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Evaluation of a direct PCR method and the Qiagen Investigator 24plex GO! Kit for typing blood, saliva and touch DNA on multiple substrates

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A Thesis Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science in Forensic Science

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Abstract

In forensic DNA typing, omitting the DNA extraction procedure and adding the sample directly to the polymerase chain reaction (direct PCR) mixture has several advantages. Without extraction and purification, there is less of a risk of sample loss, sample mix up, or contamination. This study tested the feasibility of direct PCR using the Qiagen® Investigator® 24plex GO! Kit, a megaplex kit that amplifies 22 polymorphic STR markers and the Amelogenin sex determination alleles. Test samples included blood, saliva and skin cells on porous substrates, specifically denim, white cotton, polyester fabric, and paper tissue. Glass slides were used to represent non-porous surfaces. Body fluids like blood and saliva were collected using the following: Scotch™ double-sided tape, Zots™ dots, Sellotape® and two different FLOQSwabs™ from Copan© (microFLOQ® and a nylon FLOQSwab™). The results show that utilization of Sellotape® as a sample collection method was the most successful in generating a profile. This collection yields fast PCR-STR results and is non-destructive, so that the remaining sample can be re-tested if necessary. For touch samples, collection employed a double swab technique using the nylon FLOQSwab™ from Copan, a single Fitzco CEP swab, as well as a cutting method for the fabric substrates. The use of swabs had better success than cutting, probably due to the swab covering a larger surface area, thereby collecting a larger quantity of sample. Touch samples on glass were problematic. This sample type showed some PCR inhibition for samples collected with the FLOQSwab™ and had the lowest overall success rate.

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Introduction

Forensic DNA testing has proven itself to be an advantageous technique for investigating crime and is widely accepted within the scientific community. Every individual has a genome that is unique to him or her (apart from identical twins). One-half of one's genome is inherited from an individual's mother and the other half from their father. Forensic DNA analysis targets several small regions of the genome and the genetic variation that occurs there, by testing short tandem repeats (STRs) which are patterns of nucleotides that repeat themselves, usually 2-6 base pairs in length. Polymerase Chain Reaction (PCR) amplification of short tandem repeats in large multiplexes has become the main method for forensic DNA typing. Multiplexing involves simultaneous amplification of multiple targets during PCR. STRs are sufficiently polymorphic and are of small size (2-6 bp), which makes PCR amplification easier. Differential amplification, where one target region is selected over another during PCR, also decreases because of their small size in nature (Butler, 2010).

For PCR-STR to be successful, primers need to be specific so that they only bind to flanking parts of the DNA sequence that is the target for amplification. Primers are short DNA fragments that determine which part of the DNA is to be amplified. To ensure proper annealing, ion concentrations need to be ideal. If the concentration is too low, then primer binding will be weak and PCR yields will be low. If the concentration is too high then there will be unspecific binding and unwanted PCR product. To keep ion concentrations controlled, DNA must be purified prior to its addition to the PCR reaction tube. The other concern why DNA needs to be clean is the risk of inhibition. Forensic samples may contain a number of known Taq polymerase inhibitors like humic acid, hemoglobin, or fabric dyes (Alaeddini, 2012) which could result in reduced quantities of product (amplicons).

In the event that a crime has taken place, crime scene unit personnel collect as much probative evidence as possible. Evidence can be anything from fingernails to strands of hair to carpet fibers to biological stains such as blood and semen. If a substrate or item is expected to contain DNA, the first step in DNA typing is DNA extraction. The purpose of DNA extraction is to separate the DNA from proteins and other cellular material (Butler, 2010). Three extraction techniques used in today's laboratories include organic extraction, Chelex extraction, and solid-phase extraction. The extraction method used is dependent upon the type of biological sample being examined.

Organic extraction, also known as phenol-chloroform extraction, was often used for Restriction Fragment Length Polymorphism (RFLP) and PCR testing. Organic extractions utilize the enzyme proteinase K and different reagents such as sodium dodecyl sulfate (SDS), dithiothreitol (DTT) and Ethylenediaminetetraacetic acid (EDTA) to lyse cells and release DNA. Chelex extraction is faster than organic extraction and involves fewer steps, which minimizes sample mix up and contamination. An ion exchange resin is suspended into the mixture and is used to bind metal ions, like magnesium. By removing the magnesium ions, DNases are inactivated. Unfortunately, because Chelex involves heating, the double-stranded DNA is denatured, and present as less stable, single-stranded DNA. Solid phase extraction involves binding the DNA to silica particles, which is then followed by a series of washes to remove any impurities (Butler 2010). Although beneficial to PCR and other downstream applications, extraction has been shown to cause up to 80% of DNA loss (Ottens, et al., 2013).

Direct PCR is a method where an analyst can avoid this loss. Current FBI Quality Assurance Standards state that a laboratory must "quantify the amount of human DNA in forensic samples prior to nuclear DNA amplification" (Federal Bureau of Investigation 2011). In

order to implement a direct PCR method for real-life authentic casework, this step would have to be eliminated, but this may be beneficial. Instead of treating the sample via extraction, purification and quantification, an analyst could save time, money but more importantly avoid the loss of DNA. Direct PCR involves adding the sample directly into the amplification master mix inside the PCR reaction tube and then amplifying it. This method is especially useful in cases where there is a very low amount of DNA available for testing. Templeton and Linacre (2014) were successful in generating STR profiles from touch samples on fabrics by cutting a 2 mm x 2 mm section of each fabric after volunteers handled them. Verheij, Harteveld and Sijen (2012) were also able to generate full profiles using direct PCR after tape lift collection on porous and nonporous substrates.

Implementing a direct PCR method for casework also has other advantages. Since the sample is being directly added to the PCR reaction tube, the possibility of contamination or sample mix-up is minimized, lowering the error rate. Nevertheless, there are disadvantages as well. Since we are omitting the quantification step, there is no control over input, so the DNA amount may be too low or too high. Where the DNA amount may be insufficient, stochastic effects such as allelic dropout and heterozygous imbalance are expected. Amplifying too much DNA, can result in nonspecific amplification, n + 4 stutter products and an increased baseline (Cavanaugh & Bathrick, 2018). In the case of too much DNA, diluting the PCR product can help overcome electrophoresis artefacts. Also, without extraction there is no removal of common inhibitors like indigo dye and hemoglobin, which may affect results. Lastly, the amount of evidence that can be added to the PCR tube has sample size limitations. Since the volume for the Qiagen® GO! Kit is only 20 µL, pieces of the swab or stain substrate cuttings must be small enough to not absorb too much of the reaction mix.

This study tested the feasibility of the Qiagen® Investigator® 24plex GO! Kit for direct amplification. This megaplex PCR kit looks at 22 polymorphic STR markers along with the Amelogenin sex determination alleles. The GO! Kit contains two quality sensors that let the analyst know whether amplification was successful, if DNA was present, if the sample was degraded, or if there were inhibitors present. After evaluating quality sensor results, an analyst can determine what steps to take next for sample processing. The kit's intended use is for human identity and paternity testing (Qiagen®, 2016). The developmental validation study for the GO! Kit focused on reference samples and tested blood and buccal swabs with or without prior lysis to establish success rates and detection limits (Kraemer, et al., 2017).

This study used the Qiagen® Investigator® GO! Kit to amplify mock evidence samples (blood, saliva and touch DNA) on porous and nonporous substrates. The goal of this project was to develop a technique that involved the best collection method for challenging sample types, and show that a direct PCR kit can be advantageous in real scenarios. It was expected that as a result of this study, one or more tailored recovery methods that can generate full STR profiles for each sample type would be found.

Material and Methods

Preparation of test samples

For each combination of biological material and substrate, test sets consisting of samples from 5 different donors were generated. The substrates to be tested were a pair of denim jeans, a white cotton T-shirt and a polyester T-shirt, along with glass microscope slides and Kleenex paper tissue. The three fabric substrates were cleaned using a washing machine. Protective laboratory gloves were worn for each step involved to avoid contamination of the samples. Prior

to washing the clothes, the washing machine was run empty with bleach at a hot temperature. A set of clothes hangers was cleaned with bleach and water. The white cotton T-shirt, polyester T-shirt and a mesh laundry bag were washed at 40 °C with bleach. The denim jeans were washed at 40 °C without bleach. Both washes were repeated and the clothes were left to air dry on the hangers. After drying, the clothes were transferred to the mesh laundry bag. Prior to application of biological material, cuttings of approximately 9 cm x 5 cm were made for all fabrics. Fabrics were irradiated with UV light with the Air Science® UV Box™ for 45 minutes on both sides prior to touch DNA collection. The glass slides were washed with 10% bleach, followed by a rinse with RO (reverse osmosis) water followed by a rinse with 75% ethanol.

Blood samples were obtained from a commercial provider (Lee Biosolutions, Maryland Heights, MO). Saliva and touch samples were collected from volunteers at John Jay College of Criminal Justice following IRB approval (IRB File#2016-0916). Saliva donors were asked to let saliva pool at the bottom of their mouths before spitting into a 15 mL falcon tube. Liquid blood and saliva were used to spot twenty 1 µL and ten 5 µL stains on each substrate. Stains were left to dry for at least 24 hours. Fabrics and slides were kept at room temperature in the dark until tested. Touch DNA was collected by having volunteers first wash their hands, then rub their foreheads for 15 seconds and then rub their hands together for 15 seconds. This procedure was aimed at removing non-self DNA, but mimic a real life scenario that would occur after hand washing and a period of involuntary face touching (Kwok, Gralton, & McLaws, 2015). Volunteers then grabbed the fabric with all four fingers and thumb for 5 seconds. Prior to touching another fabric, volunteers repeated the face and hand touching step. For touch DNA on glass slides, volunteers followed the same protocol as for fabrics and pressed down their right and left thumbs for 5 seconds.

Scotch® double-sided tape lifts

Biological stains were lifted from the fabrics using a Scotch doubled sided (3M, St. Paul, MN) tape cutting of approximately 3 mm x 5 mm. The cuttings were placed atop of a 0.2 mL irradiated reaction tube (Eppendorf, Hamburg, Germany). The Eppendorf tube was used to touch the stain until pink for blood and 10 times for saliva. Half of the cutting was used for PCR and the other half was left on the Eppendorf and saved.

Zots™ adhesive lifts

Biological stains were lifted from fabrics using a single Zots™ (Therm-O-Web Inc., Wheeling, IL) adhesive dot. A pair of tweezers was used to hold the dots and stamp the stain. The Zots™ tended to adhere to the fabrics quickly, so these adhesives were stamped a few times and the entire dot was used for PCR.

Sellotape® tape lifts

Biological stains were lifted with double-sided Sellotape (Henkel, Winsford, Cheshire, United Kingdom). A strip of tape approximately 6 mm x 5 mm was cut and with a pair of tweezers was used to touch the substrates until pink for blood and ten times for saliva.

Swabbing

We tested two types of flocked swabs from Copan, Italy, the microFLOQ and the regular size nylon FLOQSwab™. The microFLOQ swabs were wetted with 1 µL of irradiated H_2O and then used to swab the porous and nonporous substrates a few times (in a circular motion). The

swab head was then broken off at the swab handle breaking point and directly added to a 0.2 mL irradiated PCR reaction tube (Eppendorf, Hamburg, Germany) with the PCR reaction mix. The nylon flocked swabs were cut into 2 mm x 2 mm pieces. Two swab cuttings were used following a double swab technique employed by Templeton and Linacre (2014). The first swab was wetted with 1 µL 0.1% Triton X. The wet swab was passed across the substrate's surface 10 times horizontally and 8 times vertically. This was followed by a dry swab that was also swabbed 10 times horizontally and 8 times vertically. Both swabs were added to the PCR reaction mix.

The Fitzco® CEP® swab (Fitzco, Inc. Minneapolis, MN) was also tested. Since the swab itself is much thicker than the Copan swab, a single 2 mm x 2 mm swab piece was wetted with 2 µL of 0.1% Triton X. The same swabbing procedure was used for the Fitzco swab as for the Copan swab, (10 times horizontally and 8 times vertically).

Polymerase chain reaction (PCR)

We used the Qiagen® Investigator® 24plex GO! Kit (Qiagen, Hilden, Germany) to amplify our samples via PCR and generate a genetic profile based on 24 loci. A negative control of 2 µL 0.1 x TE (TrisEDTA) was used and 2 µL of positive control DNA from the kit, diluted 1:10 to 0.5 ng/µL, was used as a positive control. The samples were added to 20 µL of master mix consisting of 7.5 µL of Fast Reaction Mix 2.0 and 12.5 µL of Primer Mix. The thermocycler (GeneAMP® PCR System 9700, Applied Biosystems Lifetechnologies, Carlsbad, CA) was used for amplification of the samples for a total of 29 cycles as follows: 3 cycles at 98ºC for 30 seconds (s), 61ºC for 55 s, and 72ºC for 5 s, followed by 26 cycles at 96ºC for 10 s, 61ºC for 55 s, and 72ºC for 5 s, followed by a 68ºC final extension for 2 minutes and a 10ºC soak.

Electrophoresis and sample analysis

Samples were stored at 4°C prior to electrophoresis on an Applied Biosystems® Genetic Analyzer 3500. Using a 96-well plate, 1.2 µL of PCR product was added to 12 µL of the DNA BTO 550 size standard (Qiagen, Hilden, Germany) and Hi-Di formamide (Lifetechnologies, Carlsbad, CA) mixture. For every sample, 0.4 µL of size standard were mixed with 12 µL of Hi-Di formamide. For the electrophoresis, we used POP-4™ polymer and a 36 cm capillary column (both Lifetechnologies, Carlsbad, CA) were used. The injection conditions were set to 1.2 kV with a 15 second injection time. Fluorescent peaks were then analyzed with the GeneMapper® ID-X Software v. 1.5 (Lifetechnologies, Carlsbad, CA) using the Qiagen 24plex macro and a 50 relative fluorescence units (RFU) threshold.

DNA profiles were classified based on how many of the expected alleles were present (21 total autosomal STR loci). A full profile included correct genotypes at all 21 loci, a "good" partial profile included 10 or more loci correctly typed and a "bad" partial profile included 9 or less loci. A negative/not suitable for comparison profile detected either no alleles at all or only incomplete loci with alleles missing. The Qiagen® Investigator® 24plex GO! Kit also contains internal controls indicating typing success. When both Quality Sensor 1 (QS1) and Quality Sensor 2 (QS2) are present, PCR was successful. Therefore, when QS1 and QS2 are present, but no peaks were generated, it meant no DNA was present. When QS1 and QS2 are absent, it indicates that the PCR failed; when the peak of QS2 is lower than the peak of QS1, inhibitors must have been present; and, when both quality sensors are present but the overall DNA profile has a ski-slope appearance, it means that the DNA is degraded.

Results

Preliminary testing: Blood

Scotch™ versus Zots™

We tested Scotch™ double-sided tape in comparison to Zots™ clear adhesive dots. The Zots™ dots already come premade as a clear, circular dot (3/16 in) attached to a film. We simply used a set of tweezers to touch them onto the substrate for sample collection. For the Scotch™ double-sided tape, 3 mm x 5 mm of tape was set atop of a 0.2 mL PCR reaction tube. The tube was used to stamp the substrate (until light pink). We used 5 µL blood samples from a single donor on the following substrates: white cotton t-shirt (WCT), polyester t-shirt (PT) and blue denim jeans (D). For the three blood samples, full profiles were seen for both collection methods. Although the Zots™ were a bit more adhesive to the fabrics in comparison to the Scotch™ tape, they gave better results (RFU values were nearly 6x as high). Results are found in Table 1.

Table 1 Zots™ collection method in comparison to the Scotch™ collection method

Stain Type	**Zots™ collection**	**Scotch™ collection**
5 µL blood on denim	FP	FP
5 µL blood on white cotton	FP	FP
5 µL blood on polyester	FP	FP

'FP' indicates a full profile. 'GP' indicates a good partial (10 or more loci detected). 'BP' indicates a bad partial (less than 10 loci detected. 'NP' indicates a negative profile.

Zots™ versus Sellotape®

We then compared the Zots™ collection method to a collection method using Sellotape® on 1 µL blood stains from a single donor on Kleenex tissue, white cotton t-shirt and denim jeans.

The Sellotape® was cut into 6 mm x 5 mm pieces and a pair of tweezers was used to touch the substrates (until pink). The stain on Kleenex was very difficult to collect because the tissue ripped after contact with the Zots™ adhesive. On fabrics, the adhesive material can be touched down a few times but the tissue paper just tore. The Zots™ collection, however, gave a good partial profile with a good RFU value above the threshold. The Sellotape® collection method gave a full profile for the Kleenex tissue. For fabric, the Zots™ gave a higher RFU for white cotton t-shirt whereas the Sellotape® gave a higher RFU value for the denim, even though the latter was a full profile. Overall, the Zots™ gave a slightly better result than the Sellotape®. Despite better results, the Zots™ dots were more difficult to handle and the material disintegrated after multiple touchdowns. Results can be found in Table 2.

Table 2. Zots™ collection method in comparison to the Sellotape® collection method.

Stain Type	**Zots™ collection**	**Sellotape® collection**
1 µL blood on denim	FP	BP
1 µL blood on white cotton	FP	FP
1 µL blood on Kleenex tissue	GP	FP

'FP' indicates a full profile. 'GP' indicates a good partial (10 or more loci detected). 'BP' indicates a bad partial (less than 10 loci detected. 'NP' indicates a negative profile.

MicroFLOQ® versus Sellotape

The last set of collection methods that was compared was Sellotape against MicroFLOQ® on denim jeans and glass slides. With the MicroFLOQ® swabs, one is able to swab a substrate and directly add the whole swab head to the PCR reaction tube via a breaking point on the handle. We used 1 µL blood stains on glass and denim. MicroFLOQ® was an easy collection method, but the Sellotape® sample provided higher RFU values and two full profiles

(even in comparison to its previous test against Zots™). The Sellotape® performed well in this round. Results are found below in Table 3.

Table 3. Sellotape® collection method in comparison to the MicroFLOQ™ collection method.

Stain Type	**Sellotape® collection**	**MicroFLOQ™ collection**
1 µL blood on denim	FP	FP
1 µL blood on glass slide	FP	FP

'FP' indicates a full profile. 'GP' indicates a good partial (10 or more loci detected). 'BP' indicates a bad partial (less than 10 loci detected. 'NP' indicates a negative profile.

Final set of five donors: Blood

Based on the preliminary tests, we decided to test the final set of blood donors using the Sellotape®. For the blood stains, we tested 1 µL stains from five different donors deposited on a white cotton t-shirt, polyester t-shirt, denim jeans, Kleenex tissue and a glass slide. The stains on fabric were touched with the tape until a faint pink color could be seen. For the Kleenex substrates, half of a 1 µL stain was cut and directly added to the PCR reaction tube and amplified

Only two out of 25 samples showed inhibition. Both QS1 and QS2 were detected in all the Sellotape® samples for all the donors apart from the Kleenex tissue. Full profiles were seen for the white cotton t-shirt, polyester t-shirt, blue denim jeans and glass slide for all the donors. For the Kleenex tissue that had been cut and added to the PCR reaction, inhibition was seen for two of the samples (QS2=3). Nevertheless, the PCR-STR reaction still worked sufficiently because there were two good partial profiles and three full profiles. For a large multiplex with 22 polymorphic loci, a partial profile with 15-18 loci still has a very high power of discrimination. Compiled results can be found in Table 4 below.

Table 4. Final set for blood donors.

Collection/pre-treatment	Sample Type	# of full	# of good partial	# of bad partial	# of negative	QS2 detection
Sellotape	Blood on white cotton (n=5)	5	0	0	0	5
	Blood on denim (n=5)	5	0	0	0	5
	Blood on polyester (n=5)	5	0	0	0	5
	Blood on glass (n=5)	5	0	0	0	5
Cutting	Blood on paper tissue (n=5)	3	2	0	0	3

Preliminary testing: Saliva

Scotch™ versus Zots™

Scotch™ double-sided tape versus Zots™ clear adhesive dots were next tested. The Zots™ dots can be handled with tweezers to touch them onto the substrate for sample collection. For the Scotch™ double-sided tape, as before, 3 mm x 5 mm of tape was set atop of a 0.2 mL PCR reaction tube. The tube was used to stamp the substrate (1-5 times). We used 5 µL saliva stains from a single donor on the following substrates: white cotton t-shirt, polyester t-shirt and blue denim jeans. For the three saliva samples, full profiles were seen for all but one (the zot on polyester). The bad partial on the polyester could be attributed to the fact that the Zots™ stuck to the fabric once, which made stamping it a second time difficult. Results are below in Table 5.

Table 5. Zots™ collection method in comparison to the Scotch™ method.

Stain Type	Zots™ collection	Scotch™ collection
5 µL saliva on denim	FP	FP
5 µL saliva on white cotton	FP	FP
5 µL saliva on polyester	BP	FP

'FP' indicates a full profile. 'GP' indicates a good partial (10 or more loci detected). 'BP' indicates a bad partial (less than 10 loci detected. 'NP' indicates a negative profile.

MicroFLOQ® versus Sellotape

The Sellotape® collection method was compared to MicroFLOQ® on denim jeans and glass slides. For this test, 1 µL saliva stains on denim jeans and a glass slide were used. Saliva on denim produced the bad partial profiles for both collection methods with the Sellotape® having higher RFU values. Saliva on glass produced one good partial profile (Sellotape®) and a full profile for MicroFLOQ®. Overall, the Sellotape® worked better than the MicroFLOQ™. Results can be found below in Table 6.

Table 6. Sellotape® collection method in comparison to the MicroFLOQ™ collection method.

Stain Type	Sellotape® collection	MicroFLOQ™ collection
1 µL saliva on denim	BP	BP
1 µL saliva on glass slide	GP	FP

'FP' indicates a full profile. 'GP' indicates a good partial (10 or more loci detected). 'BP' indicates a bad partial (less than 10 loci detected. 'NP' indicates a negative profile.

Final set of five donors: Saliva

Based on the preliminary tests (and considering the results from our blood donors) it was decided to test the final set of saliva donors using the Sellotape®. For saliva donors, we tested 1 µL stains from five donated saliva samples that were deposited on a white cotton t-shirt, polyester t-shirt, denim jeans, Kleenex tissue and a glass slide, respectively. Since the dried saliva stains were difficult to see, circles were made on the fabrics prior to the stain being deposited. The fabric substrates and glass were touched ten times. For the Kleenex substrate, a cutting was taken from the center of the circle and directly added to the PCR mix.

Results for the Sellotape® on glass were not as good as for the other substrates. We saw three bad partial profiles and two good partial profiles. For the white cotton, denim and polyester, a majority of the profiles were full. In order to improve our results for stains on glass, we tried a new collection method using a new swab type, Fitzco® CEP® swab. For the Fitzco® swab, there were three full profiles and two bad partial profiles. The Fitzco® collection method was more successful than the Sellotape® on glass. QS2 was detected in all the saliva donors for glass, denim, white cotton and polyester. For the Kleenex tissue, inhibition was detected in three of the samples. Overall, the results for Kleenex were one full profile, one good partial profile and three bad partial profiles. Compiled results can be found in Table 7 below.

Table 7. Final set for saliva donors.

Collection/pre-treatment	Sample Type	# of full	# of good partial	# of bad partial	# of negative	QS2 detection
Sellotape	Saliva on white cotton (n=5)	4	1	0	0	5
	Saliva on denim (n=5)	5	0	0	0	5
	Saliva on polyester (n=5)	4	0	1	0	5
	Saliva on glass (n-5)	0	2	3	0	5
Fitzco CEP Swab	Saliva on glass (n=5)	3	0	2	0	5
Cutting	Saliva on paper tissue (n=5)	1	1	3	0	2

Preliminary testing: Touch DNA

Preliminary testing for touch DNA collection did not involve any tape lifting. The method selected was based on Templeton and Linacre's (2014) double-swabbing technique for nonporous substrates. A 2 mm x 2 mm cutting of a nylon 4N6FLOQSwab™ was wetted with 1 µL of 0.1% Triton X. The swab was then used to swab the denim (or white cotton) ten times horizontally and eight times vertically. This was followed by a dry swab of the same size. Both swab cuttings were added to the PCR master mix and amplified. Full profiles were seen for both of the touch DNA samples using this method (data not shown).

Final sets: Touch DNA

For the touch DNA samples on fabrics, two different methods were used. The first technique involved taking a 2 mm x 2 mm cutting of the porous fabrics and adding that cutting directly to the PCR master mix (Linacre et al. 2010). Samples on the same fabrics were also tested with the double swab technique described above (Templeton and Linacre, 2014). Touch samples were collected from five different donors on white cotton, denim and polyester and processed within 24 hours of collection. For the touch DNA samples on fabrics, QS2 was detected for all samples for both methods which indicated that there was no inhibition. Touch samples on denim and white cotton produced the best results (three full profiles), followed by polyester. The double-swabbing technique worked better than the cutting method (which only provided one full profile overall). Compiled results can be found in Table 8 below.

Table 8. Final set for touch donors using the cutting method in comparison to the double-swabbing technique.

Collection/pre-treatment	Sample Type	# of full	# of good partial	# of bad partial	# of negative	QS2 detection
Cutting	Touch on white cotton (n=5)	0	1	1	3	5
	Touch on denim (n=5)	0	0	3	2	5
	Touch on polyester (n=5)	1	1	1	2	5
Wet/dry swab Copan nylon FLOQSwab™	Touch on white cotton (n=5)	3	2	0	0	5
	Touch on denim (n=5)	3	0	2	0	5
	Touch on polyester (n=5)	2	2	1	0	5

For the touch DNA samples on glass, five donors gave left and right hand thumb prints. Two different swabbing methods were tested, the double swabbing (nylon FLOQSwab™) also used for the fabric, and the Fitzco® CEP® swab also used for saliva on glass. The latter method involved used a single 2 mm x 2 mm cutting of the Fitzco® CEP® swab wetted with 2 µL of 0.1% Triton X. For the nylon FLOQSwab™, a total of ten samples were tested. Only two of the profiles were full, two were considered a good partial profile, two were considered a bad partial profile and four were negative. QS2 was not detected in four of the samples indicating inhibition in almost half of the samples. The Fitzco® CEP® swab showed no inhibition but typing was still not successful for all samples. For the CEP swab there were two full profiles, one good partial profile, and two negative/not suitable. Results can be found in Table 9 below.

Table 9. Final set of touch DNA on glass slide samples.

Collection/pre-treatment	Sample Type	# of full	# of good partial	# of bad partial	# of negative	QS2 detection
Wet/dry swab Copan nylon FLOQSwab™	Touch on glass (n=10)	2	2	2	4	6
Fitzco CEP Swab	Touch on glass (n=5)	2	1	0	2	5

Discussion

Overall, results were promising and show that the Qiagen® Investigator® 24plex GO! Kit can be used for biological evidence as well as reference samples. Blood is an important biological specimen in forensic DNA testing. Blood is known to contain more DNA in comparison to other specimen types such as saliva or touch DNA. Hemoglobin found in blood is known to cause inhibition of PCR amplification due to degradation of the DNA primers, the target DNA or inactivation of DNA polymerase (Zhang, Kermekchiev, & Barnes, 2010). Even without DNA purification, no inhibition was seen in any of the blood samples on fabric in our study. This study used a tape-lifting method with Sellotape®, a double-sided tape that was also used in another study (Verheij, Harteveld, & Sijen, 2012). By implementing this method, these authors were able to generate full profiles for all their blood samples. They tested 3 µL of blood and 3 µL of a 1:10 dilution of blood on fabrics such as wool, nylon, course cotton, linen, viscose, fleece, fine cotton and denim. All the fabrics, minus denim, resulted in successful and complete DNA typing results. The substrates were all stamped 1-5 times. Denim was stamped 20 times and resulted in unsuccessful typing that the authors attributed to PCR inhibition due to the

presence of indigo dye. Fortunately, for our project, no PCR inhibition was seen with the denim fabric and testing resulted in successful DNA typing with full profiles for all five donors.

Another study considered the Copan microFLOQ® swab for direct PCR of blood, saliva and touch samples and came up with different results (Ambers, Wiley, Novroski, & Budowle, 2018). They tested both 10% diluted blood stains and 5% diluted blood stains. For their 10% blood stains, only 7/30 samples gave full STR profiles. They attributed their poor results to not enough sample being collected or PCR inhibition. However, their 5% diluted blood stains gave much better results and full STR profiles were seen for 28/30 samples. With their 1% diluted blood stains they were able to obtain 29/30 full STR profiles but had lower peak heights in comparison to their 5% blood stains. In all, they concluded that the Copan microFLOQ® swab was a suitable collection method for 1:99 diluted blood stains. In our limited study of only two samples, we did not see any inhibition with the microFLOQ® swab, even though our blood was undiluted.

Saliva, in comparison to blood, is known to contain less DNA (Lee & Ladd, 2001). Therefore, it was not surprising that the success rate of the saliva samples was less than that of the blood samples. This also can be attributed to the fact that it was difficult to see where exactly the saliva stain was deposited. Recovery could not be monitored, like for blood, due to the stains being very small and faint. This made stain collection less reliable and could explain some of the bad profiles. Verheij et al. (2012) looked at 57 saliva samples from chewing gum, cups or bottles, cigarette butts and tissue spit or buccal swabs. Their success rate was a little over 50% for full profiles in comparison to 63% in the present study. Thirty percent of their samples resulted in partial profiles and in 18% of their samples, they were unable to generate a profile. There were no negative profiles and approximately 37% partial profiles observed in this study.

For touch DNA samples, the double-swab technique on fabric proved successful. This was an innovation developed for this thesis. By initially using a wet swab, the biological material is loosened and can be collected. Following with a dry swab helps to collect what may have been left behind by the wet swab. Swabbing the substrates ten times horizontally and eight times vertically, a larger surface area is covered and more DNA is recovered. For Linacre et al. (2010), taking cuttings of fabric resulted in a higher success rate than it did for our work. While they had success using cuttings, this study generated mostly negative profiles, and the use of the swabbing technique was an improvement.

No inhibition was seen for any of the porous substrates and QS2 was present in all amplifications. However, on the glass slides, 4/10 profiles showed inhibition after 4N6FLOQ collection. This may be attributed to the adhesive that holds the nylon FLOQSwab™ fibers together but it is unclear why there was no inhibition for the touched fabric swabs. Despite the lack of inhibition for the porous substrates, not all DNA profiles were successful. The range from full to bad partial may be attributed to donor-to-donor variation and is consistent with previous findings (see below).

For touch DNA on clothes, Verheij et al. (2012) had 7/16 (44%) negative profiles, 5/16 (31%) partial profiles and 4/16 (25%) full profiles. In the present study, for the double-swabbing technique on fabrics, there were no negative profiles, 7/15 partial profiles (47%) and 8/15 (53%) full profiles. Our nonporous results were better as well: nearly 78% (14/18) of their results resulted in a negative profile in comparison to our 33% (5/15). As stated above, touch DNA can be difficult to detect and there is a high amount of donor-to-donor variation. Some people are naturally good “shedders” and leave more DNA behind than others. Other factors that can affect

DNA transfer include, for example, pressure and friction during contact, the surface type being handled, frequency of hand washing and donor perspiration (Cavanaugh & Bathrick, 2018).

Overall, we obtained good results using direct PCR and the Qiagen® Investigator® 24plex GO! Kit. There was little inhibition and not many over-amplified samples. One disadvantage is the fact that without DNA extraction, it may not be possible to preserve part of the sample for defense testing. Using the tape lift approach for body fluids leaves enough sample in place and the stain location is preserved. This may be a good approach in the event that pattern analysis is needed. For touch DNA, the entire sample is consumed, which may be problematic if this is the only evidence and requires careful decision making.

Concluding remarks

This study could be continued by looking at additional fabric types in different colors to explore the presence of inhibitors. For difficult to see stains such as saliva, it is possible that labelling cellular material and marking the stain location could enhance the success rate. This may also be applicable for touch DNA. Further optimizing of swab types, swabbing solutions or tape lifting methods could also be of interest.

Overall, direct PCR using the Qiagen® GO! Kit has been shown to be feasible. Direct PCR lessens the amount of time for sample preparation and thus could help laboratories to increase their throughput. The new method of swabbing touched fabric was more successful than the previous technique of cutting.

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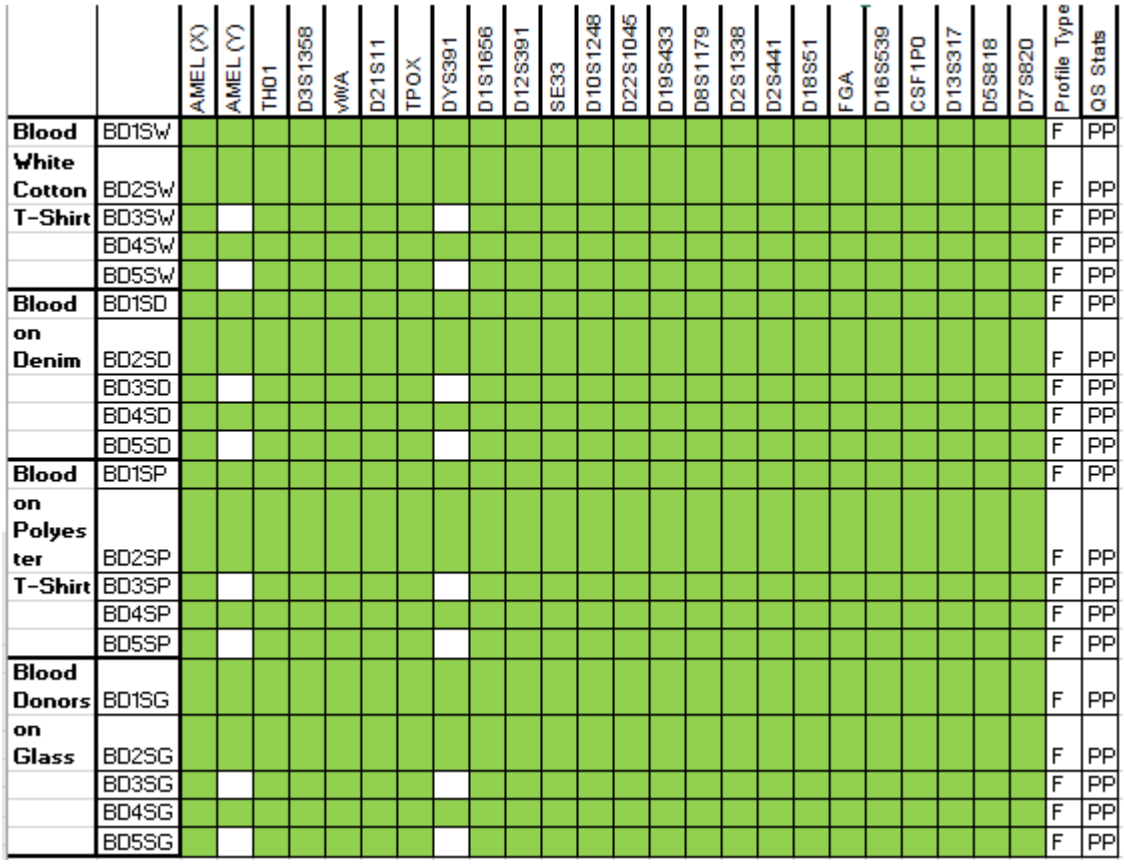
Appendix A

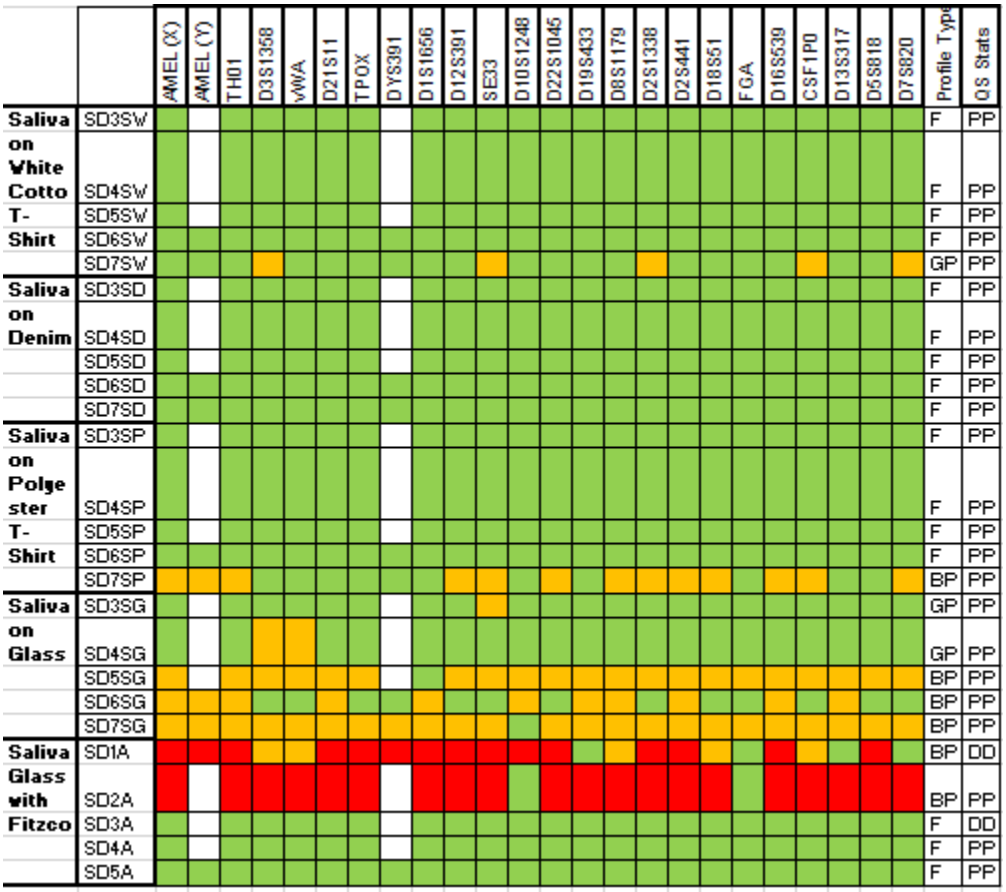
Blood Samples Heat Map



		AMEL (X)	AMEL (Y)	TH01	D3S1358	vWA	D21S11	TPOX	DYS391	D1S1656	D12S391	SE33	D10S1248	D22S1045	D19S433	D8S1179	D2S1338	D2S441	D18S51	FGA	D16S539	CSF1PO	D13S317	D5S818	D7S820	Profile Type	QS Stats
Blood	BD1SW																									F	PP
White Cotton	BD2SW																									F	PP
T-Shirt	BD3SW																									F	PP
	BD4SW																									F	PP
	BD5SW																									F	PP
Blood on Denim	BD1SD																									F	PP
	BD2SD																									F	PP
	BD3SD																									F	PP
	BD4SD																									F	PP
	BD5SD																									F	PP
Blood on Polyester T-Shirt	BD1SP																									F	PP
	BD2SP																									F	PP
	BD3SP																									F	PP
	BD4SP																									F	PP
	BD5SP																									F	PP
Blood Donors on Glass	BD1SG																									F	PP
	BD2SG																									F	PP
	BD3SG																									F	PP
	BD4SG																									F	PP
	BD5SG																									F	PP

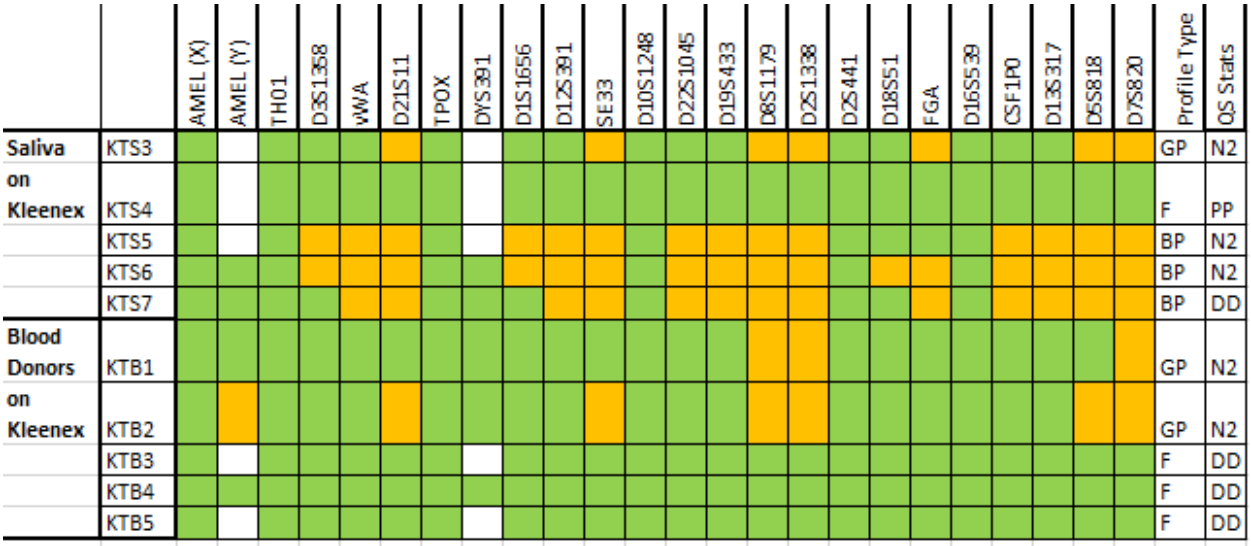
Appendix B

Saliva Samples Heat Map



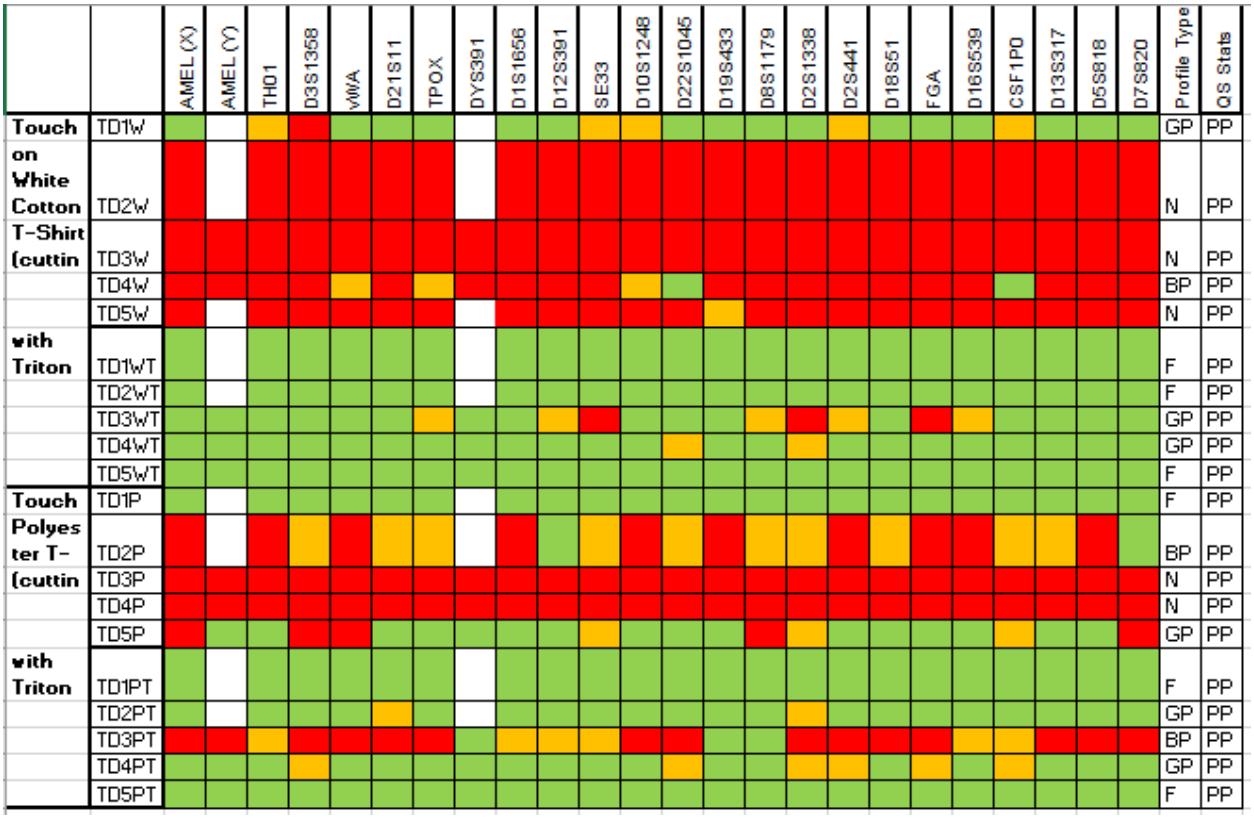
Appendix C

Kleenex Samples Heat Map



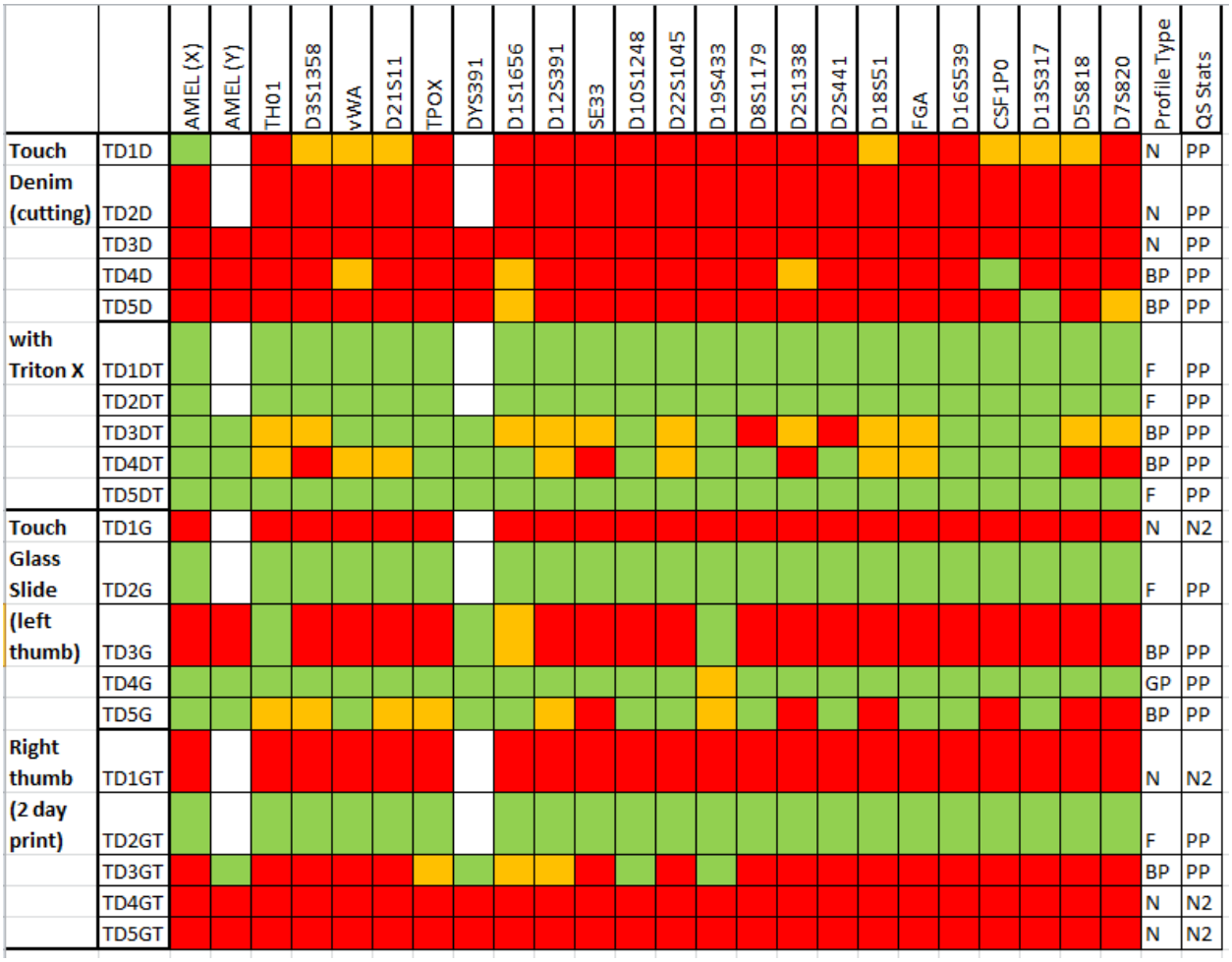
Appendix D

Touch Samples I Heat Map



Appendix E

Touch Samples II Heat Map



Appendix F

Touch Samples on Glass Heat Map

		AMEL (X)	AMEL (Y)	TH01	D3S1358	vWA	D21S11	TPOX	DYS391	D1S1656	D12S391	SE33	D10S1248	D22S1045	D19S433	D8S1179	D2S1338	D2S441	D18S51	FGA	D16S539	CSF1PO	D13S317	D5S818	D7S820	Profile Type	QS Stats
Touch	TD1A																									F	PP
Glass with Fitzco	TD2A																									N	PP
	TD3A																									N	PP
	TD4A																									GP	DD
	TD5A																									F	PP

Legend

Color Scheme		Full genotype
		Missing Allele
		Female Donor
		No allele called
Profile Type	F	Full Profile (all alleles called)
	GP	Good Partial (10 loci or more)
	BP	Bad Partial (9 loci or less)
	N	Negative/Not Suitable (No alleles called or incomplete loci with missing alleles)
QS Stats	PP	Both QS1/QS2 Present
	DD	QS2 Lower than QS1
	N2	QS2 Completely Gone (inhibition)