Optimization of DNA trace collection from firearms and effects of different gun handling scenarios

A Master's Thesis Project

By

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Abstract

More than 500 people die every day from gun violence worldwide. DNA traces may enable the identification of the shooter, but there is limited knowledge concerning where on firearms DNA can be found and how it got there. This project aimed to answer these questions as well as how the swab types and addition of detergents affect the DNA recovery.

Three swab types were examined (cotton, nylon flocked and foam swabs) by putting saliva stains (2 µL) on firearms and collecting the DNA with the swabs. The DNA recovery from these were compared after quantification with qPCR and no significant difference could be seen. The addition of the detergents SDS and Tween20 to the trace collection buffer were also studied, but as for the swab, no significant differences could be found.

When investigating the location of DNA on firearms, it could be seen that the grip and magazine sides in general gave the highest amount of DNA. The frame and muzzle + front sight gave low DNA amounts, and the barrel gave no DNA for any of the firearms. The effect of different handling scenarios on the DNA recovery was also studied. It could be seen that handling the firearm with gloves gives DNA, but lower than handling with bare hands. It was also shown that wiping the gun with a dry paper towel after firing was not efficient for removing DNA. Finally, it could also be concluded that storage of the firearm in a bag with personal items led to transfer of DNA to the gun.

The possibility of identifying the shooter of a pistol that was previously handled by other was also investigated by using the software DNAxs. One of four shooters could then be connected to its pistol.

Populärvetenskaplig sammanfattning

Var på skjutvapen finns det mest DNA och hur hamnar det där?

Mer än 500 personer världen över dör varje dag av vapenvåld och användandet av vapen i brott har ökat markant de senaste åren. Genom att lokalisera DNA-spår på brottsplatser och tillhyggen som har använts, exempelvis skjutvapen, är det möjligt att identifiera gärningsmannen.

Väldigt lite är dock känt om var på skjutvapen man kan hitta DNA och hur det hamnade där. Är det till exempel möjligt att hitta DNA om vapnet endast har förvarats i en väska? Undviker man att lämna DNA-spår om handskar används vid hantering av vapnet? Var på vapnet är det mest troligt att man lämnar efter sig DNA-spår? Dessa frågor kan spela stor roll i en utredning där skjutvapen har använts och har därför varit till grund vid utförandet av detta projekt.

Vid undersökning av var på skjutvapnet det är störst chans att hitta DNA, såg jag att kolven och sidorna av magasinet generellt gav de högsta DNA-mängderna. Stommen och pipmynningen plus kornet gav låga mängder DNA, och pipan som finnes inuti vapnet gav inget DNA för något av de undersökta vapnen. Mantelgreppet visade sig inte ha några större mängder DNA och avtryckaren och läpparna samt botten av magasinet gav mellanhöga mängder jämfört med de andra ytorna.

Jag såg också att hantering med handskar gav DNA på vapnet, men mindre mängd än hantering utan handskar. Avtorkning av vapnet med en pappersduk efter avfyrning visade sig var ineffektivt för att ta bort DNA. Förvaring av vapnet i en väska tillsammans med personliga föremål visade sig ge spår av DNA, utan att personen i fråga vidrört vapnet.

Slutligen undersöktes också möjligheten att koppla en skytt till en pistol som tidigare har hanterats av flera andra personer. Fyra vapen, som tidigare hanterats av andra, avfyrades av varsin skytt och resultatet blev att en av dessa skyttar kunde kopplas till ”sitt” vapen.

En vanlig metod för att samla in DNA-spår är genom att använda en bomullstops som är fuktad med saltlösning. Det har dock visats i andra studier att materialet på topsen påverkar hur mycket av DNA-spåren som man fångar upp. Det har därför också undersökts i projektet ifall flockad nylontops eller skumgummitops kan förbättra spårsäkringen av DNA på skjutvapen. Det visade sig dock inte vara fallet då bomulls-, flockad nylon- och skumgummitops gav likvärdiga resultat.

Detta projekt har resulterat i mer kunskap kring var och hur DNA hamnar på ett skjutvapen, vilket förhoppningsvis kan bidra till att fler brott som involverar skjutvapen kan lösas.

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Thank you!

Lisa Lindahl

List of abbreviations

BSA	Bovine Serum Albumin
CE	Capillary Electrophoresis
Cq	Quantification cycle
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleoside triphosphate
dsDNA	Double Stranded DNA
ILS	Internal Lane Standard
LR	Likelihood Ratio
NFC	Swedish National Forensic Centre
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
SDS	Sodium Dodecyl Sulfate
ssDNA	Single Stranded DNA
STR	Short Tandem Repeat
TCB	Trace Collection Buffer
TMB	Division of Applied Microbiology, Lund University

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1. Introduction

Ever since 1985, when Alec Jeffrey found that the variations and uniqueness in the genetic code could be used for identification and separation between individuals, DNA analysis has been an important tool in forensic work for capturing criminals. Thanks to DNA analysis, not only have the guilty persons been convicted, but also innocent people been freed. With today's technology, such as PCR and STR analysis, it is possible to identify individuals through their DNA and connect it to minute DNA traces found on crime scenes. This has been an important step in solving crimes, including cases involving firearms.

The use of firearms in crimes are continuously increasing. 8,500 crimes that violates the Swedish firearms law (Vapenlag (1996:67)) were reported in Sweden year 2020, which is an increase by 14 % compared to the previous year. The same year, the percentage of confirmed cases of lethal violence that included firearms was 39 %. (Brå, 2022) According to Amnesty International, more than 500 people die every day from gun violence worldwide and between the years 2012 and 2016, 1.4 million firearm related deaths were reported (Amnesty International, 2022).

When a weapon is found at a crime scene, it is of big interest to know who has handled it and it is therefore examined for DNA traces. However, a study performed at the Biology section at the Swedish National Forensic Centre (NFC) showed that only 3.4 % of all DNA traces from weapons gives a useful result, meaning that the DNA profile was evident enough to be compared to a person (Gunnar *et al.*, 2021). It has therefore been discussed at NFC how the collection of DNA traces could be optimized in order to increase the DNA recovery from weapons. A previous study showed that the use of nylon flocked swabs increased the number of useable DNA profiles compared to the standard cotton swab for cartridge cases (Jansson *et al.,* 2020) which contributes to the thought that this might be a solution for weapons as well. Before swabbing materials today, the swab is wetted with 0.9 % NaCl (Jansson *et al.,* 2019) and questions whether addition of other detergents may improve the DNA recovery have been raised. It has also been showed that different types of surfaces affect the DNA recovery as well (Akel *et al.,* 2019). When getting in contact with a firearm for criminal investigations today, there are a few recommended areas on the firearm that should be swabbed. For revolvers and pistols, the slide grip divided in left and right, the trigger, and the grip divided in left and right are swabbed. For rifles, the hand guard divided in left and right, the pistol grip divided in left and right and the trigger are swabbed. For magazines, the lips and the bottom are swabbed. One swab is used for each surface. (NFC, 2022) However, there are no clear statistics on how many useable DNA profiles that are obtained from each area. It is therefore desirable to investigate which material and areas of the firearms that are the most relevant to swab in order to generate a useful DNA profile.

1.1 Aim

The aim of this project is to optimize and evaluate the trace collection of DNA on firearms. This will be done by comparing swabs made of different materials such as cotton, flocked nylon, and foam. The effect of addition of several detergents to the trace collection buffer (TCB) will also be studied as well as which areas on firearms that are most likely to give the highest

DNA recovery. How the DNA amount is affected by different handling scenarios and the possibility of connecting the shooter of a firearm to the gun will also be examined.

The following research questions have been stated:

1. Does addition of different detergents to the trace collection buffer (TCB) affects the DNA recovery?
2. Does the swab type affect the DNA recovery?
3. Where on firearms is DNA most likely to be found?
4. How are different handling scenarios affecting the DNA amounts on firearms?
5. Is it possible to identify the shooter of firearms previously handled by others?

2. Background

2.1 DNA

Deoxyribonucleic acid (DNA) is a macromolecule consisting of two complementary strands. The strands are built of nucleotides consisting of a phosphate group and a five-carbon sugar (deoxyribose for DNA) as a core, and with one of four possible nucleobases attached, adenine (A), thymine (T), guanine (G) and cytosine (C). The two strands are bound to each other via hydrogen bonds, forming a double helix. The hydrogen bonds are formed between an adenine on one strand and a thymine on the other strand, as well as between a guanine on one strand and cytosine on the other. As the bonds are only formed between A-T and G-C, the two strands are always complementary. One pair of hydrogen bonded nucleobases is called a base pair, often shortened as bp. (Brown, 2016)

The DNA makes up the genome in all living organisms. Most of the DNA is located in the cell nucleus (nucleus DNA), but some are stored in the mitochondria as well, known as the mitochondrial DNA or mtDNA. The DNA in the nucleus is packed into 46 chromosomes in humans. The DNA molecule contains the genes which can be seen as the genetic code. As 50 % of the genes are inherited from the mother and 50 % from the father, an individual gets each gene in two different versions, i.e. an individual has two alleles. If the inherited versions of the genes are the same from both the mother and the father, the individual is said to be homozygote. If the alleles differ, the individual is called heterozygote. The order of the nucleotides with the different nucleobases determines the gene. (Brown, 2016)

2.2 DNA in Forensics

The genetic code is unique for everyone and is thus of usage for identification of individuals. DNA analysis is therefore often performed in order to identify individuals and investigate whether they are connected to a crime or not. When performing DNA analysis for identification, the entire genome is not analysed as it is an extensive amount of information, and it would take too much time. Instead, only a small part of the variation in the DNA is analysed, called short tandem repeats (STR). STRs is a big reason for the high genetic variation and are, as the name implies, a repeat of 2-6 bp in the genetic code. The number of repetitions differs between individuals, making it useable for identification. (Butler, 2010)

DNA traces can be left behind on crime scenes by the perpetrator and is then taken for DNA analysis. A person can leave DNA behind at places in several ways, for example through biological fluids such as blood, saliva, semen, vaginal secretion, and urine. A person can also leave DNA behind traces by touching items, referred to as touch DNA or trace DNA, consisting of for example skin cells. (Hess & Haas, 2016)

2.2.1 DNA Trace Collection

DNA traces can be collected in several ways, for example with swabs or tape and excision or scraping. Swabs are often moistened with trace collection buffer (TCB) in order to facilitate the release of biological material from the surface. Both one and two swabs can be used (Hedman

et al., 2020). Using two swabs, called the double swab technique, includes a swabbing with a moistened swab first, followed by a dry swab in order to pick up the remaining cells that were not collected with the moistened swab (Sweet *et al.,* 1997). The swabs can be made of different materials. Swabs have shown not to be as efficient for traces found on fabrics where tape is often used instead. The reason for this may be that swabs pick up inhibitors as well, while tape does not, at least not to the same extent, but also that it is more difficult to remove cells from the fibre using swabs. The tape is pressed on the surface multiple times, picking up the DNA. Excision refers to cutting out the area with the potential DNA trace. Scraping is done by scraping with a sterile scalpel over the material with DNA traces. (Hess & Haas, 2016)

Today at NFC, 0.9 % NaCl is used as TCB and between 1-2 drops of TCB are added to the swab prior to the sampling (Jansson *et al.,* 2020). In this project, the addition of the detergents sodium dodecyl sulphate (SDS) and polysorbate 20 (also known as Tween20) was examined. SDS is an anionic surfactant often included in cleaning products due to its amphiphilic properties (NCBI, 2022). Tween20 is a non-ionic surfactant that is effective in solubilisation and prevents surface adsorption (NCBI, 2022).

2.2.2 DNA Extraction

Once the DNA traces are collected, the DNA must be extracted before further analysis as the DNA is encapsuled in the cell. This is often done in three steps, (1) cell lysis in order to release the DNA, (2) enzymatic digestion and/or denaturation of proteins, and (3), separation of DNA from other cellular components and inhibitors. (Chong *et al.,* 2020) There are several different methods for extraction and which extraction method used today at NFC depends on the sample. Factors that affect the choice of method can be of what type the sample is, such as blood or sperm, what DNA concentration that can be expected and where the DNA trace was found. (NFC. 2022) However, the most commonly used method is an in-house Chelex-based extraction protocol, which is the method used in this project as well.

The principle of Chelex-based extraction is that cell lysis is achieved by boiling the samples at 100 °C together with a lysis buffer. The lysis buffer containing Chelex® 100 Resin, which gives the solution an alkaline property. Chelex® 100 is a chelating resin made of styrene divinylbenzene copolymers which in turn consists of paired iminodiacetate ions that binds metal ions, such as Mg^{2+}. By binding metal ions, the degradation of DNA is prevented as bivalent metal ions may act as catalysts for the DNases – the enzymes that degrade DNA. (Walsh *et al.,* 1991)

In the lysis buffer used for this project, proteinase K was also added in order to break down proteins in the cell lysate that may disrupt the DNA extraction, such as DNases and other contaminating proteins. Proteinase K facilitate the disruption of the cell membrane as well (Promega, 2017). Proteinase K has an optimal working temperature between 50-65 °C and the samples are therefore incubated at 56 °C before boiling (Dash *et al.,* 2020). Tween20 was added to the lysis buffer as well as it is a compound that prevent surface adsorption and facilitate the degradation of cell material (NCBI, 2022).

2.2.3 Amplification of DNA by Polymerase Chain Reaction (PCR)

As the collected DNA is obtained from only a few cells, it must be amplified to be quantified and analysed. This is performed by using polymerase chain reaction (PCR) which is a technique for amplifying DNA by mimicking the natural process in the cells for cell division. Only small amount of DNA is needed for PCR and as the amplification occurs exponential, a high amount of DNA is reached quickly. A PCR reaction is performed in three steps that is repeated several times. The first step is denaturation, followed by annealing with extension/elongation as the last step. For a PCR reaction, several reagents are needed. These are template DNA, primers, nucleotides, and DNA polymerases. (Brown, 2016)

The template DNA is the target DNA that is to be amplified. Primers are a short sequence of single stranded DNA complementary to a short region of the target DNA. The primers act as a starting point for the elongation, showing the DNA polymerase where to connect the dNTPs. Nucleotides are the building blocks for the PCR reaction and are added to the reaction as deoxynucleotide triphosphates (dNTPs). The dNTPs are either dATP, dTTP, dGTP or dCTP giving the nucleotides with the different nucleobases for the DNA strand. DNA polymerase is the enzyme that anneal the nucleotides together, forming the product. There are several different DNA polymerases that can be used for a PCR reaction. The different enzymes have different working temperatures, meaning that the optimal temperature for the elongation differ depending on what enzyme used. (Berg *et al.,* 2015)

During the denaturation, the mixture is heated to above the melting point of the DNA strands, which is around 95 °C resulting in separation of the two DNA strands. For the annealing, the temperature is lowered to the annealing temperature for the primers, which is around 50-60 °C. The temperature is then raised again to the optimal working temperature for the DNA polymerase used, during the elongation. This cycle is then repeated several times, and for each repetition, the number of DNA copies is doubled. (Brown, 2016) For a schematic overview for the reaction, see *Figure 1.*

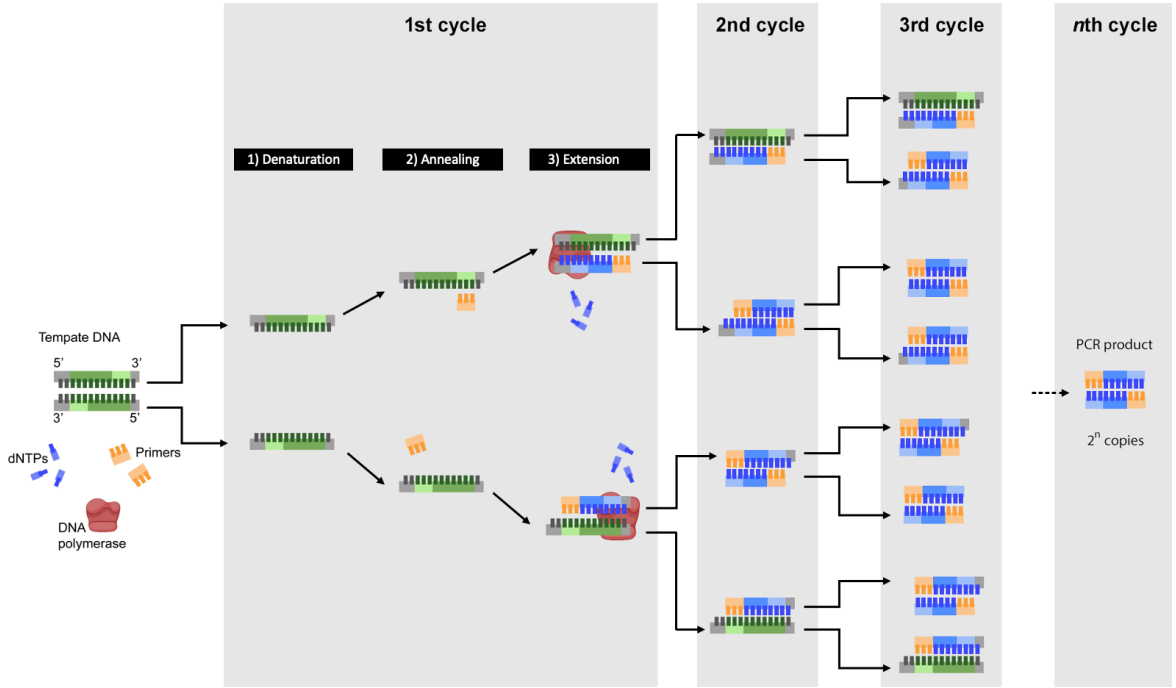

Figure1: a schematic overview of a PCR reaction, including the three steps (1) denaturation, (2) annealing and (3) extension/elongation.

In this project, the DNA polymerase Immolase was used for elongation. Immolase is inactivate in room temperature and needs to be activated for 10 min at 95 °C prior to the PCR reaction. (Bioline, n.d.). An enzyme buffer is also included in the reaction mix in order to create optimal chemical environments for the DNA polymerase. $MgCl_2$ was added to the reaction mix as well, as Mg^{2+} acts as a cofactor for the DNA polymerase. The ions catalyse the formation of phosphodiester bonds between the primer and the nucleotide which is necessary for the elongation to proceed. The cations also stabilise the negative charges of the DNA templates, preventing the strands to repulse each other. (ThermoFisher Scientific, n.d.)

There are several compounds that may inhibit the PCR reaction. These inhibitors can be for example calcium ions, bile salts, ethanol, collagen and proteinases. However, the concentration of the compounds affects to what extent they might inhibit, meaning that the presence of the compounds is not equivalent to inhibition. By including additives to the reaction mixture, it is possible to enhance the PCR reaction by preventing the inhibitory effect of the inhibitors. One example of an additive is bovine serum albumin (BSA), which is used in this project. BSA has shown having a beneficial effect on the PCR yield. The reasons are thought to be that BSA is preventing inhibitors to interact with the DNA polymerase. (Schrader *et al.,* 2012) (Farell & Alexandre, 2012)

2.2.4 Quantitative PCR (qPCR)

In quantitative PCR (qPCR), or real-time PCR, it is, as the name implies, possible to quantify the amount of DNA in a sample. This is done by real-time PCR, i.e. a PCR reaction that monitors the amplification during the reaction. There are two ways to monitor the amplification, by using either a fluorescent dye or labelled probes. The dye can bind both single stranded DNA (ssDNA) and double stranded DNA (dsDNA) and when it does, it emits fluorescent signals. By measuring this signal, the amount of DNA can be calculated. However, the fluorescence is much bigger when bounded to dsDNA than ssDNA, and the concentration obtained is therefore of dsDNA. A probe is a short oligonucleotide which, by hybridizing to the PCR product, emit light, and as for the dye, it is possible to quantify the PCR product by measuring the emitted light. However, it is not the probe itself that emit the light. The oligonucleotide probe has a fluorescent reporter molecule at one end of the strand, and a quencher compound at the other. When these are bound close, the fluorescent signal cannot be detected as the quencher inhibit the signal. However, during the elongation the DNA polymerase is hydrolysing the probes, cleaving the bond between reporter molecules and oligonucleotides. This results in a free reporter fluorochrome in the reaction, emitting light that is no longer quenched. This signal is then measured in order to obtain the concentration of DNA. As both these methods yields the concentration of generated PCR products, this must be translated into the concentration of DNA in the original samples. This is done by using standards. The standards are dilutions of known DNA concentrations before amplification, making it possible to convert the fluorescent signal to a concentration. (Brown, 2016) In this project, a probe with the dye label FAM was used.

The data outcome from a qPCR are Cq values, which is a measure on how many quantification cycles that were needed to be able to detect the signal. A qPCR analysis can be seen in *Figure 2*. The x-axis is the number of PCR cycles, and the fluorescence is shown on the y-axis. A PCR reaction consists of two phases, the exponential phase, and the plateau phase. During the exponential phase, the products are doubling for each cycle, but eventually, the reagents will run out and no more strands can be amplified, and this is when the plateau phase is reached. In

every reaction, there will be background fluorescence. The Cq value indicates how many cycle that is needed to be able to distinguish the amplicon fluorescence from the background fluorescence, i.e. when the threshold line is reached. (BIO-RAD, n.d.)

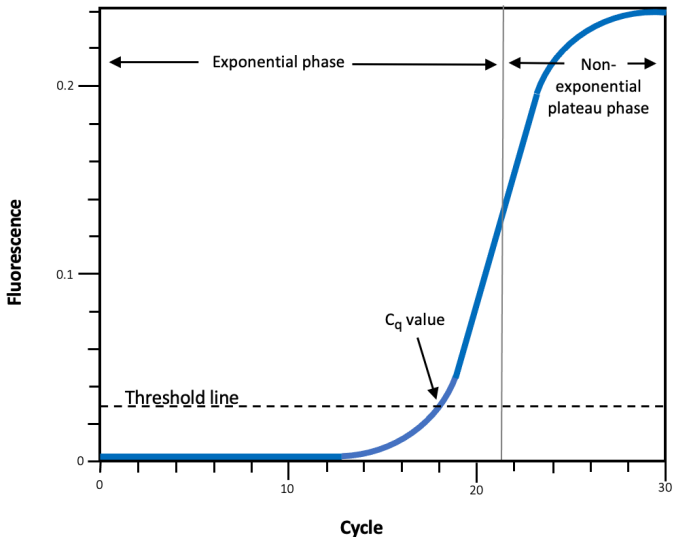

Figure 2: a schematic overview of a qPCR analysis.

The Cq value is directly correlated with the concentration, as a sample with a higher DNA concentration will give a stronger signal earlier in the reaction compared to a sample with low concentration. (Pabinger *et al.,* 2014)

NFC has a concentration limit of 0.01 ng/µL for samples to be further analysed in their standard routine flow. If samples have a concentration between 0.001-0.0099 ng/µL, it is said that not enough amount of DNA could be detected. If the concentration is < 0.001 ng/µL, it will be said that no detectable DNA could be detected. For some samples, example cartridges and cartridge cases, or if it is heavier crimes that have been committed, the samples are then analysed in a LCN-flow (low copy number). The concentration limit is then 0.001 ng/µL for the samples to be further analysed. (NFC, 2022)

2.2.4.1 RB1-Assay

The RB1-assay was the assay used in this project. It targets the human retinoblastoma-1 gene (RB1 gene) which has been reported for having low variation, and is validated for high accuracy, specificity and sensitivity. The primers and probes used in the project were therefore designed for the RB1 gene. (Andréasson *et al.,* 2002)

2.2.5 Short Tandem Repeat (STR) Profiling

When the DNA is quantified and it is stated that the concentration is high enough for further analysis, the samples is further processed for DNA profiling. This means to find out from whom the DNA originates which is possible with the help of the STRs. STRs are located in the non-coding regions of the DNA and stands for approximately 3 % of the human genome. However, only 15 specific loci in the DNA are used today for STR profiling at NFC, together with an extra for gender determination. (NFC, 2022) As 15 loci are used, and the number of repetitions

varies within the loci, the likelihood that two persons have the same number of repetitions on every locus is very low.

The DNA samples are first amplified by a PCR reaction, as described above. In this project the samples were amplified using PowerPlex® ESX 16 fast, which is a DNA profiling kit developed by Promega Corporation. It amplifies 16 different loci, including the amelogenin gene for gender determination. The PowerPlex® ESX 16 system is robust and sensitive, making it suitable for forensic DNA samples. The amplification gives amplicons that varies between 79 bases and 410 bases. The kit includes all needed reagents for the PCR reaction, mixed in a master mix and a primer pair mix. The master mix and the primer pair mix are mixed in each well together with the DNA sample. The primers are conjugated to fluorescent dyes which is then used for identification of the products. (Tucker *et al.,* 2012)

The amplicons are then separated by size using either gel electrophoresis or capillary electrophoresis (CE). The latter was used in this project. The samples are, together with internal lane standards (ILS) and formamide, then loaded into a capillary with an applied voltage. Due to the length of the STR products, they will vary in charge making them migrate different lengths in the capillary when the voltage is applied. The products are then detected, using the fluorescence labelled dyes of the primers. The different dyes emit the light at different wavelengths, making it possible to distinguish the different dyes and thus the STRs. The fluorescent signals are shown as peaks, where the height corresponds to the initial amount of DNA in the sample. The ILS is a mix of several fragments with known size. The fragments are labelled with a dye and by comparing the fragments from the unknown sample with the ILS, the sizes of the fragments from the unknown sample are given. The sizes of the STRs are then compared to a ladder, which contains all common STR lengths, giving the lengths of the STRs from the sample which results in a DNA profile of all the 16 STRs. (Bright *et al.,* 2014) Formamide is a strong denaturant and is therefore often used in order to create single stranded DNA (ssDNA) before CE (Butler *et al.,* 2004).

2.2.6 Likelihood ratio (LR)

The STR profile obtained after CE analysis is then compared to the DNA of the suspect for a match. The STR analysis can show that the DNA was from a single person (single donor) or from multiple, a then so-called mixed profile. When comparing the obtained STR profile, an evidentiary value of the match is obtained, called the likelihood ratio (LR). The LR is a ratio between the probabilities of two hypotheses. The first hypothesis, Hp, is that the match is due to the suspect has left his/her DNA on the crime scene and the other hypothesis, Hd, is that the DNA match is just a coincidence, i.e. the suspect has not left his/her DNA at the crime scene and the DNA belongs to someone else. The probability of Hp is always 1, as it is always the prosecutions hypothesis that the defendant committed the crime. Hd, on the other hand, is calculated from the genotype frequency of the obtained STR profile from the crime scene. As the different alleles are different common among the human population, each allele variation in the genes has been given a value. A match between certain alleles can be more common than others and a match in an uncommon allele is therefore increasing the LR, i.e. increasing the probability that the DNA belongs to the suspect. (Butler, 2010)

These calculations have before been performed manually at NFC, but since the 2nd of May 2022 they have started to use a software called DNAxs. The software has been developed by the

Netherlands Forensic Institute (NFI) which has been using the software in routine analysis since 2017. (NFC, 2022)

2.3 Different DNA Trace Collection Methods for Firearms

Information about trace collection methods that are used in other countries is difficult to find. It is not always clear whether studies concerning swab types, detergents to TCB, location on firearms to swab etc. are the same methods used in the national forensic laboratories of the country. The information in the articles can, however, give an idea of other methods of trace collection that can be used.

In a study described in an article from Germany regarding transfer DNA on firearms, the areas swabbed for revolvers were the trigger, the grip, and the cylinder. For pistols these areas were the trigger, the grip, and the slide. The firearms were swabbed using 4N6FLOQSwabs® Genetics (Copan) and 10-25 µL of DNA free water was used as TCB. (Gosch *et al.,* 2019)

An article from the Illinois State Police Forensic Science Center at Chicago describing a study aimed to investigate whether swabs from different areas of the same weapon should be combined before extraction and further analysis or analysed separately. At the time the article was written, it seemed like swabs were analysed separately which often resulted in low concentration of DNA and in turn gave weak DNA profiles if any at all. However, the swabs were analysed separately in order to avoid as much mixed DNA profiles as possible. The areas that were swabbed were trigger, grip, hammer, cylinder release, magazine, slides, straps and front sites. However, a crime scene investigator told that the number of areas that were swabbed could range from two to six. The swabs used were sterile white cotton swabs, moistened with distilled water as TCB and the entire swab head was taken from the stick for extraction. (Richert, 2011)

A study performed in Switzerland describes that the DNA sampling in Switzerland at the time the article was written was by using the Prionics cardboard evidence collection kit. This kit included cotton swabs, sterile water ampoules (as TCB), cardboard and adhesive seals. (Comte *et al.,* 2019)

Only cotton swabs were used before at NFC, but after a study performed by Jansson *et al*. (2020), the standard swab was exchanged to nylon flocked swabs for cartridges and cartridge cases. They performed a study where they evaluated factors that might affect the DNA recovery on cartridge cases. They compared the DNA recovery obtained with three different type of swabs (cotton, nylon flocked and foam) and three different detergents in the TCB (SDS, Tween20, TritonX-100). When examining the detergents, they saw a beneficial effect of using Tween20 and TritonX-100 for nylon flocked swabs when the DNA amount was known to be high. When the DNA amount was lower, no positive effect was seen with the detergents. As cartridges and cartridge cases often have a low DNA concentration, they thought that detergents were not necessary for these objects.

Hedman *et al*. (2021) performed a study where they examined the influence of swabbing technique and swab type for different kind of surfaces. 13 different swabs were examined, divided into the following four categories: cotton, flocked nylon, small foam, and large foam. All 13 swabs were initially tested on three different surfaces, window glass, ridged plastic, and wood. The swabs with the best potential from each swab group were then tested on a plastic

bag, oilcloth, aluminium, steel, brass, and leather. The different surfaces were aimed to represent the different types of surfaces, smooth/non-absorbing, ridged/non-absorbing, and smooth/absorbing. By comparing the DNA yield after swabbing controlled saliva stains on the surfaces, they saw that the 13 swabs gave equal DNA yields for smooth/non-absorbing surfaces and that large foam swabs gave higher DNA yield for an absorbing wood surface. They also concluded that an angle of approximately 60° between the swab and surface was beneficial as well as rotating the swab during sampling. When sampling wood surfaces, they also saw that a larger volume of TCB was beneficial.

Gosch *et al*. (2019) performed a scenario study where they examined the DNA transfer during four different scenarios. For every scenario, background DNA was deposited on the firearm by a person, hereafter referred to as owner, for four days. The owner stored the firearm in his living environment and handled the firearm on two different days of these four days he stored it. On the fourth and last day, the owner mimicked loading the firearm and transported it back to the research facility where one of the four scenarios took place.

The scenarios were the following:

1. The owner mimicked a shooting by taking out the firearm from the bag, pulling back the hammer/slide, pulling the trigger and put down the firearm immediately.
2. As scenario 1, but with another person, hereafter referred to as shooter.
3. The shooter handled the firearm for a longer time, 5 min., and also carried the firearm in his waistband for 15 min. prior the shooting as performed in scenario 1-2.
4. As scenario 2, but the shooter also used a dry cotton towel to wipe off the firearm for 15 s. afterwards.

They performed these scenarios on a blank pistol and a blank revolver. The areas that were sampled the trigger, grip and cylinder for the revolver, and for the pistol, trigger, grip and slide. The swabbing was performed with flocked nylon swabs and 10-25 µL of DNA free water as TCB. From scenario 1, the owner stood for 92 % of traces from all the surface sampled. In scenario 2, it was a mixed profile of the owner and the shooter, but with a higher amount of the shooter's DNA on the pistol slide and the grip of both firearms. However, on the cylinder, where no contact was needed for the shooting, more of the owner's DNA was found. In scenario 3, the shooter's DNA stood for the largest relative proportion in 83 % of trace profiles. Scenario 4 showed that a wipe off with a dry cotton towel reduced the DNA recovery but did not prevent the yielding of a DNA profile.

A study investigating the DNA recovery from firearms performed by Polley *et al*. (2006) tells that the TCB used was 50 % methanol and the areas were swabbed with a linear fashion in a cross (hatch pattern), rotating the swab periodically. The study included revolver, pistol, single barrel break-action shotgun and a pump-action shotgun. The areas swabbed were the following:

Revolver: hammer spur, trigger/trigger guard, cylinder release, grip (both sides)
Pistol: hammer spur, trigger/trigger guard, slide serrations, grip (both sides)
Single barrel break-action shotgun: hammer spur, trigger/trigger guard, break-open lever, grip (both sides)
Pump action shotgun: safety switch/catch, trigger/trigger guard, forestock – pump arm, grip (both sides).

The highest DNA recovery was found on the grip and the slide.

3. Materials and Methods

The project was divided into several sub-studies which examined different aspects of DNA recovery. These sub-studies investigated the addition of different detergent to the TCB, different swab types for different types of surfaces, and where to find DNA on firearms. Some procedures where the same throughout all studies, and these are explained below.

3.1 Firearms and Cartridges

Firearms used in the project were aimed to represent the firearms that are used in criminal circles. The firearms chosen for this were the rifle Kalashnikov, and the two pistols Glock and Zastava M70. The police's service weapons today are a SIG Sauer, which was used as well. The cartridges used were V-crown JHP cartridges from SIG Sauer. The different firearms and the cartridge can be seen in *Figure 3*.

Figure 3: a) Kalashnikov, b) Glock, c) Zastava M70, d) SIG Sauer, e) V-crown JHP cartridges from SIG Sauer.

3.2 Decontamination

Before any sampling, the surfaces were cleaned from old DNA and dirt. Cartridges were cleaned by rubbing a paper towel wet with 70 % ethanol and then dried in room temperature. Firearms, magazine included, were first rubbed with 1 % sodium hypochlorite by rubbing a moistened paper towel, a sponge, or a toothbrush on the surface. This was followed by 70 % ethanol on a paper towel in order to remove excess sodium hypochlorite.

3.3 Preparation of DNA Traces

DNA traces were created by either adding saliva or by handling the objects, leaving touch DNA. Saliva was used in order to have a relatively controlled amount of DNA on the surfaces. As the cartridges and firearms were cleaned from old DNA before any of the studies, it was known that the collected DNA was from that specific amount of saliva. When using touch DNA, it is more difficult to control the deposition of DNA that ends up on the surface as different persons shed differently (Jansson *et al.,* 2022). It is then difficult to say whether the amount of collected DNA is due to the collection method or the shedding status of the donor.

Saliva was donated by a voluntary donor and the same batch of saliva was used during the entire project. The saliva was aliquoted into 200 µL batches in Eppendorf tubes in order to avoid thawing the entire batch every time. When saliva was used for DNA traces, it was diluted with DNA free water to an optimized volume for the particular surface, always containing 2 µL of saliva. For example, for cartridges 20 µL of saliva solution was needed to cover the surface. The saliva was then diluted 10 times to a solution of 0.1 µL saliva/µL. The saliva solution was put on the surface as small drops using a pipette (see *Figure 4*). The saliva stains were then dried in room temperature until no wet traces could be seen (approximately 1 h).

Figure 4: when using saliva, the DNA traces were put on the surface as small bubbles covering the surface.

When using touch DNA for the DNA traces, voluntary persons handled the firearms according to instructions created for the particular study.

3.4 Sampling Procedure

Swabs used in this project were cotton swab (Selefa), 4N6FLOQSwabs Crime Scene (Copan), 4N6FLOQSwabs Genetics (Copan) and Critical Swab (VWR Collection), see *Figure 5.*

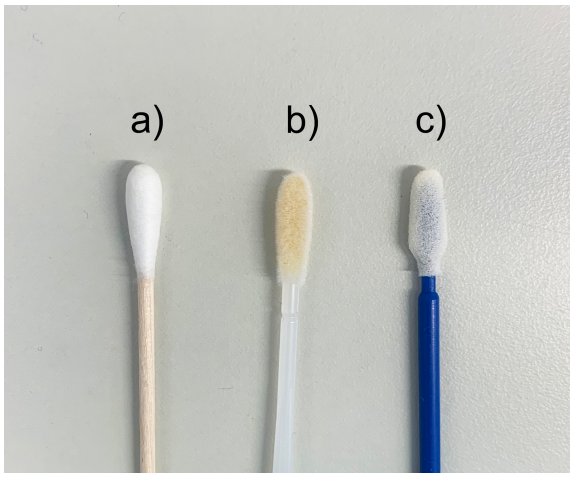

Figure 5: a) cotton swab (Selefa), b) 4N6FLOQSwabs Genetics (Copan) and c) Critical Swab (VWR Collection).

The cotton swab (Selefa) is hereafter referred to cotton swab, the 4N6FLOQSwabs Crime Scene (Copan) and 4N6FLOQSwabs Genetics (Copan) to nylon flocked swab and Critical Swab (VWR Collection) to foam swab. 4N6FLOQSwabs Crime Scene was used as the nylon flocked swab during the study of addition of detergents, but as the stock run out, this swab was exchanged to 4N6FLOQSwabs Genetics for the other studies.

Before swabbing any surfaces, the swabs were moistened with TCB. Due to the different properties of the material of the swabs, the swabs were moistened with different volume of TCB. Cotton swabs were moistened by adding 100 µL of TCB directly to the swab and nylon flocked swabs were moistened by adding 30 µL to one side of the swab and the swab was then rotated on the surface with a light pressure in order for the TCB to get into the swab. The foam swabs were moistened by dipping half of the swab-head into an Eppendorf tube with TCB and excess of TCB was removed by pushing the swab against the edges of the tube. If nothing else is stated, 0.9 % NaCl was used as TCB.

If nothing else is stated, the cotton and nylon flocked swabs were rotated while swabbing and the swabbing covered the area twice. As the foam swab is not round, this technique was not possible for that swab. Instead, one side of the swab was swabbed over the area once, and then flipped and the other side was used for swabbing the same area again. For cotton swabs, only the top of the swab was pushed against the surface. For nylon flocked swabs, the entire swab was used if possible, otherwise only the tip. For the foam swab, the entire swab was used. One swab was used per area.

The swabs were directly cut into Eppendorf tubes according to Appendix I. If samples were not extracted directly, they were stored in -20 °C until further analysis.

3.5 DNA Extraction

The DNA extraction was performed using the ChelexDirect protocol used at NFC for routine analysis. The ChelexDirect solution was prepared according to *Table 1* in a clean 100 mL glass bottle together with a clean stirring magnet.

Table 1: reagents and amounts in the ChelexDirect base used for DNA extraction.

ChelexDirect base	
Reagents	**Amount**
Chelex® 100 Resin	1 g
Super-Q water	18.6 g
Tween20 (10 %)	400 µL

The Chelex buffer was put on a magnetic stirrer in order to get an even amount of Chelex beads for every sample. Depending on what type of swab and study, different volumes of Chelex buffer was added to the samples. The volume added differed as the swab varied in size and the Chelex buffer should cover the entire swab when vortex. Either 100, 200 or 300 µL was used. 1 µL proteinase K (10 mg/mL) was then added per 100 µL Chelex buffer. For the studies were only 100 µL of Chelex buffer was used, 2 µL of proteinase K was added as 2 µL is the lowest amount of proteinase K to be added. The samples were vortexed for 10 s. and then incubated in a thermomixer for 1 h. at 1500 rpm and 56 °C. The samples were then incubated in a heat oven, 100 °C, for 20 min. and then cooled down in room temperature for approximately 15 min. Lastly, they were centrifuged at 11,000 rcf for maximum 1 min. If the samples were not quantified directly, they were stored in -20 °C freezer for further analysis.

3.6 DNA Quantification

The DNA quantification was performed using the qPCR with the RB1-assay and Immolase. The master mix was prepared according to *Table 2* in an Eppendorf tube. Depending on how many samples to be analysed, the total volume of the master mix was adjusted accordingly. As the reagents were stored in a freezer, they were first thawed in room temperature and then briefly vortexed and lastly spun down with a microcentrifuge.

Table 2: reagents in the master mix used for qPCR RB1-assay with Immolase.

Master mix qPCR RB1-assay			
Reagents	**Stock conc.**	**Conc./well**	**µL/well**
Super-Q water			5.9
Immolase (buffer)	10x	1	2
dNTP	2 mM	0.2	2
MgCl2	25 mg/mL	4	3.2
Primer RB1_80F	10 µM	0.3	0.6
Primer RB1_235R	10 µM	0.3	0.6
Probe RB1 FAM	10 µM	0.2	0.4
BSA	20 mg/mL	0.1	0.1
Immolase DNA pol HS	5 U/µL	0.005	0.2
Total			**15**

The master mix was vortexed before 15 µL of the master mix was added to each well together with 5 µL of the extracted samples. If the samples were stored in a freezer, they were first thawed in room temperature, quickly vortexed and then spun down at 11,000 rcf for maximum 1 minute.

The DNA standards used were prepared from a stock solution of 2800M DNA with a concentration of 10 ng/µL (Promega), with a dilution factor of 5, obtaining the following concentration: 5, 2, 0.2, 0.04 and 0.008 ng/µL. The standards were loaded into the wells as duplicates. A negative control was also always included containing DNA free water instead of sample.

The instrument was programmed according to *Table 3.*

Table 3: PCR programme for qPCR with RB1-assay with Immolase.

	Temp. (°C)	**Time (sec)**	
Initial denaturation	95	600	
Denaturation	95	10	45 cycles
Annealing	60	20	
Extension	72	30	

3.7 STR Profiling

As the samples were stored in a freezer, they were first thawed in room temperature, quickly vortexed and then spun down at 11,000 rcf for 1 minute. Samples with a concentration >0.05 ng/µL was diluted with DNA free water to 0.05 ng/µL or slightly less.

3.7.1 DNA Amplification with PowerPlex® ESX 16 Fast

A reaction mix was prepared according to *Table 4* in an Eppendorf tube. Depending on how many samples to be analysed, the total volume of the reaction mix was adjusted accordingly. The reagents were first vortexed before added.

Table 4: reagents in the reaction mix for amplification using PowerPlex® ESX 16 Fast kit.

Master mix PowerPlex ESX 16 Fast	
Reagents	**µL/well**
PowerPlex ESI/ESX Fast 5X Master Mix	5
PowerPlex® ESX 16 Fast 10X Primer Pair Mix	2.5
Total	**7.5**

The reaction mix was vortexed before 7.5 µL of the reaction mix was added in each well together with 17.5 µL of the extracted samples. A positive and negative control were also included. For the positive control, 0.05 ng/µL DNA sample was used. This was prepared from a stock solution with a concentration of 10 ng/µL by first taking 4 µL of the stock solution and mixing it with 76 µL of DNA free water (1:20 dilution). Secondly, taking 50 µL of the 1:20 dilution and mixing it with 450 µL of DNA free water (1:10 dilution) resulting in a 1:200 dilution. The solutions were vortex before and in between any mixing. The negative control contained DNA free water.

The instrument was programmed according to *Table 5*.

Table 5: PCR programme for amplification with PowerPlex® ESX 16 Fast system.

	Temp. (°C)	**Time (sec)**	
Initial denaturation	96	60	
Denaturation	96	5	30 cycles
Annealing	60	35	
Extension	72	5	
Final extension	60	120	
Hold/cooling	4	∞	

3.7.2 CE Analysis

The software was programmed according to the manual. A CE mix was mixed according to *Table 6*. Depending on how many samples to be analysed, the total volume of the CE mix was adjusted accordingly. The reagents were first vortexed before mixed.

Table 6: reagents in the reaction mix for the CE analysis.

CE mix	
Reagents	**µL/well**
Formamide	9.6
WEN ILS 500 ESS	0.4
Total	**10**

The CE mix was vortexed before 10 µL of it was added in each well of the CE plate together with 1 µL of the samples amplified with PowerPlex® ESX 16. Except for the positive and negative controls that were amplified with the PowerPlex® ESX 16, new controls were added to the CE plate. The positive control was already amplified DNA obtained from NFC with a known DNA profile. The negative control was DNA free water. 1 µL of Allelic ladder was also added to some wells. Allelic ladders were always in the first well, and in the last well if the samples were more than 20 in total. If the samples were more than 20, an allelic ladder was placed between every 24 samples.

The CE plate was then placed in the ABI 9700 at 94 °C for 3 min. The plate was then immediately put on an ice block for at least 2 min. A septa was then put on the plate and the plate was put in a CE retainer which was then put into the instrument for analysis. The analysis was then performed according to the manual and the STR profiles were obtained using the software GeneMapper ID-X.

3.8 Data Analysis

Data analysis, including statistical calculations and creating diagrams was performed using Microsoft Excel. One-way ANOVA followed by Bonferroni *post hoc* t-test were used to find

out whether the differences were statistically significant or not. The confidence interval used was 95 %.

3.9 Study Design

The sub-studies will be described more detailed in this section.

3.9.1 Addition of Detergents to the TCB

The examination of the detergents was performed in three rounds: round 1 with cotton swabs, round 2 with nylon flocked swabs and round 3 with foam swabs. The TCB used by NFC today, 0.9 % NaCl, was compared to 0.2 % SDS and 1 % Tween20. These detergents were prepared by diluting the stocks with NaCl.

For this study, cartridges were used as objects to be swabbed. 18 cartridges were cleaned as described in section 3.2. The saliva was thawed and vortexed before it was diluted 10 times with DNA free water by taking 900 µL of water and 100 µL of saliva in an Eppendorf tube. The saliva solution was vortexed and 20 µL was then put on 15 cartridges each. The remaining three cartridges acted as negative controls. The cartridges were dried in room temperature for 3 h.

Five cartridges were then swabbed with cotton swabs moistened with 100 µL NaCl, five cartridges were swabbed with cotton swabs moistened with 100 µL 0.2 % SDS and five cartridges were swabbed with cotton swabs moistened with 100 µL 1 % Tween20. One cartridge was used as a negative control for each TCB, i.e. no DNA was put on them in order to see if the TCB was free from contaminating DNA as well as the cartridges were cleaned from DNA residues.

The cartridges were cleaned once again, and the procedure was repeated with both nylon flocked swabs and foam swabs. The samples were then extracted with 300 µL ChelexDirect and 3 µL proteinase K and were treated as described above in section 3.5. The samples were then quantified with qPCR described in section 3.6.

3.9.2 Different Swab Types on Different Type of Surfaces

This study was performed in three parts: part I with saliva stains on Glock, part II with saliva stains on Kalashnikov, and part III with touch DNA on Glock for confirmation of the saliva test. The aim of this study was to examine what swab type is the best on the different type of surface, rather than where on the firearm the DNA can be found.

3.9.2.1 Part I – Glock and Saliva

Three Glock were used for this part, and each Glock was cleaned and swabbed three times resulting in triplicates for each swab type and area. The firearms were cleaned as described in section 3.2. Between the first cleaning and the first addition of DNA traces, negative controls

were taken from one of the firearms, as well as in between the last cleaning and the last addition of DNA traces in order to confirm the cleaning efficiency.

As mentioned, saliva was used for creating the DNA traces. The saliva was diluted differently for every area as their respective sizes differed in order to get an optimized volume of saliva containing 2 µL of saliva for each area. First, one 1:10 dilution with a total volume of 1 mL and one 1:5 dilution with a total volume of 500 µL were prepared. The final dilutions were prepared according to *Table 7.* In *Table 7*, it can also be seen how much of each saliva solution that was put on the surfaces. The stains were dried in room temperature for 1 h before sampled.

Table 7: Volumes of each dilution to create the final dilution, as well as volume added to the different areas for saliva study on Glock. For the trigger and the levers/controls, one saliva-dilution was made as the two areas were going to have the same volume, 10 µL on each.

Area	**From 1:10 (µL)**	**From 1:5 (µL)**	**H2O (µL)**	**Total volume (µL)**	**Final concentration (µL saliva/µL)**	**Volume added to the surfaces (µL)**
Grip	300		2700	3000	0.01	200
Slide area		150	1350	1500	0.02	100
Slide grip	300		900	1200	0.025	80
Magazine lips	300		300	600	0.05	40
Trigger and levers/controls		350	0	350	0.2	10

Three different swabs were used for this study, the cotton swab, nylon flocked and foam swab. One type of swab was always used for one firearm at the time and one swab was used for each area. In order to avoid as much bias as possible, the following schedule was created, see *Table 8*. The negative control samples were swabbed with cotton swabs. The swabbing was performed as described in section 3.4.

Table 8: Swab schedule created for the swab type study on Glock using saliva, in order to avoid as much bias as possible.

	Glock 1	**Glock 2**	**Glock 3**
Sampling 1	Cotton	Nylon-flocked	Foam
Sampling 2	Foam	Cotton	Nylon-flocked
Sampling 3	Nylon-flocked	Foam	Cotton

The areas that were samples were grip, slide area, slide grip, trigger, levers/controls, and magazine lips, see *Figure 6.* These areas were chosen as they represented different kind of surfaces, such as smooth and ridged surfaces in different sizes.

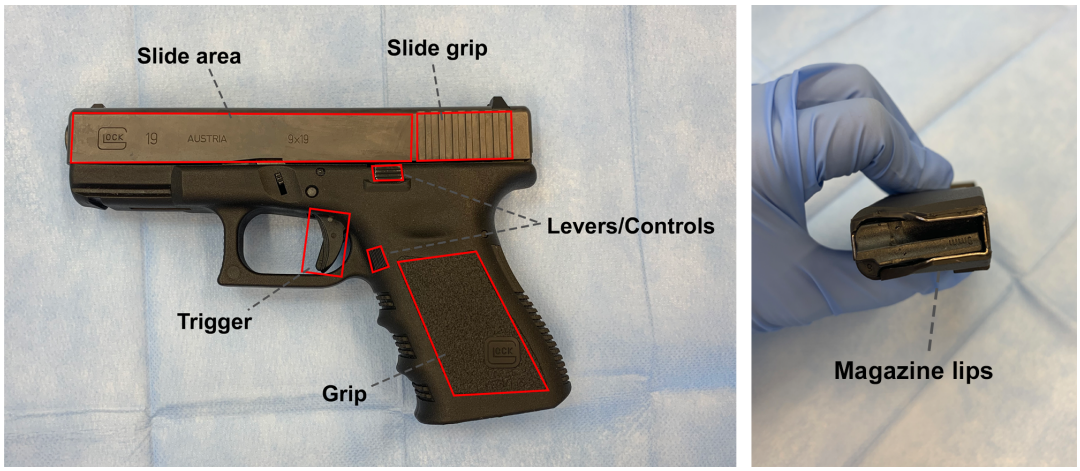

Figure 6: the different areas of the Glock that were swabbed for the swab study with saliva.

3.9.2.2 Part II – Kalashnikov and Saliva

Three Kalashnikov were used for this part, and it was performed as Part I, except for the negative controls that were only taken in between the last cleaning and the last addition of DNA traces. The volume of TCB was also larger for the hand guard as it was made of wood. For cotton, 120 µL TCB was used for the hand guard, and for the nylon flocked, 60 µL was used for the hand guard. The foam swab was dipped into the TCB as usual.

As the firearms were quite rusty at some areas, two rust samples were taken where DNA traces had not been put, in order to see whether the rust may inhibit the PCR or not. Two cotton swabs moistened with 100 µL each, were used to swab the rust.

The saliva for this part was prepared by firstly making one 1:10 dilution with a total volume of 1.3 mL and one 1:5 dilution with a total volume of 350 µL. The final dilutions were prepared according to *Table 9*. In *Table 9,* it can also be seen how much of each solution that was put on the surfaces. The stains were dried in room temperature for 1 h before sampled.

Table 9: Volumes of each dilution to create the final dilution, as well as volume added to the different areas for the saliva study on Kalashnikov. For the trigger and the catch for shoulder support, one saliva-dilution was made as the two areas were going to have the same volume, 10 µL on each.

Area	**From 1:10 (µL)**	**From 1:5 (µL)**	**H2O (µL)**	**Total volume (µL)**	**Final concentration (µL saliva/µL)**	**Volume added to the surfaces (µL)**
Hand guard	200		4800	5000	0.004	500
Shoulder support	200		1800	2000	0.01	200
Pistol grip	200		2300	2500	0.008	250
Receiver	200		1400	1600	0.0125	160
Magazine lips	240		360	600	0.04	50
Trigger + catch for shoulder support		250	0	250	0.2	10

The sampling procedure was performed as in Part I with the same type of schedule in order to avoid as much bias as possible, see *Table 10.*

Table 10: Swab schedule created for the swab type study on Kalashnikov using saliva, in order to avoid as much bias as possible.

	Kalashnikov 1	**Kalashnikov 2**	**Kalashnikov 3**
Sampling 1	Cotton	Nylon-flocked	Foam
Sampling 2	Foam	Cotton	Nylon-flocked
Sampling 3	Nylon-flocked	Foam	Cotton

The areas that were sampled were the hand guard, shoulder support, pistol grip, receiver, magazine lips, trigger, catch for shoulder support, see *Figure 7.* These areas were chosen as they represented different kind of surfaces, such as smooth and ridged surfaces in different sizes.

Figure 7: the different areas of the Kalashnikov that were swabbed for the swab study with saliva.

3.9.2.3 Part III – Glock and Touch DNA

Three Glock were used for this study and the decontamination and negative control sampling were performed as in Part II. Each firearm was cleaned and swabbed three times each, as in Part I and Part II.

Instead of saliva, touch DNA was used for creating DNA traces in this part. One person, a high shedder, handled each firearm three times. Between these handlings, the firearms were swabbed and cleaned. The person picked up the firearm, removed the magazine, loaded the magazine with 3 cartridges, put down the loaded magazine on the bench, picked up the firearm once again, pretended to shoot repeatedly three times by pulling the slide back and pressing the trigger, grabbed the slide area for five seconds before the person put down the firearm and removed the cartridges from the magazine. Between handling the firearms, the person rubbed himself in the neck with the hands in order to pick up some epithelial cells to shed on the firearm.

The sampling procedure was performed as in Part I and II, with the same type of schedule in order to avoid as much bias as possible, see *Table 11.*

Table 11: Swab schedule created for the swab type study on Glock using touch DNA, in order to avoid as much bias as possible.

	Glock 1	**Glock 2**	**Glock 3**
Sampling 1	Cotton	Nylon-flocked	Foam
Sampling 2	Foam	Cotton	Nylon-flocked
Sampling 3	Nylon-flocked	Foam	Cotton

The areas that were sampled were the slide area (left+right), grip (left), grip (right), slide grip (left), slide grip (right), trigger, levers/controls, magazine lips, magazine side (left) and magazine side (right), see *Figure 8.* These areas were chosen as they represented different kind of surfaces, such as smooth and ridged surfaces in different sizes.

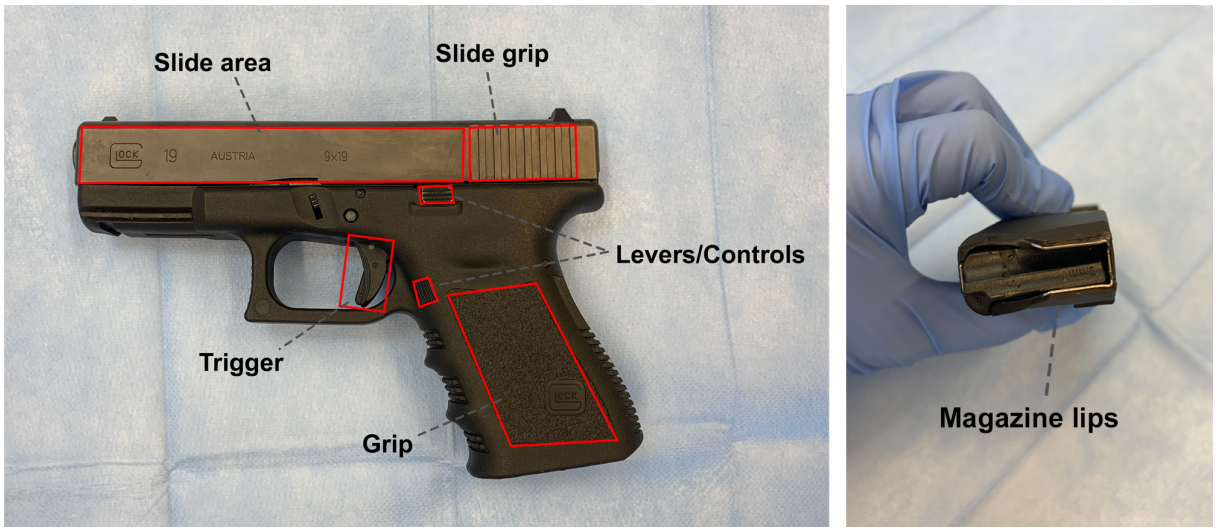

Figure 8: the different areas of the Glock that were swabbed for the swab study with touch DNA. The figure shows the left side of the pistol.

3.9.2.4 Extraction and Quantification

For Part I and II, the samples were extracted with 300 µL ChelexDirect and 3 µL proteinase K and were treated as described above in section 3.5. For Part III, 200 µL ChelexDirect and 2 µL proteinase K was used instead. The samples were then quantified with qPCR described in section 3.6 above.

The rust samples were extracted as the other samples but prior to the quantification, they were diluted with DNA free water so the final dilutions of each rust samples were 1:1, 1:2 and 1:4. This in order to see to what extent the rust may inhibit. A master mix was prepared as usual, but instead of 5.9 µL water in each well, 0.9 µL of 1 ng/µL DNA and 0.9 µL of water was put in each well. The DNA was prepared from a stock solution with 10 ng/µL diluted 10 times. 5 µL of the rust dilutions was added to the wells together with 15 µL of master mix. Two positive controls were used with only water together with the master mix in order to obtain Cq values of samples without any inhibition. The PCR was programmed as usual and the obtained Cq values were compared in order to see if the rust inhibited the PCR.

3.9.3 Location of DNA on Firearms

The aim of this study was to identify the location on firearms where DNA can be found after "normal" usage. This study was divided into the following sub-studies: (1) the crime scene investigators' service weapons and (2) Police officers' practice weapons.

3.9.3.1 (1) Crime Scene Investigators' Service Weapons

The firearms of 12 crime scene investigators were collected (SIG Sauer). Before sampling, nobody was allowed to touch the firearms except the owner.

The weapons were swabbed for the following areas: grip (left/right); slide grip (left/right); slide area (left/right); frame (left/right); trigger; levers/controls; muzzle + front sight; hammer; magazine lips; magazine bottom; magazine side (left/right) and barrel resulting in 17 different areas, see *Figure 9*. Only cotton swabs were used for this study and the sampling procedure was performed as described in section 3.4.

Figure 9: the different areas of the crime scene investigators' service weapons that were swabbed. The figure shows the left side of the pistol and the right side of the magazine. The barrel is found inside the pistol.

In order to compare the DNA profile from firearm with the DNA profile of the owner, each crime scene investigator gave a reference DNA sample. This was done by taking a cotton swab and rub the inside of the cheek for approximately 30 s.

The extraction was performed as described in section 3.5 with 100 µL ChelexDirect and 2 µL proteinase K for the firearm samples, and 300 µL ChelexDirect and 3 µL proteinase K for the reference samples. The quantification was performed as described in section 3.6 and the STR profiling as described in section 3.7.

As all barrels gave no DNA concentration after the qPCR, but some showed peaks during the STR analysis, an inhibition test was performed for some of the samples. The samples examined

were the barrel of firearm 5, 6, 7 and 12. Some other areas that gave 0 ng/µL after qPCR was also tested for inhibition. These were the left slide area and right side of the frame of firearm 5, the trigger and muzzle + front sight of firearm 7, the right side of the grip and the controls/levers of firearm 8 as well as the right side of the slide grip of firearm 9.

The inhibition test was performed with the extracted samples. The barrel samples were diluted so the final dilutions of each barrel samples were 1:1, 1:2 and 1:4. This is to see to what extent they might be inhibited. The qPCR master mix was prepared as in the inhibition test for rust described in section 3.9.2.4. Three positive controls were used with only water together with the master mix in order to obtain Cq values of samples without any inhibition. The PCR was programmed as usual and the obtained Cq values were compared in order to see whether the samples were inhibited or not.

An additional inhibition test was also performed using ToughMix instead of the reagents used for the other studies, in order to see if the inhibition could be prevented by using other reagents, and not only by diluting the samples. The following reagents were then mixed in each cell, see *Table 12,* together with 5 µL of the samples. The samples chosen to be analysed were left slide area and right side of the frame of firearm 5, the barrel of firearms 5, 6 and 7, and the right grip and the controls/levers of firearm 8.

Table 12: reagents in the master mix used for the inhibition test with ToughMix, using RB1-assay.

Master mix inhibition test with ToughMix			
Reagents	**Stock conc.**	**Conc./well**	**µL/well**
Super-Q water			2.4
DNA			1
ToughMix	2x	1	10
Primer RB1_80F	10 µM	0.3	0.6
Primer RB1_235R	10 µM	0.3	0.6
Probe RB1 FAM	10 µM	0.2	0.4
Total			**15**

Three positive controls were used with only water together with the master mix in order to obtain Cq values of samples without any inhibition. The PCR was programmed according to *Table 13* and the obtained Cq values were compared in order to see if the inhibition were prevented using ToughMix.

Table 13: PCR programme for qPCR with RB1-assay with ToughMix.

	Temp. (°C)	**Time (sec)**	
Initial denaturation	95	180	
Denaturation	95	10	45 cycles
Annealing/Extension	60	50	

3.9.3.2 (2). Police Officers' Practice Weapons

This study was performed by sampling the practice weapons at the facility where police officers are practicing. This means that several persons had handled these firearms (SIG Sauer). Four

different persons were instructed to load one of these weapons each, with five bullets, and the fire these. After the shooting, nobody else touched the weapons before they were sampled.

The weapons were then swabbed for the following areas: grip (left/right/front/back); slide grip (front/back/top); slide area (left/right); frame (left/right); trigger; levers/controls; muzzle + front sight; hammer; magazine lips; magazine bottom; magazine side (left/right) and barrel resulting in 20 different areas, see *Figure 10.* Only cotton swabs were used for this study and the sampling procedure was performed as described in section 3.4.

Figure 10: the different areas of the police officers' practice weapon that were swabbed. The figure shows the left side of the pistol and right side of the magazine. The barrel is found inside the pistol.

In order to compare the DNA profile from firearm with the DNA profile of the shooter, each shooter gave a reference DNA sample. This was done by taking a cotton swab and rub the inside of the cheek for approximately 30 s.

The extraction, quantification and profiling were performed as in 1. Crime scene investigators' service weapons. Samples from the grip and the trigger were also sent to NFC for calculating the LR of the samples. The calculations were performed with DNAxs.

3.9.4 DNA Recovery After of Different Handling Scenarios

The aim of this study was to investigate how different handling scenarios effected the DNA amounts on the firearm. The study was divided into four scenarios and the firearms used was two Zastava M70 and two voluntary persons (high shedders) handled the firearms. The scenarios were the following:

1. Storage of the firearm in a bag
The firearm was first cleaned as described in section 3.2 and was then put, together with at least five personal items (such as a sweater, a headwear, socks etc.), in a bag. The bag was then either transported or shaken for at least 10 min. every day for at least 5 days. None touched the firearm after the cleaning and before the sampling.

2. Handling with gloves
The two voluntary persons was given one pair of new gloves each (work gloves bought from JULA, item number 016521). These gloves where then used for at least 15 min. every day for at least 5 days in order to get into used condition prior to the handling. The firearms were cleaned as described in section 3.2 and the persons handled one firearm each wearing the gloves. The high shedders picked up the firearm and put in the empty magazine and put the firearm in the pocket while transporting itself and the firearm to the shooting room (8 floors down, with elevator). Once in the shooting room, they took out the firearm from their pockets, removed the magazine, put down the firearm on a bench, loaded the magazine with one cartridge, put back the magazine in the firearm, walked about 3 meters, unsecured the firearm, pulled back the slide and fired the firearm. This was repeated three times, in order to touch the levers and controls not only once.

3. Handling without gloves
The firearms were cleaned as described in section 3.2 and then handled by the two volunteers as in scenario 2, but without gloves.

4. Handling without gloves and a quick wiping
The firearms were cleaned as described in section 3.2 and then handled as in scenario 3, followed by a quick wiping with a dry paper towel before sampling. The wiping was to try mimicking a cleaning of the firearm that might happen on the streets by the criminals. The magazine was not taken out and cleaned.

The weapons were then swabbed for the following areas: grip (left/right/front/back); slide grip (left/right); slide area (left/right); frame (left/right); rear sight, levers/controls; muzzle + front sight; trigger; hammer; magazine side (left/right/front/back); magazine bottom and magazine lips resulting in 21 different areas (see *Figure 11*).

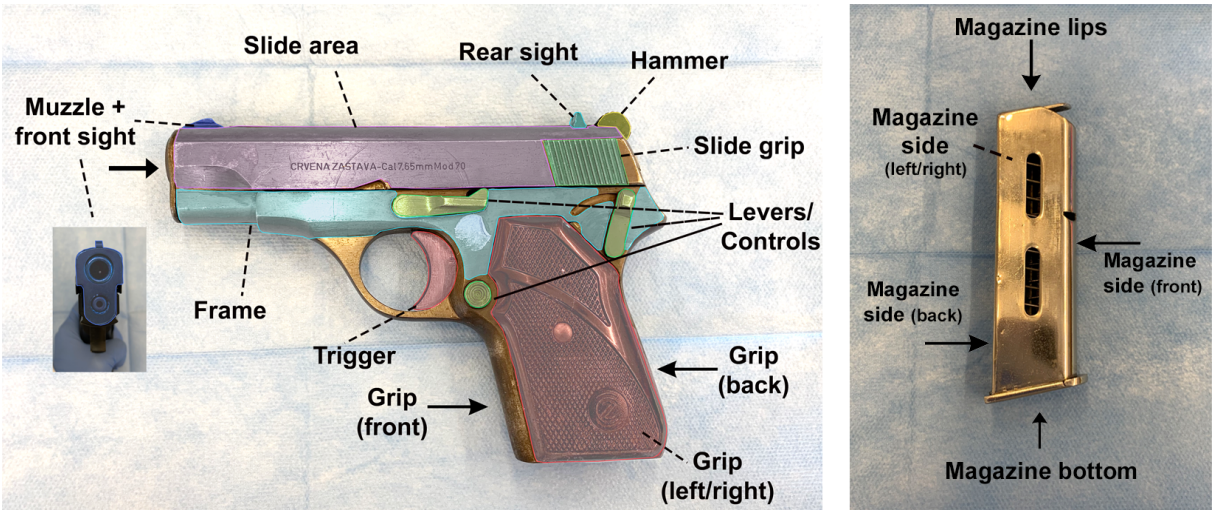

Figure 11: the different areas that were swabbed of the firearms used in the scenario study. The figure shows the left side of the pistol, and the right side of the magazine.

The extraction was performed with 100 µL ChelexDirect and 2 µL proteinase K and the samples were treated as described in 3.5. The quantification was performed as described in section 3.6.

4. Result & Discussion

4.1 Does Addition of Different Detergents to the TCB Affect the DNA Recovery?

With one-way ANOVA, the differences for the addition of the detergents were not significant for any swab type (p=0.94 for cotton swab, p=0.32 for nylon flocked swab, and p=0.17 for foam swab). The average DNA recovery was very similar for all detergents using cotton swabs, but the variation was smaller for Tween20compared to NaCl and SDS (see *Figure 12a*). As can be seen in *Figure 12b,* the DNA recovery were gathered lower for the SDS-samples, i.e. the interquartile range is having a lower concentration span, and with the first quartile and median values very close, when using nylon flocked swab. However, the maximum value of SDS was 0.028 ng/µL and increased the concentration span. Jansson *et al.* (2020) showed an improve DNA recovery when using Tween20 for nylon flocked swabs, but only for ≥5 µL of saliva. When having ≤2 µL of saliva, no differences were shown, as in this study. When using foam swab, the DNA recovery for Tween20 was in general slightly higher than for NaCl and SDS and the variation was the biggest for SDS (see *Figure 12c*). However, as p>0.17 for all swab types making the differences to not be statistically significant, these differences may occur by chance and no conclusion can be drawn. The number of replicates were only 5 and if a higher number of replicates were used, bigger differences might have been observed.

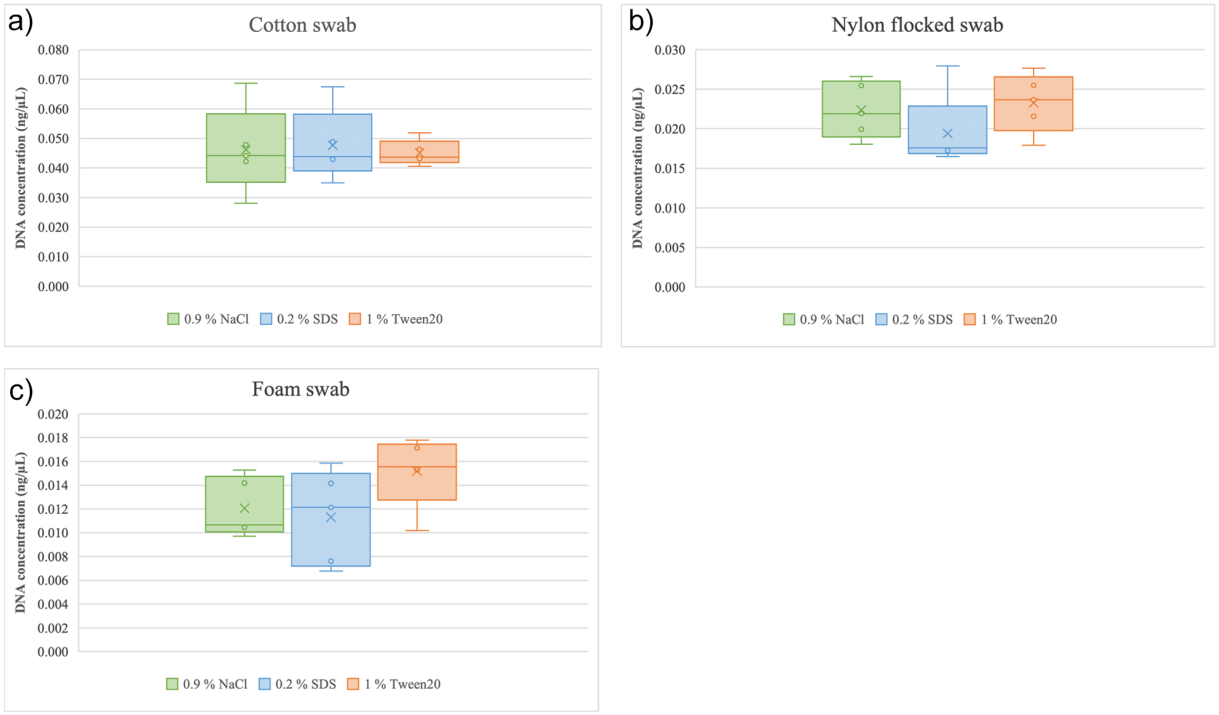

Figure 12: DNA recovery for a) cotton swabs, b) nylon flocked swabs and c) foam swabs with the addition of the different detergents compared to the standard TCB (NaCl) on cartridges. 2 µL of saliva was diluted in 20 µL as DNA traces (n=5). DNA concentrations are shown as boxplots visualizing the minimum, first quartile, median, third quartile and maximum values as well as mean values (x).

The addition of detergents did not seem to improve the DNA recovery, but the concentrations of the detergents were relatively low. A higher concentration might have given a higher DNA recovery. However, a higher concentration may inhibit the PCR reaction as both SDS and

Tween20 have shown to have inhibitory effects in higher concentrations. (Schrader *et al.,* 2012).

This study only included SDS and Tween20, i.e. no conclusions if the concept of adding detergent to TCB is efficient or not, can be drawn. TritonX-100 has for example, shown to improve the DNA recovery for nylon flocked swabs when the DNA amount is higher (Jansson *et al.,* 2020). As the differences were small on the cartridges using saliva, no more studies were performed on the detergents and focus was put on swabs and location of DNA on firearms instead. However, the efficiency of the addition of detergents may be higher on other surfaces than brass, and with other types of DNA traces than saliva. However, the results indicate that 0.9 % NaCl is as effective as SDS or Tween20.

4.2 Does the Swab Type Affect the DNA Recovery?

As can be seen in *Figure 13,* no swab type was clearly the best for all surfaces on a Glock using saliva and the variation between replicates were high in general. Using one-way ANOVA followed by Bonferroni *post hoc* t-test, the only significant difference was found on the trigger between the nylon flocked swab and the foam swab ($<0.05/3 \approx 0.017$) where nylon flocked swab gave higher DNA amounts than foam swab. For all other areas, $p>0.09$ indicating that there were no statistically significant differences between the different swabs. Cotton swabs did not give the highest mean DNA recovery for any surface. For the slide area, the nylon flocked swab had a higher mean DNA concentration compared to cotton and foam swabs. The slide area is a big smooth area, similar to the surface of a cartridge where nylon flocked swabs previously have proven to be the best. (Jansson *et al.,* 2020) The foam swab gave the highest mean DNA concentrations for the slide grip, while nylon flocked swabs gave the highest mean DNA recovery for the levers/controls and the trigger. This was quite surprisingly, as it was not expected due to the structure of the swabs and the surfaces. As all these surfaces had a ridged structure in some way, it can be thought that cotton should reach the corners and pits easier as the structure of the cotton swab is softer and more flexible than the other swab types. However, as mentioned, $p>0.09$ for all areas except the trigger, indicating that the DNA concentrations may be due to chance. As no other significant differences for small edgy surfaces, the difference between the nylon flocked and foam swab for the trigger, might have happened by chance as well. $n=3$ for all surfaces, and by using a higher number of replicates, more differences might have occurred.

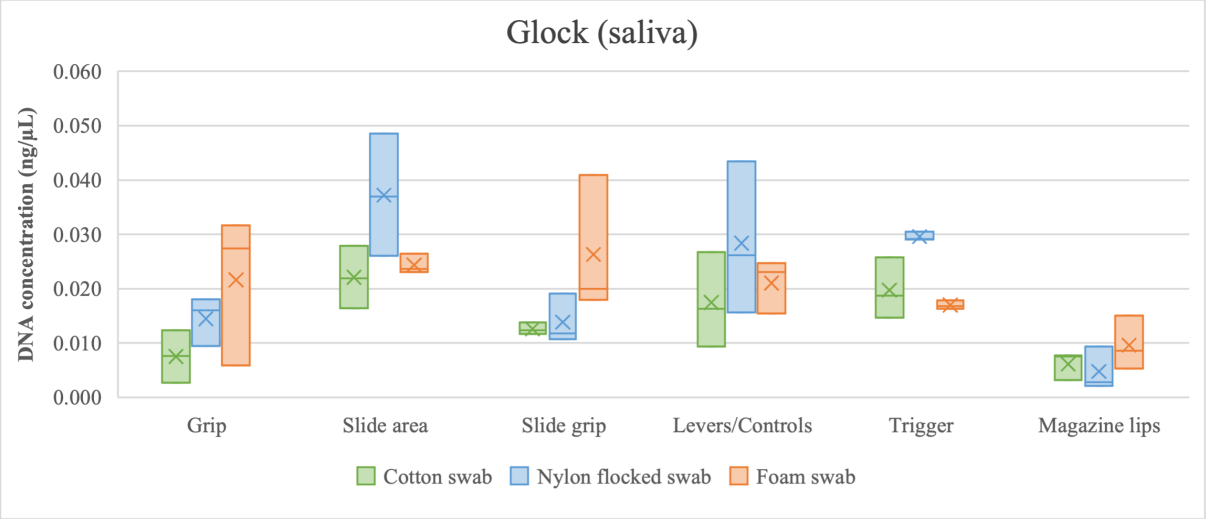

Figure 13: DNA recovery for the different type of surfaces on a Glock, using 2 µL of saliva diluted to optimal volume for each area as DNA traces (n=3). DNA concentrations are shown as boxplots visualizing the minimum, median, maximum and mean values (x).

Using one-way ANOVA for the surfaces on Kalashnikov using saliva as DNA traces, no statistically significant differences were found between the different swabs ($p>0.13$). Cotton swabs gave the lowest DNA recovery on the hand guard, which is a wooden surface (see *Figure 14*). Cotton swabs has shown to be less efficient on wooden material before, where foam swab performed better. (Hedman *et al.,* 2021) However, the foam swab did not give the highest DNA amounts in this study for the hand guard. Wood is an absorbing material, making it absorb not only the saliva, but also the TCB. The cotton swab had quickly a dry appearance when swabbing the hand guard, which might have contributed to the low DNA recovery. DNA might had been absorbed and located further into the wood as well, making it difficult for the swab to reach. The DNA recovery for foam swab at the trigger at the Kalashnikov was almost the same for all replicates. This can be explained as the trigger for the Kalashnikov was smooth and therefore easy to cover in the same way for all replicates with the foam swab. Due to the angle of trigger, it was more difficult to swab in the same way for all replicate for the other swab types. The DNA concentrations, may however, occur by chance as $p>0.13$. As for the Glock, the number of replicates were only three and with a higher number of replicates, other trends might have been seen.

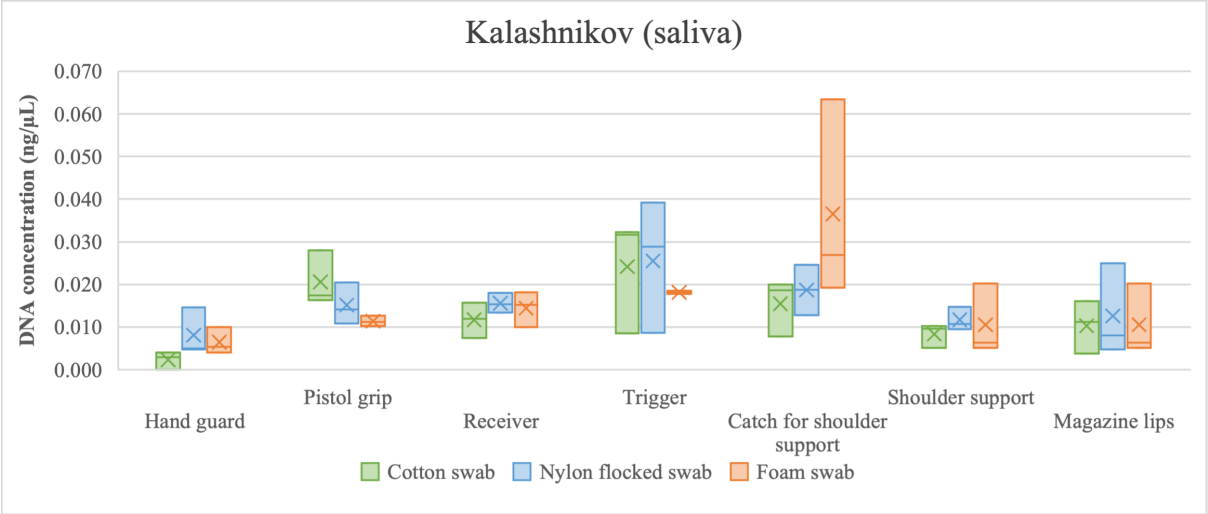

Figure 14: DNA recovery for the different type of surfaces on a Kalashnikov, using 2 µL of saliva diluted to optimal volume for each area as DNA traces (n=3). DNA concentrations are shown as boxplots visualizing the minimum, median, maximum, and mean values (x).

As can be seen in *Figure 15*, the differences in DNA recovery within the areas for the different swabs were small using touch DNA on Glock as well. One-way ANOVA showed that no differences were significant, with a $p>0.27$ for all surfaces. Several swabs gave no DNA at all, especially on the trigger, levers/controls, and magazine lips where there were at least one of all swab types that did not gave any DNA. The common property of these surfaces is the small size. On the levers/controls, none of the three cotton swabs gave any DNA. However, as for the saliva test on the Glock and Kalashnikov, the differences were not statistically significant, and it can therefore not be said that the differences occurred by chance or not.

As the amount of deposited DNA on the surfaces is more difficult to control when using touch DNA, as well as the replicates are only three, it is very difficult to draw any conclusion from the swab study on Glock using touch DNA. It is difficult to say if the DNA recovery is due to the swab type, or the amount of deposited DNA, for example, multiple areas had no DNA recovery at all.

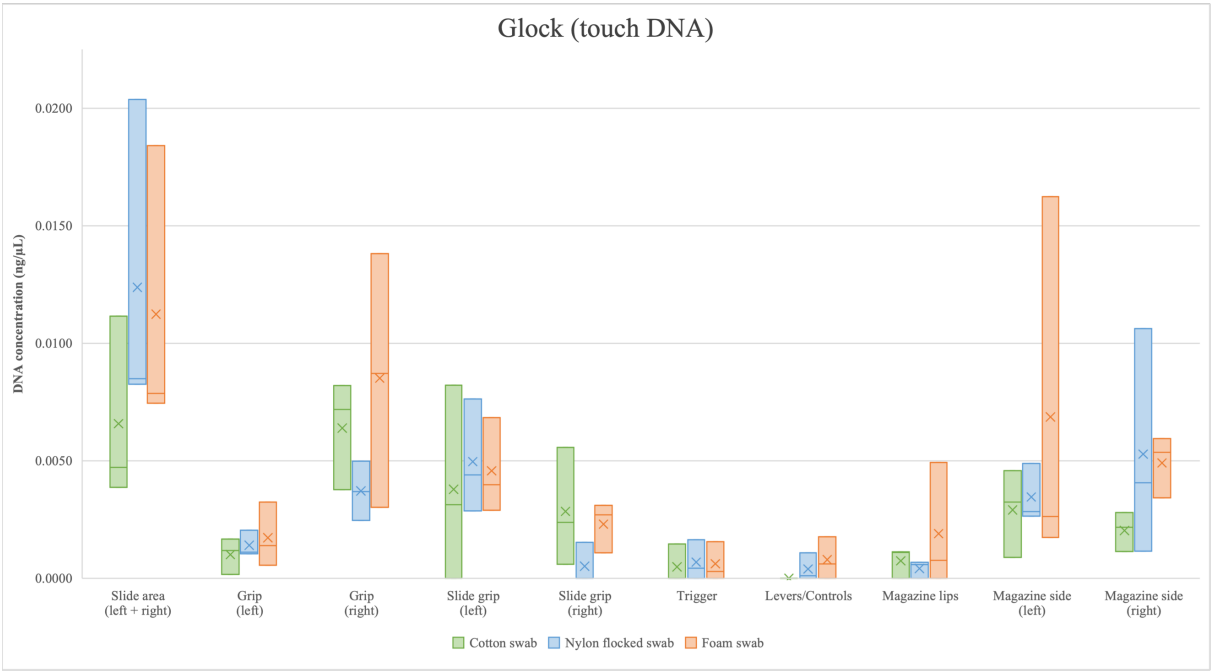

Figure 15: DNA recovery for the different type of surfaces on a Glock, using touch DNA as DNA traces (n=3). DNA concentrations are shown as boxplots visualizing the minimum, median, maximum, and mean values (x).

The DNA recovery is affected both by the absorption and release of the cells. Cotton and nylon flocked swabs are thought to have similar absorption ability, but due to the open fibre structure of the nylon flocked swab, it has shown to have greater release during extraction of DNA. Cotton swabs, on the other hand, have a more compact structure with fibres more tightly wounded. Which extraction method that is most suitable may also differ for the different swab types and does not necessarily be the one used in this project, i.e. the different swab types may perform better than what is shown in this project. (Jansson *et al.,* 2020)

4.3 Where on Firearms Can DNA Be Found?

The result from the different sub-studies of the location of DNA of firearms will shortly be presented separately below in section 4.3.1 and 4.3.2. A general discussion regarding the result of where on firearms the DNA can be found will be found in section 4.3.3 below.

4.3.1 Pistols handled by one individual

As can be seen in *Table 14*, the DNA recovery differs a lot between the different weapons belonging to the crime scene investigators. When looking at *Figure 16*, the magazine sides gave in general the highest DNA recovery. The magazine sides, the grip and controls/levers had more than 33 % of the samples with a concentration >0.01 ng/µL. Both sides of the slide grip, and both side of the frame gave a low DNA recovery compared to the other areas. They, as well as the muzzle + front sight had more than 66 % of their samples with a concentration <0.001 ng/µL. The barrel gave no DNA recovery for all weapons. The other areas were quite similar.

Table 14: DNA recovery for the different areas of the crime scene investigators' service weapons given in ng/µL, using qPCR RB1-assay with Immolase. The darkest green colour indicating a concentration ≥0.1 ng/µL, the second darkest green colour indicating a concentration between 0.01-0.09 ng/µL, the light green indicating a concentration between 0.001-0.009 ng/µL and the yellow colour indicating a concentration <0.001 ng/µL.

	Firearm 1	Firearm 2	Firearm 3	Firearm 4	Firearm 5	Firearm 6	Firearm 7	Firearm 8	Firearm 9	Firearm 10	Firearm 11	Firearm 12
Grip (left)	0.014	0.0080	0.0074	0.090	< 0.001	0.0033	0.077	0.022	0.0010	0.0081	0.0040	< 0.001
Grip (right)	0.0066	0.0048	0.017	0.047	0.044	0.0023	< 0.001	< 0.001	< 0.001	< 0.001	0.011	< 0.001
Slide grip (left)	0.0037	< 0.001	0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Slide grip (right)	0.0094	< 0.001	0.0017	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Slide area (left)	0.0058	< 0.001	< 0.001	0.011	< 0.001	0.0033	0.047	< 0.001	0.0012	0.0025	0.014	< 0.001
Slide area (right)	< 0.001	< 0.001	0.0016	0.017	< 0.001	0.0056	0.044	< 0.001	0.0032	< 0.001	0.021	< 0.001
Frame (left)	0.0026	< 0.001	0.014	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.010	< 0.001
Frame (right)	0.0046	0.0023	0.0051	0.043	< 0.001	< 0.001	< 0.001	< 0.001	0.0018	< 0.001	0.0019	< 0.001
Trigger	< 0.001	0.0011	< 0.001	0.024	0.016	0.0022	0.0093	< 0.001	0.022	< 0.001	0.0020	< 0.001
Levers/Controls	0.0020	< 0.001	0.013	0.016	0.054	< 0.001	0.011	< 0.001	0.0088	< 0.001	< 0.001	0.0011
Muzzle + front sight	< 0.001	< 0.001	0.027	0.019	< 0.001	< 0.001	0.030	< 0.001	< 0.001	< 0.001	< 0.001	0.0011
Hammer	< 0.001	< 0.001	< 0.001	0.019	0.031	0.0033	0.0087	0.12	0.0019	< 0.001	0.0026	0.0010
Magazine lips	< 0.001	< 0.001	< 0.001	0.0019	0.0056	< 0.001	0.014	0.0067	0.0016	0.0075	< 0.001	< 0.001
Magazine bottom	0.0021	0.0099	0.0029	0.0087	0.0091	0.0017	0.035	0.060	0.0084	0.0013	0.012	< 0.001
Magazine side (left)	0.0015	0.0091	0.0058	0.032	0.059	0.010	0.18	< 0.001	0.012	0.0065	0.0022	0.0040
Magazine side (right)	0.0069	0.0084	0.0011	0.042	0.082	0.013	0.11	< 0.001	0.0093	0.0023	< 0.001	0.0076
Barrel	-	-	-	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

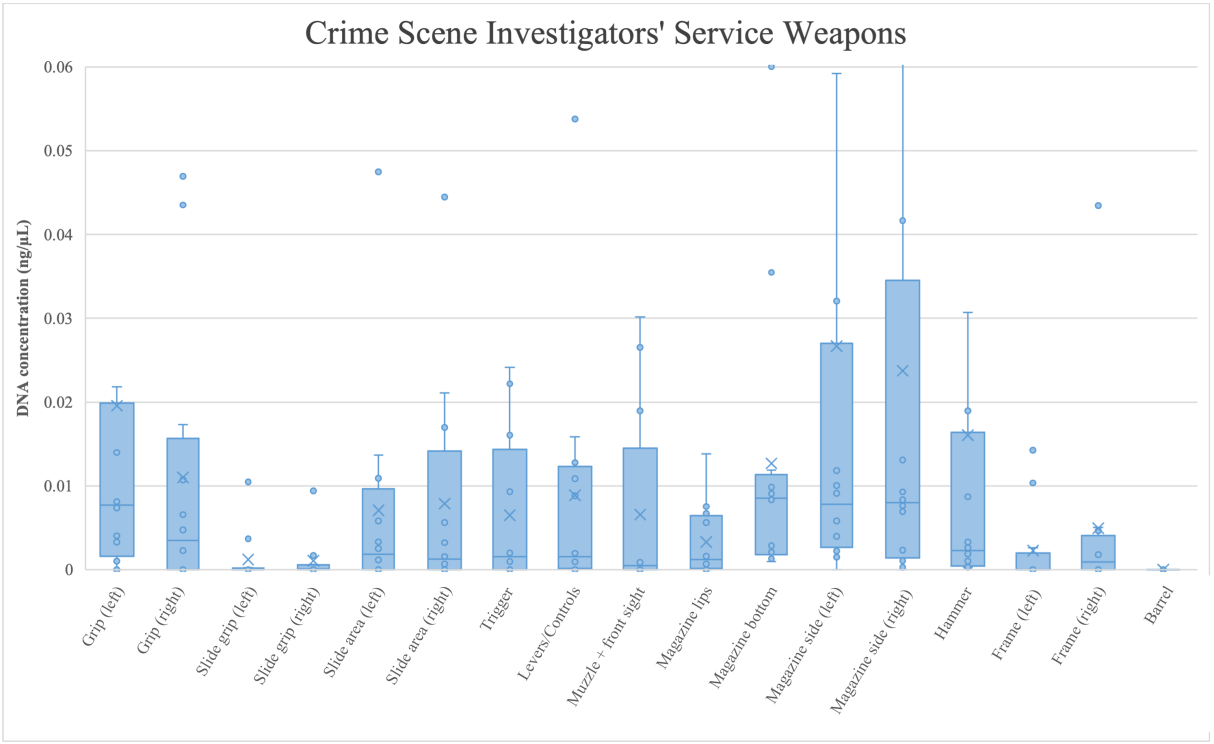

Figure16: DNA recovery for the different areas of the crime scene investigators' service weapons given in ng/µL, using qPCR RB1-assay with Immolase. n=9 for the barrel, and n=12 for all other areas. DNA concentrations are shown as boxplots visualizing the minimum, first quartile, median, third quartile and maximum values. As the diagram is cropped and centered to the mean values, several values are not shown in the diagram which are the following: grip (left): 0.077, 0.090; magazine side (left): 0.18; magazine side (right): 0.082, 0.11; hammer: 0.124 ng/µL.

The STR profiling of the crime scene investigators' service weapons showed both single donors and mixed profiles for the samples, as well as some blank profiles (see *Table 15)*. The barrel was either blank, or had a mixed DNA profile, with one exception for firearm 7 which had a single donor. Firearms 4, 5 and 7 were only single donor except for the barrel of 4 and 5 which were blank and the right side of the frame of firearm 4 that had a mixed profile. Firearm 6 had predominantly mixed profiles. The areas with the most samples with a single donor are the levers/controls and the magazine bottom with 8 sample each. The area with the highest number of mixed profiles is the right side of the frame with 6 samples showing a mixed profile. Firearm 9-12 belongs to women, but all gave at least one sample with a STR peak for the Y allele on the amelogenin gene. The single donors do not necessarily be the owner of the weapons' DNA, as it was not compared due to time limitations. The mixed profiles are either due to that several persons have handled the weapons, or the owner of the firearm have some cells from other persons on its hands as well.

Table 15: an overview of the result from the STR analysis of the crime scene investigators' service weapons. The green indicating a single donor (S), the blue indicating a mixed profile (M), the orange indicating a blank result, i.e. no peaks were obtained (B). The white (-) are samples without any result due to the areas were not sampled or something went wrong in the STR analysis.

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Grip (left)	M	S	M	S	S	B	S	S	B	B	S	B
Grip (right)	S	S	M	S	S	M	S	S	B	B	M	S
Slide grip (left)	B	B	B	S	S	B	S	S	S	B	S	S
Slide grip (right)	S	B	B	S	S	M	S	S	B	B	B	M
Slide area (left)	B	B	B	S	S	M	S	S	S	S	S	B
Slide area (right)	B	B	B	S	S	M	-	M	S	M	S	M
Frame (left)	B	M	M	S	S	S	S	M	S	B	S	B
Frame (right)	M	M	M	M	S	S	S	M	M	B	S	B
Trigger	M	S	B	S	S	M	S	S	S	B		B
Levers/Controls	S	S	M	S	S	M	S	S	S	B	S	M
Muzzle + front sight	M	S	S	S	S	S	S		M	B	B	M
Hammer	S	B	S	S	S	S	S	S	M	M	M	B
Magazine lips	S	B	B	S	S	M	S	S	M	S	S	B
Magazine bottom	S	S	S	S	S	M	S	S	M	S	M	M
Magazine side (left)	B	B	S	S	S	M	S	B	M	S	M	M
Magazine side (right)	B	B	S	S	S	M	S	-	M	S	S	M
Barrel	-	-	-	B	B	B	S	B	M	B	B	M

4.3.2 Pistols handled by several individuals

As can be seen in *Table 16*, the frame, muzzle + front sight, and the barrel gave no DNA recovery for all firearms of the practice weapons. The grip (left), on the other hand, gave a DNA recovery >0.01 ng/µL for all firearms. The grip in general gave a relatively high DNA recovery. All areas of the magazine gave a DNA recovery between 0.001-0.09 ng/µL for all firearms, as well as the levers/controls. The side of the slide grip, on the other hand, gave only DNA concentration ≠0 for firearm 1.

Table 16: DNA recovery for the different areas of the police officers' practice weapons given in ng/µL. The dark green colour indicating a concentration between 0.01-0.09 ng/µL, the light green indicating a concentration between 0.001-0.009 ng/µL and the yellow colour indicating a <0.001 ng/µL.

	Firearm 1	Firearm 2	Firearm 3	Firearm 4
Grip (left)	0.069	0.033	0.027	0.025
Grip (right)	0.059	< 0.001	0.002	0.027
Grip (front)	0.049	0.013	0.002	0.057
Grip (back)	0.088	0.014	0.002	0.008
Slide grip (left)	0.028	< 0.001	< 0.001	< 0.001
Slide grip (right)	0.071	< 0.001	< 0.001	< 0.001
Slide grip (top)	0.007	< 0.001	0.001	0.011
Slide area (left)	0.055	< 0.001	< 0.001	0.027
Slide area (right)	0.002	< 0.001	0.008	< 0.001
Frame (left)	< 0.001	< 0.001	< 0.001	< 0.001
Frame (right)	< 0.001	< 0.001	< 0.001	< 0.001
Trigger	0.011	< 0.001	0.003	0.014
Levers/Controls	0.020	0.008	0.004	0.007
Muzzle + front sight	< 0.001	< 0.001	< 0.001	< 0.001
Hammer	0.005	< 0.001	< 0.001	0.014
Magazine lips	0.003	0.002	0.003	0.010
Magazine bottom	0.018	0.008	0.005	0.015
Magazine side (left)	0.029	0.009	0.005	0.059
Magazine side (right)	0.029	0.023	0.001	0.042
Barrel	< 0.001	< 0.001	< 0.001	< 0.001

4.3.3 Discussion regarding the location of DNA on firearms

The areas that are swabbed on a pistol at NFC today are the slide grip, the trigger, the grip and the lips and bottom of the magazine in the standard analysis. However, the result from this project did not show a high DNA recovery at the slide grip compared to the other areas. The trigger did not give a remarkably high DNA recovery as well, but not low either. The same applies for the lips and bottom of the magazine. The grip, however, were often one of the areas with the highest DNA recovery. This was also shown in the study performed by Polley *et al.* (2006). Which is not surprising as it is an area that is needed to be hold quite steady and tight when firing. The structure is often ridged, which may contribute to shedding cells. The magazine sides, on the other hand, is an area not swabbed today at NFC due to it is an area often examined for fingerprints. The study of the crime scene investigators' service weapon gave a high DNA recovery of the magazine sides. However, the DNA recovery from the magazine sides was not remarkedly high for the studies of the police offers' practice weapons. The hammer is not an area swabbed in standard analysis as well but gave approximately the same DNA recovery in the study of service weapons as the trigger and muzzle + front sight.

The frame was not the area that gave highest DNA recoveries. This area is not included in the ones needed to touch during firing a weapon, which can explain the low DNA recovery. Where to swab for DNA traces is always a consideration between DNA traces and fingerprints. Fingerprints are often found on smooth surfaces, as the pattern is difficult to distinguish on a ridged surface. The frame, as well as the slide area and magazine sides are therefore common surfaces to look for fingerprints on, which is good as it seems to not be that much DNA traces there, at least not at the lower.

As can be seen in *Table 14,* the DNA recovery varies a lot between the firearms. For example, firearms 8 and 12 gave no DNA recovery for the majority of the areas, while firearm 4 gave a DNA recovery >0.01 for almost all areas. This is due to the concept that the amount of shedded cells varies between individuals. The amount of shedded cells depends on the shedder status of the donor, which means how prone the persons is to leave DNA behind. There are good shedders and poor shedders, meaning that the individual is prone to leave a high or low amount of DNA. However, there are several factors that affect the amount of shedded cells. For example, by washing hands, the amount of shedded cells will decrease, and by touching other parts of the body, the amount will increase. It has also been shown that men are often better shedders than women. (Jansson *et al.,* 2022)

All the handlings of the firearms have been performed by persons employed by the Swedish Police, meaning that all handlings were performed with high safety and in controlled ways. This might not be the case among the criminals. They might for example store the firearm in the waistband, which was however, tried to be mimicked during some studies. The service weapons are for example almost always stored in a locked cupboard or in the firearm holster of their police belt, if it is not used for firing.

4.4 How does different handling scenarios affect the DNA recovery?

As can be seen in *Figure 17*, the amount of DNA increased when handling the weapon without gloves compared to when gloves were worn. However, even though the shooter wore gloves during the entire scenario 2, it gave detectable amounts of DNA. It can also be seen that only storage of the firearm in a bag together with several personal items gave a DNA recovery for

all areas except for some areas of the magazine. However, the magazine was located inside the firearm during the entire storage, making no parts of the magazine except for the bottom, to touch the bag or the items inside the bag. The result is also showing that a quick wipe off with a dry paper towel does not remove any major amount of DNA, as in the study performed by Gosch *et al.* (2019). As can be seen, no DNA was found on the magazine lips for neither of the persons in the scenario with the wipe off. However, the wiping was not performed on the magazine, except for the bottom, as it was put in the firearm, i.e. the non-existence of DNA is not due to the wiping with the paper towel. The differences between the sides of the areas (left vs right) were smaller for the firearms stored in a bag compared to the handled firearms, at least for person 1, which also left a higher amount of DNA in general.

Table 17: DNA recovery for the different areas of the firearms used in the scenario study given in ng/µL. The dark green colour indicating a concentration between 0.01-0.09 ng/µL, the light green indicating a concentration between 0.001-0.009 ng/µL and the yellow colour indicating a concentration <0.001 ng/µL.

	Handling without gloves		Handling without gloves + wiping		Handling with gloves		Storage in bag	
	Person 1	Person 2	Person 1	Person 2	Person 1	Person 2	Person 1	Person 2
Grip (left)	0.23	0.010	0.042	0.009	0.010	0.004	0.012	0.003
Grip (right)	0.046	0.025	0.041	0.011	0.017	0.002	0.016	0.006
Grip (front)	0.33	0.012	0.050	0.019	0.009	< 0.001	0.005	0.003
Grip (back)	0.085	0.008	0.025	0.020	0.004	0.002	0.007	< 0.001
Slide grip (left)	0.10	0.002	0.077	0.004	0.005	< 0.001	0.005	0.007
Slide grip (right)	0.094	0.005	0.030	< 0.001	0.001	< 0.001	0.008	< 0.001
Slide area (left)	0.076	0.002	0.059	< 0.001	0.009	< 0.001	0.020	0.008
Slide area (right)	0.028	0.002	0.011	0.004	0.011	0.002	0.017	0.008
Frame (left)	0.034	< 0.001	0.034	0.002	0.0059	0.004	0.006	0.008
Frame (right)	0.080	< 0.001	0.011	0.002	0.013	0.002	0.006	0.004
Rear sight	0.003	< 0.001	0.002	< 0.001	< 0.001	< 0.001	0.003	0.002
Levers/Controls	0.060	0.004	0.018	< 0.001	0.004	< 0.001	0.006	0.009
Muzzle + front sight	0.003	< 0.001	0.005	< 0.001	0.004	< 0.001	0.005	0.001
Trigger	0.010	0.005	0.005	< 0.001	< 0.001	< 0.001	0.002	< 0.001
Hammer	0.033	0.002	0.006	0.002	0.008	< 0.001	0.007	0.005
Magazine side (left)	0.038	< 0.001	0.030	< 0.001	0.004	0.002	0.001	< 0.001
Magazine side (right)	0.005	0.002	0.011	0.002	0.006	0.001	< 0.001	< 0.001
Magazine side (front)	0.011	< 0.001	0.011	0.006	0.006	< 0.001	< 0.001	< 0.001
Magazine side (back)	0.026	0.002	0.003	0.002	0.002	< 0.001	< 0.001	< 0.001
Magazine bottom	0.096	0.019	0.055	0.004	0.014	< 0.001	< 0.001	0.003
Magazine lips	0.007	0.002	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

4.5 Is It Possible to Identify the Shooter of Firearms Previously Handled by Others?

As can be seen in *Table 18*, one of the shooters could be connected to the gun he fired applying DNAxs. Three samples gave partial result and the remaining samples harboured more than four contributors and were thus not proceeded with calculations in DNAxs in this project. LR-calculations were performed on four samples, giving one sample with the person of interest as main donor (trigger). The grip is larger with a more ridged structure, which may contribute to more shedded cells. The ridged structure may also prevent the cells from falling off as well, compared to the trigger which has a smooth structure. More contact has also been established with the grip. These factors may lead to more accumulation of DNA from previous shooters.

Table 18: result using DNAxs of the grip and trigger from the police officers' practice weapons. POI stands for "Person of Interest" and mismatches means how many STR locus of the 15 analysed that did not show any peaks. Person 1-4 is the shooters, and thus the desirable DNA to be distinguished. Result value is the NFC's scale of conclusions.

POI	Sampled area	No. of contributors	No. of mismatches	Likelihood ratio	Result value	Comment
Person 1	Grip, left	>4	1	-	-	Too many contributors, no calculation performed
	Trigger	>4	0	-	-	Too many contributors, no calculation performed
Person 2	Grip, left	4	1	0.1	0	Partial result
	Trigger	4	0	37040000000	+4	Person of interest is the main donor
Person 3	Grip, left	>4	2	-	-	Too many contributors, no calculation performed
	Grip, right	4	5	0.006	0	Partial result
	Trigger	3	8	0.4	0	Partial result
Person 4	Grip, left	>4	0	-	-	Too many contributors, no calculation performed
	Grip, right	>4	1	-	-	Too many contributors, no calculation performed
	Trigger	>4	0	-	-	Too many contributors, no calculation performed

4.6 Inhibition

The inhibition test for the rust obtained from the Kalashnikov shows that rust is not inhibiting the PCR reaction as the obtained Cq values are only varying with 0.11 from the positive control, as a maximum (data not shown). The rust might have occurred due to the cleaning of the firearms with sodium hypochlorite, as it is a strong oxidizing agent. However, the rust showed no inhibitory effects and as the firearms were cleaned before sampled, no other inhibitors should have been present. The result presented in *Figure 14* should thus be trustworthy in aspect of the DNA concentration obtained from the qPCR.

During the study using the crime scene investigators' service weapon, the firearms were opened in order to swab the barrel. The barrel was then found to be very dirty and oily. This, together with the fact that the barrel showed STR peaks in STR analysis even though the qPCR gave a concentration of 0 ng/µL, raised thoughts regarding inhibition. An inhibition test was therefore performed as described in section 3.9.3.1. For Barrel 7 and 12, the inhibition is removed by dilution of the samples. The barrel of firearm 7 showed inhibition until the 1:4 dilution, and the inhibition of the barrel of firearm 12 disappeared already for the 1:2 dilution. However, the barrel of firearms 5 and 6 still gave a Cq value of 0, even after dilution, indicating a strong inhibitory effect during PCR. The left slide area and right side of the frame of firearm 5, and the grip and levers/controls of firearm 8 gave no DNA recovery during qPCR, and a Cq value of 0 during the inhibition test, i.e. they were inhibited as well. The trigger and muzzle + front sight of firearm 7 gave DNA recovery during qPCR, and also a Cq value lower than the positive controls during the inhibition test. This is showing that they were not inhibited, during the qPCR. The result from the inhibition test makes it difficult to fully trust the result from the service weapons, as some of these samples may be inhibited. They can both be fully inhibited, resulting in no DNA recover, but also only partly inhibited, resulting in a DNA recovery, but not as high as in fact it is. However, inhibition is also a problem in "the real life", making the barrel not to be the best area to search for DNA traces, at least not with the methods used in this project.

As could be seen in the inhibition test of the service weapons, the inhibition disappears when diluting the samples as the concentration of the inhibitors contributing to the inhibition effect. However, the DNA in the samples is of course also diluted and a low amount of DNA is not always detectable. Dilutions is therefore not always a solution of inhibitors. The inhibition test with ToughMix showed a decreased inhibition (data not shown). All samples except the barrel of firearm 5 showed that the inhibition was defeated, at least for some extent. The barrel of

firearm 5, gave still a Cq value of 0. What reagents the ToughMix contains is not known, more than it is developed in order to be more robust against pollutants and inhibitors during PCR. This is showing that the inhibitors in the samples are possible to defeat in another way than dilutions. Other solution for removing inhibitors may be the use of other extraction methods or extra purification steps, or by the use of another DNA polymerase that are more tolerant to inhibitors or by PCR additives. (Schrader *et al.,* 2012)

4.7 General discussion

Another factor that may have affected the result is the way the cotton swabs are cut. When swabbing, it was tried to only use the tip of the cotton swab, with an angle of approximately 60°. However, it was not always possible, and the side of the swab was probably used sometimes. However, when the swab was cut, only the tip was cut off (see Appendix 1) as it is the way it is done at NFC today. This might lead to that DNA is left on the pieces of the swab that is not cut off, giving a lower DNA recovery than it could have been from that surface.

The sensitivity of the qPCR may also affect the result. However, the method should be as sensitive for all samples making it possible to perform comparison between all samples. If the sensitive is low, it can, however, give a DNA concentration of 0 ng/µL for samples containing a low DNA amount, but still not 0. Conclusion may then be drawn that no DNA was found on those areas.

5. Conclusion

As the differences for the addition of detergents using the various swab types were not significant, 0.2 % SDS and 1 % Tween20 does not seem to improve the DNA recovery at cartridges. The study on swab types did not show any significant differences as well, except for the trigger where the nylon flocked swab performed better than foam swab. On the remaining surfaces, the swab types performed equivalent.

The studies investigating the location of DNA on firearms after handling showed the highest DNA amounts on the grip and magazine sides, while the barrel did not give DNA for any pistol. However, the inhibition on the barrel showed to be a big problem. The frame and muzzle + front sight gave low DNA amounts on the police officers' practice weapons as well. High variations between the pistols could also be observed for the handled firearms, which is due to the different shedder properties of the persons. The levers/controls and the magazine bottom gave highest number of single donors when profiling the samples, while the right side of the frame gave most mixed profiles. However, inhibition was not only seen on the barrel, but on other areas as well, i.e. the samples might have been inhibited during PCR reaction.

The scenario study showed that handling with gloves gives DNA, but lower than handling with bare hands. It could also be seen that wiping the gun with a dry paper towel after handling was not efficient for removing DNA. Finally, it could also be concluded that storage of the firearm in a bag with personal items led to transfer of DNA to the gun, but in small amounts.

Applying DNAxs on 10 samples from the practice weapons (from the grip and the trigger), led to that one shooter could be connected to its pistol (trigger). Remaining 9 samples gave either partial result or had too many contributors to proceed the DNAxs calculations.

This project has resulted in more knowledge regarding the location of DNA on firearms as well as the effect of various handling scenarios. It has been shown that the areas swabbed today at NFC, do not always yield the highest DNA amounts. Hopefully, the result of this project can be a part of solving more cases involving firearms.

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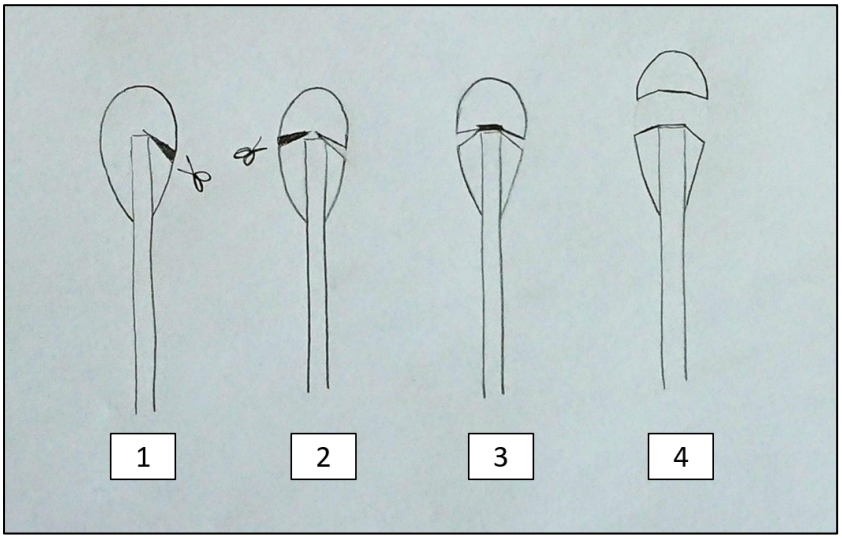
7. Appendix

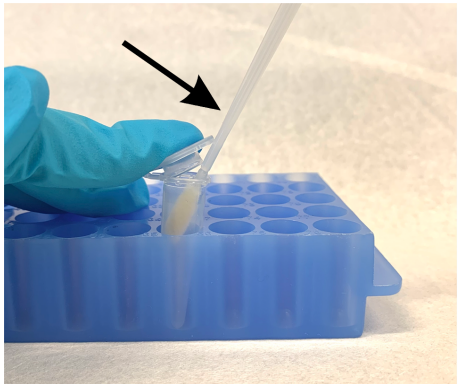
Appendix I – How to cut the swabs

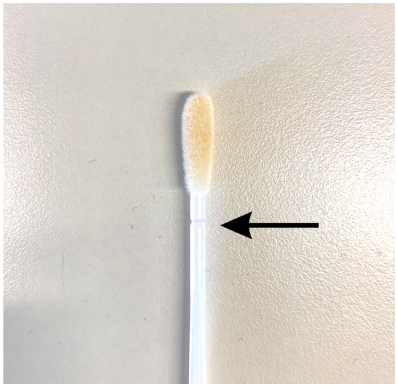
Cotton swabs



1. The goal is to cut the top of the swab as straight as possible, just above the wooden stick, without fluffing the cotton. Place the scissor where you think the stick ends and cut diagonally upwards, so you end up just by the end of the stick. Feel with the scissor all the time so you will find right. This will result in a diagonally cut from one of the sides, shown as black in figure above.
2. Now when you know where the stick ends, you can cut a little bit more horizontal from the other side.
3. Cut of the rest of the piece in the middle, shown as black in figure above, by putting the scissors blade in the already cuts openings.
4. Cut the swab directly into an extraction tube. It is important to cut the cotton completely, as the cotton will fluff if you pull off the cotton. Fluffy cotton will need a larger amount of volume in the extraction and will give lower DNA-recovery.

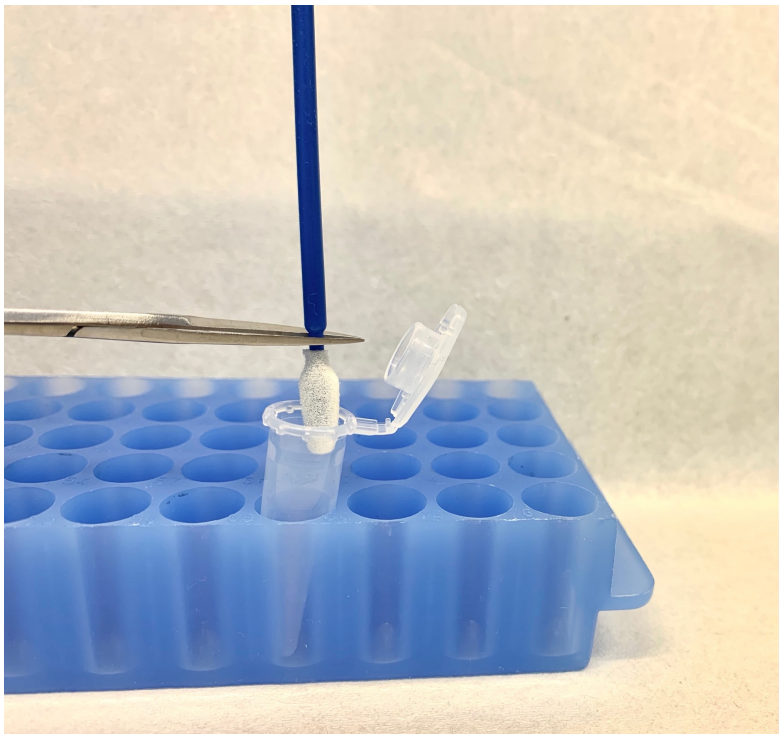
Nylon flocked swabs





1. Place the swab in an Eppendorf tube as in figure above (left) and align the small groove (marked with a black arrow in left figure above) on the swab with the hat of the tube as in figure above (left).
2. Push the stick downwards until the stick breaks.

Foam swabs



1. Place the swab in an Eppendorf tube and, with a scissor, cut the stick just above the swab head.