconds at 96˚C and extension at 60˚C for 1 minute succeeded by a final elongation at 60˚C for 10 mins and samples were held at 4˚C for 5 minutes.

2.2 DNA Electrophoresis

Capillary electrophoresis was performed using the AB 3500 Genetic Analyzer. 1 µL of PCR product were added with 9.5 µL HIDI formamide and 0.5µL WEN ILS and run using the Applied Biosystems 3500 Genetic Analyzer following the manufacturers' recommended amplification protocol.

A cocktail was created in a 1.5mL microcentrifuge, consisting of 9.5 µL HIDI formamide and 0.5µL WEN ILS for each sample. Once created, the cocktail was vortexed and then centrifuged. 10.0µL of this mixture was dispensed into each reaction well containing 1 µL of sample, standard or control into appropriate wells. The plate covered with the septa and centrifuged briefly before loading onto the AB 3500 Genetic Analyzer using the Data Collection Software V.3.0. The analyzed data was exported from the Data Collection Software V.3.0 and processed using the Genemapper IDX Software version 1.4.

3. Results and Discussion

3.1 Qualitative Assessment

Qualitative analysis was performed on electropherograms generated during genetic analysis. Data was assessed based of three criteria namely: completeness, RFU level and peak balance. Completeness refers to the capacity to generate full profiles. A profile is considered full if 100% of the expected alleles are reported. Alleles are considered reportable if allele is correctly called and true allele peak can be distinguished from artifact peaks like pull up and stutter.

RFU value refers to relative fluorescence signals generated during electrophoresis and is directly proportional to the amount of amplified products. High amount of PCR products results to high fluorescence signals that saturates the camera and elevates the level of artifacts. Very low amounts of PCR products results to peak height imbalances and allelic drops. A sample is considered to have low RFU values if most of the alleles have RFU values lower than 500. It is considered acceptable if RFU values are between 1000 to 25000. Samples are categorized to have high RFU values if some of its alleles have RFU values higher than 25000.

Peak balance refers to the value of peak height ratio of the heterozygote alleles. A well balanced profile has a peak height ratio higher than 0.60 as established in the validation studies performed in the laboratory. An imbalance is said to be observed if sister alleles of heterozygote displays peak height ratio lower than 0.6.

Table 1 reflects the results of qualitative assessment performed on different samples. Full reportable DNA profiles were generated in all cigarette butt samples extracted with DNA Swab Solution™ while none was generated on DNA IQ™ extracted cigarette butt samples. Profiles generated on cigarette butt samples were found to have RFU values that are within the range and exhibited well balanced alleles. For facemasks, all swab solution extracted samples generated partial profiles while only one of the five samples generated a partial profile from DNA IQ extracted face masks. Profiles generated on facemasks were found to have RFU values that are relatively low and exhibited allele imbalances which was a characteristic of a sample with low amount of PCR products. Thus, qualitative assessment of electropherograms for both cigarette butt and face masks demonstrates that Swab Solution™ performs better than DNA IQ™.

Qualitative analysis of samples involving bloodstains on different substrates revealed that both methods are able to produce full reportable DNA profiles. While imbalances were observed in some of the DNA IQ extracted samples, well balanced profiles were obtained in all Swab Solution Extracted samples. An elevated level of RFU values has been observed on blood extracts processed with swab solution.

Qualitative assessment of electroherograms on nail clippings presented a different observation. While all of the DNA IQ™ extracted samples were generated full, well balanced profiles, 2 out of 5 samples yielded partial results for samples processed from Swab Solution™. This implies that lower amounts of PCR

Table 1. Qualitative assessment of cigarette butt, face masks, bloodstain on different substrate and samples processed with Swab Solution™ versus DNA IQ™

Swab Solution™			DNA IQ™		
COMPLETENESS	RFU values	PEAK BALANCE	COMPLETENESS	RFU values	PEAK BALANCE
CIGARETTE BUTTS					
Full	Acceptable	Well Balanced	NO PROFILE		
Full	Acceptable	Imbalance	NO PROFILE		
Full	Acceptable	Well Balanced	NO PROFILE		
Full	Acceptable	Well Balanced	NO PROFILE		
Full	Acceptable	Well Balanced	NO PROFILE		
FACE MASKS					
Full ; 41	Low	Imbalanced	NO PROFILE		
Partial; 33	Low	Imbalanced	Partial; 17	Low	Imbalanced
Partial; 30	Low	Imbalanced	NO PROFILE		
Partial; 8	Low	Imbalanced	NO PROFILE		
Partial; 28	Low	Imbalanced	NO PROFILE		
BLOODSTAIN ON DIFFERENT SUBSTRATES					
Full	High	Well Balanced	Full	Acceptable	Well Balanced
Full	Acceptable	Imbalanced	Full	Acceptable	Imbalanced
Full	Acceptable	Well Balanced	Full	Acceptable	Imbalanced
Full	High	Well Balanced	Full	High	Well Balanced
Full	High	Well Balanced	Full	Low	Imbalanced
NAIL CLIPPINGS					
Partial; 98%	LOW	Imbalance	45; Full	Acceptable	Well Balanced
47; Full	Acceptable	Imbalance	47; Full	Acceptable	Well Balanced
Partial; 70%	Low	Imbalance	47; Full	Acceptable	Well Balanced
42; Full	Acceptable	Imbalance	42; Full	high	Well Balanced
42; Full	Acceptable	Imbalance	42; Full	Acceptable	Well Balanced
Full	High	Well Balanced	Full	HIGH	Well Balanced
Full	Acceptable	Well Balanced	Full	HIGH	Well Balanced
Full	Acceptable	Well Balanced	Full	Acceptable	Well Balanced
Full	High	Well Balanced	Full	HIGH	Well Balanced
Full	High	Well Balanced	Full	HIGH	Well Balanced

Table 2. Quantitative assessment of cigarette butt samples processed with Swab Solution™ versus DNA IQ™

Sample name	Swab Solution™ (ng/µL)	DNA IQ™ (ng/µL)
CIGARETTE BUTTS		
CB2	1.020	0.005
CB6	0.572	0.002
CB10	0.343	0.004
CB11	0.473	0.014
CB12	0.701	0.007
Mean	0.622	0.006
Bloodstain on Different Substrate		
A034 (denim)	3.472	0.897
A035 (plastic)	0.279	1.171
A036(concrete)	1.963	0.260
A037(cloth)	2.415	4.464
A038(paper)	0.599	0.221
Mean	1.745	1.403
Nail Clippings		
ASC1	0.505	1.265
JPM 1	2.661	4.728
JPM 2	1.165	3.741
SBTA1	0.372	2.691
SBTA2	1.747	1.288
Mean	1.290	2.743
Hair Samples		
HH5	7.389	11.232
HH6	3.296	17.464
HH7	1.988	2.076
HH8	10.858	16.550
HH9	14.884	12.377
MEAN	4.887	7.095

products were amplified on Swab Solution™ than DNA IQ.

Full and well balanced DNA profiles were produced in all single hair stands with roots. Electropherogram comparison shows that results were comparable in terms of completeness, RFU Values and Peak Balance.

3.2 Quantitative Assessment

Table 2 reflects the results of quantitative assessment performed on different samples. To determine if there is a significant difference on the performance of Swab Solution™ extraction and DNA IQ™ magnetic bead extraction, t-test was performed on the DNA quantities obtained in real time PCR. The t-computed value from cigarette butt samples and mixed blood samples were found to be greater than the t-tabular value at 0.05 level of significance. This observation implies that there is a significant difference between the quantities obtained from the swab solution extraction and DNA IQ magnetic bead extraction on cigarette butt samples. In addition, a positive value for t-calculated suggests that Swab Solution™ method performed better than DNA IQ on cigarette butt samples.

For bloodstains from various substrates, it was observed that a higher amount of DNA was extracted when swab solution is used. However, statistical assessment revealed that differences between quantities obtained were not significant.

Quantitative assessment on nail clippings and hair samples gave negative T- value indicating that the quantities of DNA obtained from DNA IQ™ magnetic bead extraction is slightly higher than Swab Solution™ extraction. However because the t-value obtained from both nail clippings and hair are within the critical range, it can be said that statistically speaking, quantities of DNA obtained from Swab Solution™ extraction are not significantly different to the amounts of DNA obtained when samples are processed using DNA IQ™ magnetic bead extraction.

Upon analyzing the results from both quantitative and qualitative examination, it was observed that most of the samples processed with Swab Solution resulted in interpretable genetic profiles. The obtained profiles were found to be in agreement with the expected allele calls. Higher amounts of DNA were particularly observed in extracting DNA from cigarette butt samples and face masks. This may be explained by the fact that this method does not require subsequent transfers and repeated washing that may have resulted to loss of DNA.

For nail clippings and hair samples, although a slight increase in the amounts of DNA was observed when DNA IQ™ is used than Swab Solution™, no statistical significance between their values exists. This suggests that performance-wise, the capacity to extract DNA using both method were comparable.

3.3 Efficient Use of Resources

Another factor that may be used in choosing the method to be used in the laboratory involves efficient use of resources. Table 3 reflects a comparison on the use of resources between Swab Solution and DNA IQ™. Based on the resource comparison, Swab Solution™ is favored because of low cost, shorter processing time, ease of use and less waste.

Table 3. Quantitative assessment of cigarette butt samples processed with Swab Solution™ versus DNA IQ™

Resources	Swab Solution™ (ng/μL)	DNA IQ™ (ng/μL)
Cost per sample	P 36.40	145.39
Processing Time	35 mins	1 hr
Disposable plastics used		
1.5 ml tube	3	1
200 μL pipette tips	14	2
Cost per sample	P 36.40	145.39

4. CONCLUSIONS

Information and data gathered from this research verified the feasibility of using Swab Solution™- Powerplex Fusion™ in casework samples. Experiments showed its ability to produce reportable allelic calls that may be used for profile interpretation. Comparison of Swab Solution™ method with the currently used DNA IQ™ magnetic bead method displays its superior performance on cigarette butts and face masks which contains epithelial cells. Also, method comparison reveals that Swab Solution™ have comparable results on simple and bloodstains, nail clippings and hair samples. Furthermore, low cost, shorter processing time, ease of use and minimal production of ecological waste makes

the use of this direct amplification using Swab Solution™ method more beneficial.

5. ACKNOWLEDGMENTS

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