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Prepared by:

Dean P. Hildebrand, Ph.D.
Fareen Shamji, M.Sc.
Brian Yamashita, Ph.D.
Della Wilkinson, Ph.D.

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For additional copies or further information contact:

Canadian Police Research Centre (CPRC) Building M-55, 1200 Montreal Road Ottawa, ON K1A 0R6 Telephone: (613) 990-8577 Fax: (613) 949-3056 **www.cprc.org**	Centre canadien de recherches policières (CCRP) Édifice M-55, 1200, chemin de Montréal Ottawa (Ontario) K1A 0R6 Téléphone : (613) 990-8577 Télécopieur : (613) 949-3056 **www.cprc.org**

DNA SAMPLING FROM THE TRIGGER AND HANDGRIP OF DISCHARGED FIREARMS

A Report submitted to:

Canadian Police Research Centre

Dean P. Hildebrand[1.], Ph.D.

Fareen. Shamji[2.], M.Sc.

Brian Yamashita[3], Ph.D.

Della Wilkinson[3], Ph.D.

[1]B.C. Institute of Technology. 3700 Willingdon Ave., Burnaby, B.C., Canada, V5G 3H2.

[2]Kings College London. School of Health Sciences. 150 Stamford St., London, England, SE1 9NN

[3]R.C.M.P. Forensic Identification Research and Review Section. 1200 Vanier Parkway, Ottawa Ontario, Canada, K1A 0R2.

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SUMMARY

Minute quantities of DNA can now be analyzed using PCR-based DNA technology. Since many criminal acts employ the use of firearms, methods for obtaining DNA-based identifications from firearms need to be developed. In the past, DNA has been successfully obtained from skin cells deposited on handled objects that have simply been touched by an individual. We describe here an approach to maximize the recovery of DNA from handled firearms in order to generate a profile of the handler. The *DNA IQ*™ (Promega) extraction protocol was applied to 69 swabs collected from firearms belonging to 23 recruits after three consecutive classes using 25% ethanol, 50% ethanol or distilled water for each swabbing. Swabs that were taken using 25% ethanol and distilled water yielded the most DNA. DNA (trace amounts to 1ng) was successfully isolated from 11 samples swabbed using 50% ethanol, 18 samples swabbed using 25% ethanol and 17 samples swabbed using distilled water. PCR-based DNA profiling was carried out on each sample using the AmpF*I*STR® Profiler Plus ™ PCR Amplification Kit and the ABI PRISM 310 Genetic analyzer. PCR amplification was attempted on all samples regardless of whether DNA was seen on the slot-blot. Five additional cycles were added to the protocol (33 cycles total) in order to facilitate the matching process. This, however, did result in minor contaminating alleles visible in many of the gun swab samples. Profiles generated were compared to the profiles of the recruits obtained from buccal swabs on FTA ™ cards. Of the 69 sample swabs that were tested, 32 of these amplified at enough loci for a conservative match (4-9 loci).

METHODOLOGY

1. RCMP samples

Dr.'s Brian Yamashita and Della Wilkinson, RCMP Forensic Identification Research and Review Section, arranged for the Ontario Police College to receive instructions, swabs as well as the distilled water, 25% and 50% premixed ethanol solutions for this study. 25 individuals from the Basic Cadet Training Class # 312 at the college volunteered to take part in the study. The volunteers were broken up into three groups and all three solutions were sampled on the three occasions. In order to verify recruit identity buccal swabs were taken from the volunteers using foam swabs and FTA paper. All samples were done in a clean environment and changed on each occasion to prevent contamination. Weapons were swabbed according to the protocol provided as outlined below. Students did not clean their weapons after shooting and the samples were taken on three consecutive classes. The Forensic Identification Specialists at the Ontario Police College were responsible for taking all swabs. Upon collection, swabs were packaged in a cardboard box and sent to Dr. Hildebrand.

i. Swabs from weapons

The swab container was labelled with the volunteer's identification number. Wearing a clean pair of gloves, about 1-2cm of the end of the swab container was cut off and about 5cm of the shaft of the swab was broken off. A few drops of distilled water was added to a clean cotton swab shaking off any excess water. After shooting, the weapon's grip and trigger surface was vigorously rubbed with the moistened swab. The swab was then placed back into the labelled container and sealed. Swabs were allowed to air dry in the fume hood and placed into dry, labelled exhibit bags that were then stored in a clean, dry place. The process was repeated using swabs moistened with 25% ethyl alcohol and distilled water swabs as well as 50% ethyl alcohol and distilled water.

ii. Buccal swabs

The swab container was labelled with the volunteer's identification number. Wearing a clean pair of gloves, about 1-2cm of the end of the swab container was cut off and about 5cm of the shaft of the swab was broken off. The swab was rubbed against the inside surface of the

volunteer's cheek and applied to the FTA card by dabbing the surface and allowing it to dry completely in a fume hood. The dried FTA cards were placed into dry, labelled exhibit bags and stored in a clean, dry place.

2. DNA extraction

i. FTA Cards

Reference samples were obtained from each recruit used in this study. DNA was extracted from the FTA cards (buccal swabs) using the standard FTA card DNA extraction protocol employed by the BCIT Forensic Lab. The FTA cards received from the RCMP were verified against the list of volunteer's provided. All packages were sealed. The FTA cards were analyzed in two groups; volunteer's 1 to 12 and volunteer's 13 to 25. A clean Harris punch and punch pad was used. The FTA card was removed from its packaging and placed onto the self-sealing punch pad. A 2mm diameter sample was punched using the Harris punch from the centre of the sample area on the FTA card and ejected into a labelled, autoclaved PCR tube. The FTA punches were soaked in 200µl of FTA purification reagent (Gibco BRL® Life Technologies) at room temperature for 5min. The reagent was decanted and the process repeated twice more. Some samples were difficult to decant without loosing the punch therefore a pipettor was used instead. The FTA punches were then soaked 200µl of FAD water and left at room temperature for 5min. The buffer was removed with a pipettor and the procedure repeated one more time. The tubes were then incubated at 50ºC, with lids open, for 20min to dry. The protocol indicates that the tubes be left to dry at 60ºC; however, this is not important as the process is a drying process only. Once dry, the samples were stored at 4ºC till ready to amplify. Note, we were unable to obtain profiles for two of the recruits (Simpson and Phillips) and, therefore, they were excluded from further analyses because confirmation of their correct profiles on the gun swabs could not be made.

ii. Gun swabs

Upon receipt of the swabs from the RCMP, names, packaging and labelling was verified against the provided volunteer's list. The small sample casework protocol for the *DNA IQ*™ system by Promega was followed to extract DNA from the swabs taken from the weapons. The

same procedure was followed as was for the validation studies. For the initial lysis reaction, 500µl of prepared lysis buffer was used. Extracts were eluted with 25µl of elution buffer and stored at –20 ^{O}C.

iii. DNA quantification, amplification and fragment analysis

The extracted DNA samples from the weapon swabs were quantified using the *ACES 2.0+ Human DNA Quantification System* according to the standard protocol in place at BCIT.

All stages in the amplification procedure were carried in separate labs using dedicated equipment, gloves, lab coat and sleeves. The remainder of each swab sample (15 µL after slot-blot analysis) was dried in a Speed-Vac and resuspended in 10 µL of sterile water in order to maximize the amount of DNA in the PCR reaction. Most samples in this study contained less DNA than the 1ng optimum suggested by the manufacturer. Samples were amplified using the AmpF*I*STR® *Profiler Plus* ™ PCR Amplification Kit**.** The gun swab samples were amplified for 5 more cycles to give a total of 33 cycles.

FTA cards were subjected to amplification directly without quantification. DNA from the FTA cards were resuspended in 10µl of FAD water prior to transferring it to the PCR reaction tubes for amplification (28 cycles).

Fragment analysis was conducted on an ABI 310 Genetic analyzer using GeneScan™ 3.5 Software and Genotyper® 2.5 Software using standard conditions in place at BCIT.

RESULTS AND DISCUSSION

The RCMP samples used for this study were collected from firearms belonging to and handled by 25 recruits from the Ontario Police College (Table 2). Each recruits' firearm was swabbed once on three different occasions using one of three different solutions. Swabs were taken on the 26/06/03, 27/06/03 and 02/07/03 using either 25% ethanol, 50% ethanol or distilled water. Promega's *DNA IQ™System* was employed in this study. The aim was to determine how much DNA could be recovered from firearms using each of the solvents and whether DNA profiling could be conducted. Note, only 23 sets of recruit samples were used due to the failure of 2 of the reference FTA cards. The DNA analysis performed on the RCMP gun swab samples where then used to compare to the known (reference) samples. PCR was performed on each gun sample regardless of whether or not quantification indicated the presence of human DNA. Initial results indicated that the standard 28 cycle PCR method would be insufficient to obtain results in this study. It was decided, therefore, that 33 cycles would be used and that every sample would be tested. The additional cycles added may have resulted in low level contamination appearing in these results.

Of the 69 RCMP samples tested (3 solvents x 23 recruits), DNA (trace to 1ng) was recovered from 46 samples (Table 2**).** DNA was recovered from 78.3% (18 of 23) of the guns swabbed with 25% ethanol, 47.8% (11 of 23) of the guns swabbed with 50% ethanol and 73.9% (17 of 23) of the guns swabbed with distilled water (Figure 1). DNA was not recovered from 33.3% (23 of 69) of the samples. Although previous studies have documented the difficulty in obtaining DNA profiles from handled objects, we were surprised at the relatively poor yield of human DNA in this study. Although no study on handled objects using *DNA-IQ™* has been reported, this system has been tested by the manufacturer and us on a variety of sample types. The paramagnetic resin will bind up to 100ng of DNA under the conditions used in this study. Experiments done in BCIT's forensic laboratory have also shown this system to be very effective at isolating DNA from degraded samples like bones and teeth which are expected to have low quantities of DNA.

Table 2. *Quantity of DNA extracted from RCMP gun swab samples using ACES 2.0+ Human DNA Quantification System. A total of 69 samples were quantified*[†]

Recruit	50% ethanol			25% ethanol			Water		
	26/6/03	**27/6/03**	**2/7/03**	**26/6/03**	**27/6/03**	**2/7/03**	**26/6/03**	**27/6/03**	**2/7/03**
1									
2	0.04[††]				0.04				0.2
3			0.2	0.04				0.04-0.2	
4					0.04				0.04
5							0.04		
6			0.2	0.04-0.2				0.04	
7									
8						0.04	0.4		
9			Trace[†††]	Trace				0.04	
10					0.04				0.04
11				0.04					
12									
13	Trace				0.04				
14			0.04					0.04	
15		Trace			0.04		0.04		
16			Trace	0.04				0.2	
17	0.4				1				0.8
18				0.04				0.04-0.2	
19	0.04-0.2				0.04				0.04
20				0.04				0.04	
21		Trace				0.04	0.04		
22					0.04				0.2
23					Trace				

[†] A total of 75 gun swabs and 25 reference buccal swabs were received. Two reference buccal swabs did not generate profiles; therefore, were excluded from all further analyses.

[††] ng of human DNA.

[†††]"Trace" indicates a visual band on the slot-blot film that was less intense than the lowest quantity standard (40 pg).

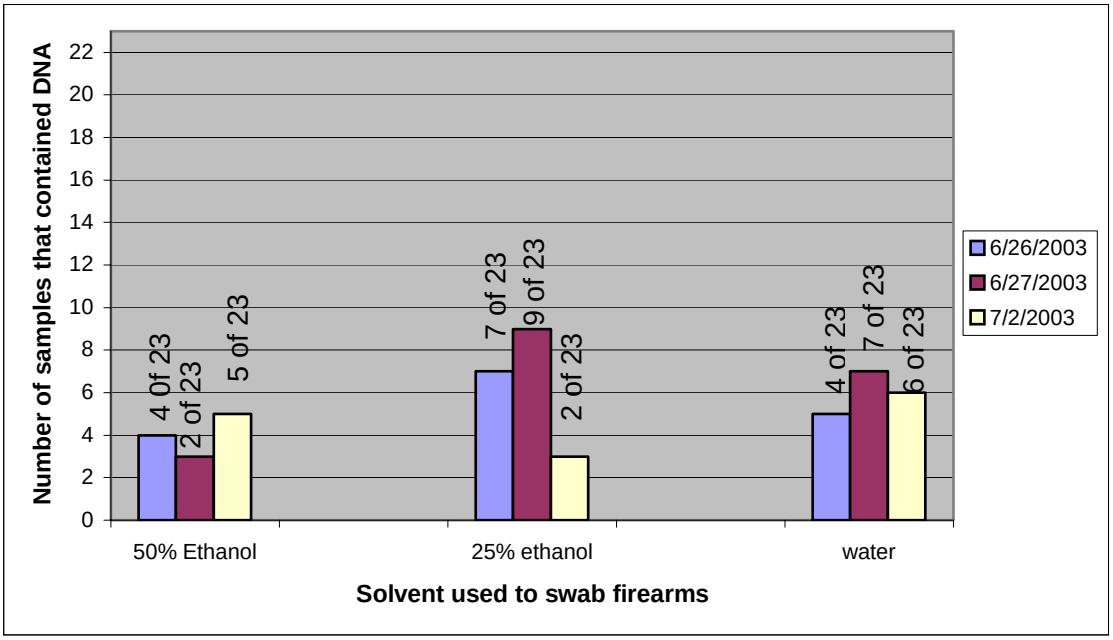

Figure 1. The number of RMCP gun swab samples that contained DNA after slot blot analysis and illustrated. 25 recruits were divided into three groups. It should be noted that samples from 23 recruits were analyzed. Firearms from each group were swabbed once on three different days 26/6/03, 27/6/03 and 2/7/03, using either 25% ethanol, 50% ethanol or distilled water.

These results suggest a benefit, in terms of DNA yield, if water or 25% ethanol is used for swabbing the firearms. This is somewhat misleading, however, due to the high number of samples swabbed with 50% ethanol that showed no observable DNA on the slot-blot but yielded a positive PCR result (see below).

Ultimately it is whether or not a given sample yields a usable DNA profile that is of most importance to the forensic biologist. In this study 32 (of 69) of the firearms swabbed yielded a positive match (defined as 4-9 amplified loci that match the reference sample). Eleven (of 69) resulted in no amplification. The remaining 26 samples resulted in amplification at 1-3 loci and were not called a match due to low level contamination that was observed in this study. The contamination was of unknown origin but is presumably due to the low-copy PCR amplification conditions used in this study (less than the 1 ng optimum DNA amount and increased cycle number). A representative sample electropherogram of a gun swab is shown in figure 2.

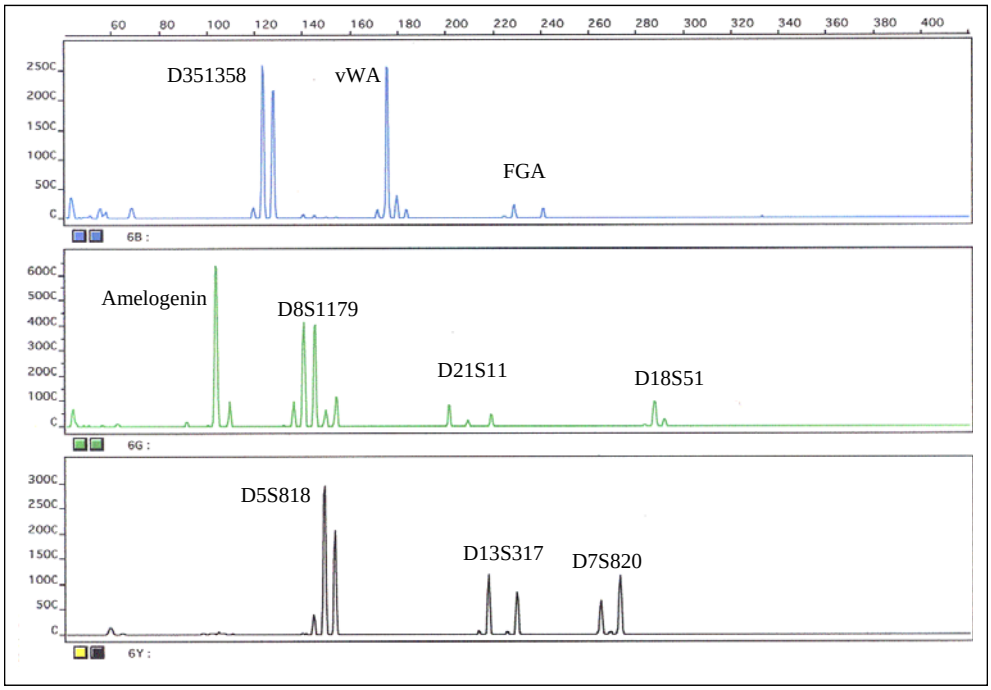

Figure 2. A representative profile obtained from one of the RCMP gun swabs. The AmpF*l*STR Profiler Plus Kit was used that amplifies Amelogenin plus 9 STR loci: D351358, vWA, FGA. D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820. The arrows indicate which alleles are contributing to the contamination. Contamination, indicated by minor alleles are clearly visible in this sample and was observed in many of the samples in this study.

Likelihood ratios were calculated using the Canadian Caucasian database for all samples that matched at 4 or more loci (Table 3). Values ranged from $4.8x10^4$ for a 4-locus match to 3.70×10^{13} for a 9-locu match.

Of the 32 positive matches, 14 were from samples swabbed with 50% ethanol, 12 from 25% ethanol and only 6 from water. In this study, firearms swabbed with water were more likely to yield DNA although many more of these samples failed to amplify. Conversely, samples swabbed with 50% ethanol were less likely to yield DNA, but a higher proportion of these resulted in usable profiles and this group had more samples that amplified in spite of no observable DNA on the slot-blot. These results make it difficult to draw definitive conclusions concerning the best method of swabbing handguns DNA evidence and hence develop SOP's for field specialists. We recommend that a repeat round testing be considered and time and funding permits.

Table 3. *Combined genotype frequencies for obtained profiles that matched the recruit reference profiles at a minimum of four loci*♦

Sample	Likelihood Ratio (LR) ♦	Number of Loci used to calculate the LR
2 (25%)	2.31×10^{9}	8
3 (50%)	2.65×10^{9}	7
4 (50%)	9.11×10^{10}	8
5 (50%)	3.96×10^{9}	7
6 (25%)	4.8×10^{4}	4
8 (50%)	2.82×10^{9}	7
9 (50%)	7.80×10^{4}	4
9 (25%)	7.16×10^{6}	5
9 (Water)	7.49×10^{6}	5
10 (50%)	1.07×10^{7}	8
10 (25%)	1.07×10^{9}	8
10 (Water)	2.43×10^{7}	7
12 (50%)	2.01×10^{5}	4
13 (50%)	2.61×10^{10}	7
13 (25%)	3.70×10^{13}	9
14 (25%)	6.49×10^{6}	4
15 (50%)	1.72×10^{8}	7
15 (25%)	9.03×10^{6}	6
16 (50%)	1.26×10^{11}	9
16 (25%)	5.12×10^{9}	8
16 (Water)	6.63×10^{6}	6
18 (50%)	4.11×10^{11}	8
18 (25%)	2.76×10^{10}	7
19 (25%)	9.82×10^{4}	4
19 (Water)	2.05×10^{5}	4
20 (50%)	6.41×10^{9}	8
21 (25%)	2.44×10^{8}	6
21 (water)	6.23×10^{5}	5
22 (50%)	3.67×10^{8}	7
22 (25%)	1.61×10^{7}	6
23 (25%)	2.69×10^{11}	9
23 (Water)	1.72×10^{8}	6

♦ Inverse of the combined genotype frequencies (or random match probabilities). Calculated using the Canadian Caucasian Database