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Spot On Sciences, Inc. has recently developed a new device, the HemaSpot™, which allows for samples to be stored safely at ambient temperatures. The UNTHSC FGEN program was contacted to conduct a research study to determine its feasibility for use as collection and storage media with trace samples. Extractions of 108 samples were conducted with QIAGEN® QIAamp DNA Investigator Kits, a 3130xL Genetic Analyzer, and GeneMapper® *ID-X* software. A hypersensitivity study worked with sub-optimal amounts of control DNA in order to observe the quality and variation of the generated profiles. The trace study swabbed items found at typical crime scenes and determined the device's ability to generate readable profiles. Results uncovered that all samples either contained large portions of allelic dropout or contamination. Relatively similar partial profiles were produced for both cartridge types in the hypersensitivity study. In addition, readable trace profiles were compared to one another to conclude that the HemaSpot™-HD had the most success, however this may have been the cause of limited size and sample variation. Both products should be tested further.

ANALYSIS OF HEMASPOT™-HF AND HEMASPOT™-HD SAMPLING KITS USING TRACE DNA

THESIS

Presented to the Graduate Council of the

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By

Rachel McGehee, B.S.

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CHAPTER I

INTRODUCTION

The identification of criminals based on their individualizing features has long been sought after by forensic professionals. Many decades of trial and error have brought researchers closer than ever to the most reliable and convenient technologies. Ideal characteristics of such technology would include the ability to identify unique features of the perpetrators that they may not realize themselves. These features would not change over time and would be available for testing at the researcher's convenience. An early example of this ideal system was believed to be fingerprinting. In those instances where fingerprints are not available, DNA could be used to identify the perpetrator. The future of forensic identification would require the evidence to be recognizable to investigator and not the perpetrator. Trace DNA could be the solution to this dilemma.

Trace DNA, unlike other samples, is not visible to the naked eye. Also known as touch DNA, it is left behind in varying amounts as loose skin cells that come in contact with an object. Trace DNA is also left behind in much less quantity than that of blood, semen, or saliva. [1] Roughly 20 years ago, it was nearly impossible to detect or obtain any useful profile data from touch samples. However, using today's advanced STR (short tandem repeat) methods, analysts are now able to obtain DNA profiles from trace DNA unknown to crime scene investigators. [1] Trace DNA has seen a dramatic expansion in media coverage over the past few years as with famous cases like that of JonBenét Ramsey, in which the Ramsey family was exonerated after analysts found unidentified male touch DNA on her long johns. [1] Another stunning case came

from the Juliana and Alan Grna murder in which the touch DNA from Johnnie Cook was discovered on the inside of a toilet paper roll left behind by Cook after an attempt to clean up the crime scene.

Intuitively, with every contact of skin to an object's surface, a small fraction of sloughed cellular material will be left behind. Also known as Locard's Exchange Principle, this concept is the driving force for trace DNA analysis in forensics. As the largest human organ, skin also has the highest potential yield for DNA containing cells. The average person sheds roughly 400,000 epithelial cells per day, each one holding approximately 5 picograms of nuclear DNA. [2] To increase the success of touch DNA analysis, a storage medium which can protect such miniscule samples is crucial.

Spot On Sciences, Inc. in Austin TX has recently developed a groundbreaking new device, the HemaSpot™-HF sampling kit, which allows for biological samples to be stored at ambient temperatures for extended periods of time in a contamination-free, moisture-tight environment. [3] These qualities could make the HemaSpot™-HF a valuable asset to the field of forensics. Based on educational background with STRs and DNA profiling, the UNTHSC FGEN program was contacted to see if the HemaSpot™-HF sampling kit was feasible for use as a collection and storage media in forensic trace analysis. The information collected from this study serves as a helpful tool for crime labs and police departments. In addition, the study provides important information into how well the HemaSpot™-HF kit can be used as a collection device for forensic media.

The first restriction fragment length polymorphisms (RFLPs) used to exclude an innocent man were utilized by Jeffreys et al. in 1985 during the infamous Colin Pitchfork murder case. [3] RFLPs also uncovered critical information to the lead to the eventual arrest of the perpetrator.

The results from this finding started a ripple effect in the fields of forensic DNA and forensic serology. These "DNA Fingerprints" still held meaningful information to forensic serologists regarding suspect elimination. However, it was evident that far less questionable crime scene evidence could be evaluated for DNA research purposes. This also demonstrated the higher stability and reliability of DNA than that of the proteins used in serological testing. Another breakthrough quality of RFLPs was based around the low (250ng) sample size requirement. This was expanded in the 1990's, when as little as 30ng of high molecular weight DNA template could be used to create a usable profile. [3]

Over the past 20 years short tandem repeats (STRs) began to take the place of RFLPs in the field of forensic DNA. This genetic material of choice provides analysts with a plethora of individualizing loci, more than sufficient for individual discrimination. Even though the level of polymorphism per locus is less than that of RFLPs, the smaller STR fragments allow for a greater likelihood of successful DNA profiles. This new advancement is also paired with internal lane standards, and fluorescence to incorporate allele sizing and loci multiplexing. [3] The potential for high discrimination in generated profiles using smaller amounts of evidence sample has become a reality over the last few decades in the forensic field. With the use of polymerase chain reaction (PCR) STR profiling, analysts now only require 0.1-1.0ng of purified DNA per sample to yield a full genetic profile. [3] With new cutting-edge testing such as rapid DNA, analysts can drop a swab into a machine and get DNA results back within roughly 90 minutes. Extremely small and degraded pieces of evidence now hold the potential to put cold cases to rest after decades of inscrutability.

With new technology should come new collection devices that alleviate the pressure of storage size, and contamination threats. HemaSpot™-HF is a simplistic, durable, blood sampling

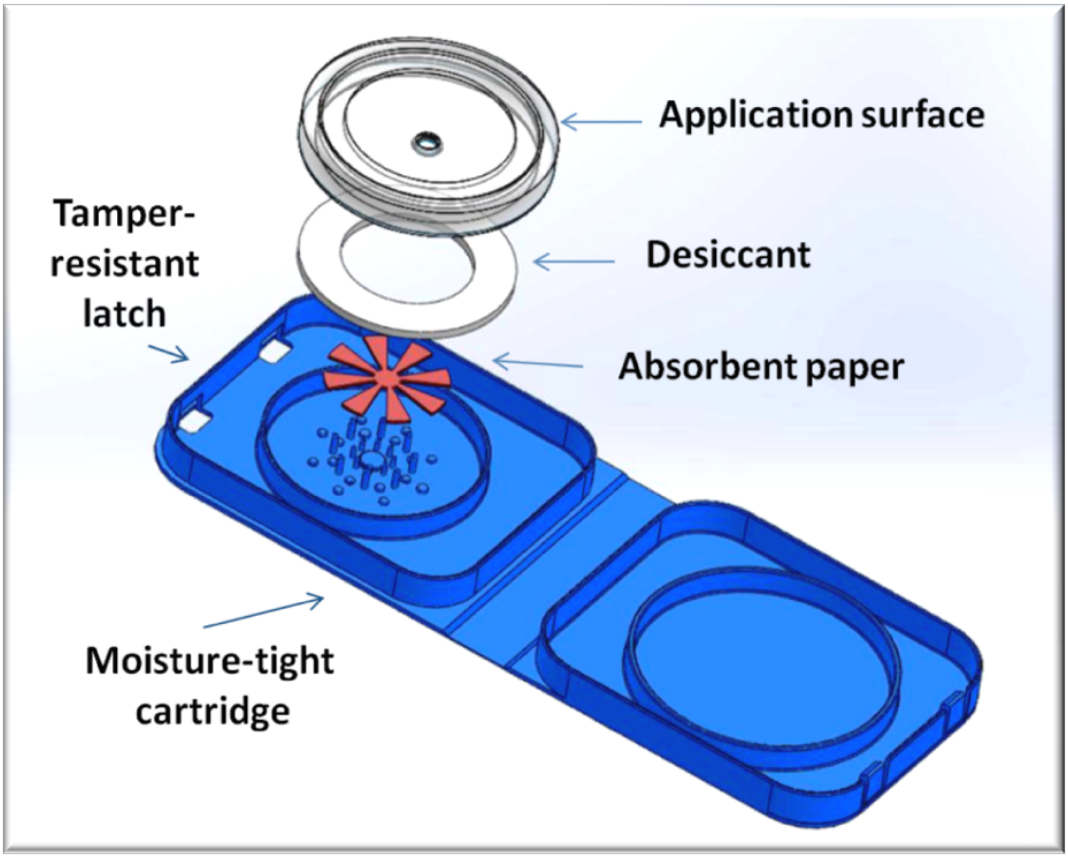
device developed and patented by Spot On Sciences, Inc. that does just that. The device works like a storage and shipping container, protecting the sample inside from contamination, degradation, and harmful environmental factors. The device's ability to store a clean sample at ambient temperatures has made it a recent hit in the scientific community; even gathering attention and funding from DARPA, the Defense Advanced Research Projects Agency in Arlington County, VA. [4] The HemaSpot™-HF sampling kit comes with many parts including a finger-stick to gather blood. When the sample is set, and ready for transport, it is safe to be transported to the designated research institution for analysis. [5] The HemaSpot™-HF has enabled critical medical studies to take place when they were not previously feasible, due to the sheer inconvenience for the test subjects alone.

Jeanette Hill, founder and C.E.O of Spot On Sciences, Inc. had her mother in mind when designing the concept for the HemaSpot™. She wanted to find a way for elderly and disabled patients to collect blood samples at their own convenience. Her patented design allows for blood samples to be safely stored until the patient is ready to ship, eliminating the hassle of routine, in person lab visits. [4]

The device itself (*Figure 1*) contains an absorbent paper and desiccant covered with a plastic top that allows for the application of three drops of sample. After the sample has been deposited into the center of the card, the desiccant quickly dries it. The cartridge can then be sealed shut and is ready for transport at the user's earliest convenience. The moisture-tight, tamper resistant collection shell protects the sample inside from becoming compromised. The HemaSpot™ mobile device application (currently in development) also allows for the user to capture the QR barcode information on the back of the device, stamping the date and time in

which the sample was taken. In total, the collection device is approximately the size of a credit card and stands 1 cm high. Its size makes it extremely convenient for shipping and storage. [5]

Figure 1: HemaSpot™-HF design [5] *The following image is a depiction of a deconstructed HemaSpot™-HF cartridge.*



The HemaSpot™-HF device comes with an individualizing barcode on the back for unique sample identification. The code is read by a patent pending application available for smartphones, laboratory scanners, and other basic readers (*Figure 2)*. The application will also alert the lab that the code containing sample has been shipped, along with basic information such as patient name, date, and time of collection. [5]

Figure 2. HemaScan™ application. *The following image depicts a mobile phone reading the HemaSpot™-HF QR code on the HemaScan™ application. [5]*

Once transported safely to the designated laboratory, an analyst may open the cartridge using a provided tool. One of the largest benefits of the HemaSpot™-HF device is its ability to be shipped and stored at ambient temperature. In addition, the HemaForm™ absorbent paper inside of the cartridge (Figure 3) allows for the sample to be tested and stored safely in a moisture-tight environment for short or long-term analysis. The patented eight blade paper design allows for improved sample quality and handling, as tested by the Spot On Sciences team. [4] So far, the HemaSpot™-HF device has mainly been used for blood samples and medical research purposes. This project tested the device's ability to store trace amounts of DNA from a forensic perspective. This includes items one might collect at a typical crime scene such as bullet casings, knife handles, and steering wheels.

Figure 3. HemaForm™. *The following image depicts the HemaForm™ breakthrough patented design. [5]*

Applying the HemaSpot™-HF device to the field of forensics has the potential to benefit many groups of individuals. From collection to analysis the moisture-tight, tamper-resistant environment allows for sample security in just about every forensic setting. Crime scene investigators could collect biological samples more efficiently due to the small size, and contamination-resistant container that surrounds the sample. The collection device also allows for the stored sample to be kept at ambient temperatures, eliminating the need to plastic wrap and cool the sample immediately, as in the case with swabs. A device of comparable qualities is the FTA® (Flinders Technology Associates) card. [6] Classic FTA® cards (Whatman®BioScience), are small coated sheets with four circles designed to store around 100µL of whole blood each. [6] These drops of blood are protected by the effective matrix, which preserves the sample until it is processed via the isolation of high molecular-weight genomic DNA. FTA cards were

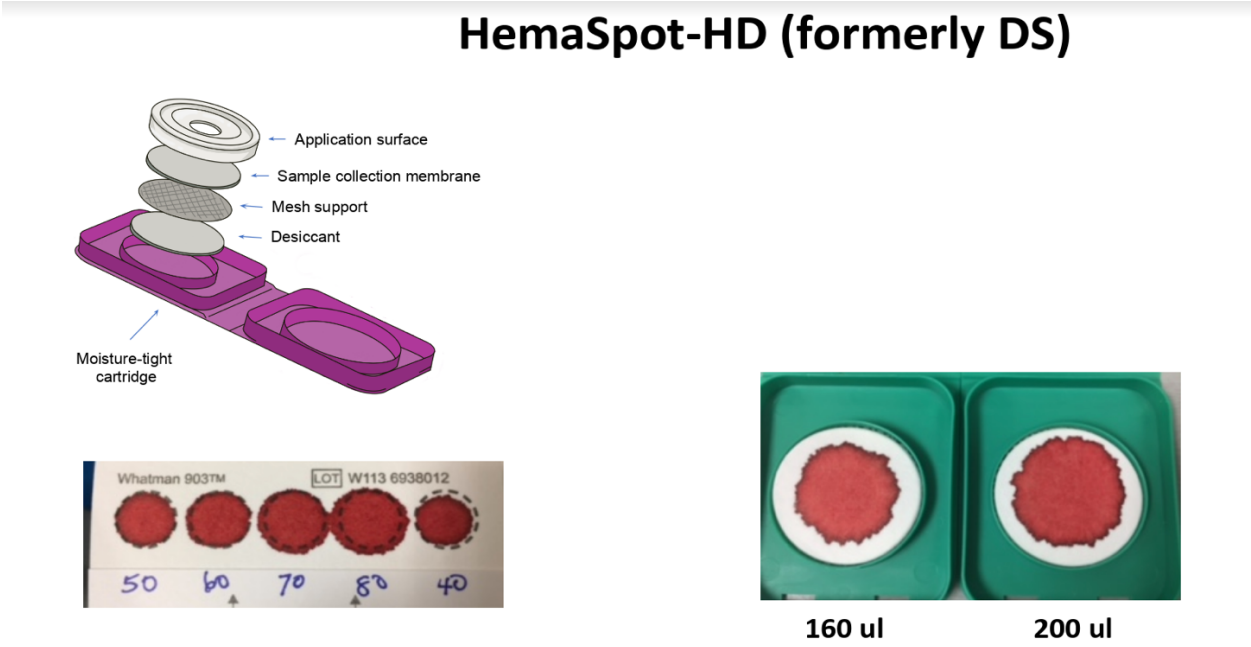
developed with DNA purification, storage, RNA isolation, and PCR in mind. For blood samples, several drops at room temperature dry fairly quickly, and are used in a wide variety of laboratory studies including paternity testing, human cancer studies, and neonatal screening. Recent labs have applied this device to the field of forensic science as well [6]. However, one drawback to the FTA cards is their need to be stored in plastic bags to avoid contamination. [6]

The HemaSpot™-HF device was developed from scratch, including the design of the filter paper inside of the collection cartridge. On normal collection paper, the sample disperses randomly, with very little control. This makes sampling one particular hole punch difficult to yield reproducible results on a molecular level. With the HemaSpot™-HF patented eight blade star design, the deposited sample disperses evenly in all directions, significantly increasing the chance of accurate readings no matter which blade is plucked. [5] Users will also find sample organization to be easier with the scannable barcode on the back of each collection device. The Spot On Sciences phone application (patent pending) captures the code information and records the donor's name, date, and time of collection. This also means less paperwork for the analyst and department. Storage and transport of the sample is also made simpler electronically, since the barcode helps keep an organized library of information for labs and sample storage facilities.

As a last-minute addition to the study, UNTHSC was able to acquire a brand-new product from Spot On Science's Lab known as the HemaSpot™-HD. This design change was found to be beneficial because of the similarities to its sister project, the HemaSpot™- HF. The devices were shipped in by Shelley Hossenlopp, Founder Poca International LLC, who was a help in gathering the supplies for the project. She stated: "The HemaSpot™-HD (Formerly DS) is a large circle of TFN paper inside of a similar cartridge and includes an internal desiccant. It will hold 200µL of blood or other tissue, but only 160µL is recommended due to drying of the sample." According

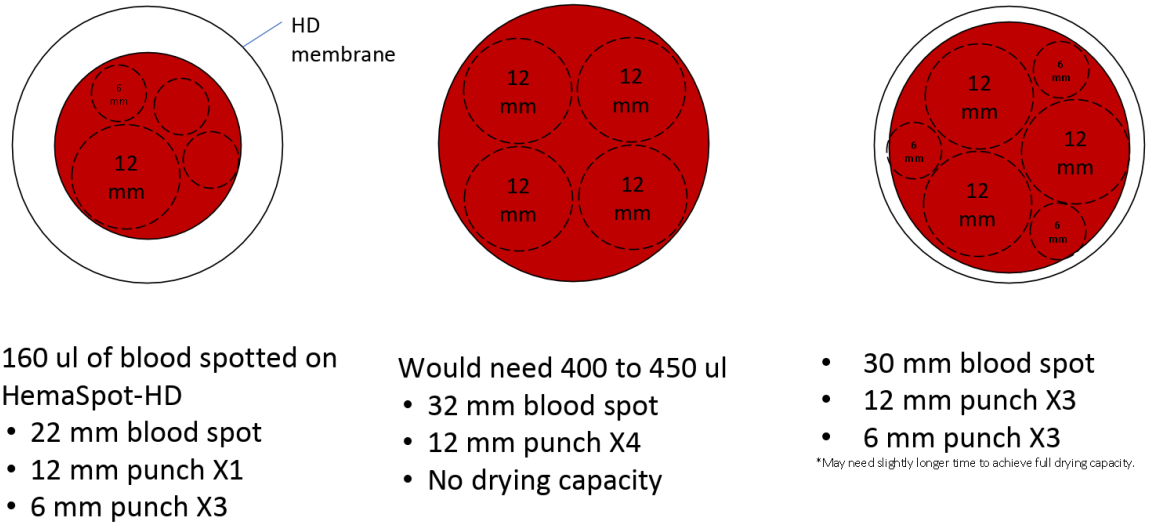
to her, the HemaSpot™-HD has had successful use with not only blood but nasopharyngeal wash as well as diarrhea fluids, and it may even work well with vaginal wash fluids for future studies. The purpose of including this product in the project was to compare the two devices and see if either worked well for forensic trace sampling and analysis.

Figure 4. HemaSpot™-HD design. *The following image depicts a deconstructed HemaSpot™-HD cartridge and its ability to store blood samples at varying volumes.* [5]



Much like the HF, the HemaSpot™-HD is a plastic cartridge containing an application surface, sample collection membrane, mesh support, and desiccant all inside of a moisture-tight cartridge. Unlike the HemaSpot™-HF however, the HD can hold up to 200µL of sample, a much larger volume. This would benefit samples with a larger collection volume. However, the HD does not contain a patented star design collection membrane, making it more difficult to apply and sample in a precise and accurate fashion.

Figure 5 HemaSpot™-HD Sampling. *The following image depicts the collection membrane of a HemaSpot™-HD cartridge and its ability to be sampled in various sized punches.*



160 ul of blood spotted on HemaSpot-HD
- 22 mm blood spot
- 12 mm punch X1
- 6 mm punch X3

Would need 400 to 450 ul
- 32 mm blood spot
- 12 mm punch X4
- No drying capacity

- 30 mm blood spot
- 12 mm punch X3
- 6 mm punch X3

*May need slightly longer time to achieve full drying capacity.

The HemaSpot™-HD does however allow for different sized sample punches to be made, which can be useful when working with smaller quantities of DNA in cases like that of trace analysis.

CHAPTER II

MATERIALS AND METHODS

Evaluation of the HemaSpot™-HF and HD sampling kits was completed in the UNTHSC student lab 370 and computer room 311. The hypersensitivity study took place in two segments. First, all HemaSpot™-HD cartridges were prepped, extracted, and analyzed. This was followed by the same set of steps for the HemaSpot™-HF. To start, three HD cartridges (A, B, and C) were allotted to each of the following ng/μL concentrations. For better statistical results, each cartridge was run in triplicate for a total of 9 samples per DNA concentration.

Part 1: Hypersensitivity Study

HD Sensitivity

Table 1 HemaSpot™-HD samples. *The following table represents the labeling system for the hypersensitivity study HemaSpot™-HD samples. Three cartridges (labeled A, B, and C) were distributed to each of the 6 DNA concentrations. These samples were run in triplicate for better results.*

DNA Concentration (ng/μL)	Tube 1: 0.5	Tube 2: 0.25	Tube 3: 0.125	Tube 4: 0.0625	Tube 5: 0.0313	Tube 6: 0.0156
Sample Count (1 cartridge = 3 samples)	T1HD1/3A T1HD2/3A T1HD3/3A T1HD1/3B T1HD2/3B T1HD3/3B T1HD1/3C T1HD2/3C T1HD3/3C	T2HD1/3A T2HD2/3A T2HD3/3A T2HD1/3B T2HD2/3B T2HD3/3B T2HD1/3C T2HD2/3C T2HD3/3C	T3HD1/3A T3HD2/3A T3HD3/3A T3HD1/3B T3HD2/3B T3HD3/3B T3HD1/3C T3HD2/3C T3HD3/3C	T4HD1/3A T4HD2/3A T4HD3/3A T4HD1/3B T4HD2/3B T4HD3/3B T4HD1/3C T4HD2/3C T4HD3/3C	T5HD1/3A T5HD2/3A T5HD3/3A T5HD1/3B T5HD2/3B T5HD3/3B T5HD1/3C T5HD2/3C T5HD3/3C	T6HD1/3A T6HD2/3A T6HD3/3A T6HD1/3B T6HD2/3B T6HD3/3B T6HD1/3C T6HD2/3C T6HD3/3C

T=tube, HD=HemaSpot™-HD

HF Sensitivity

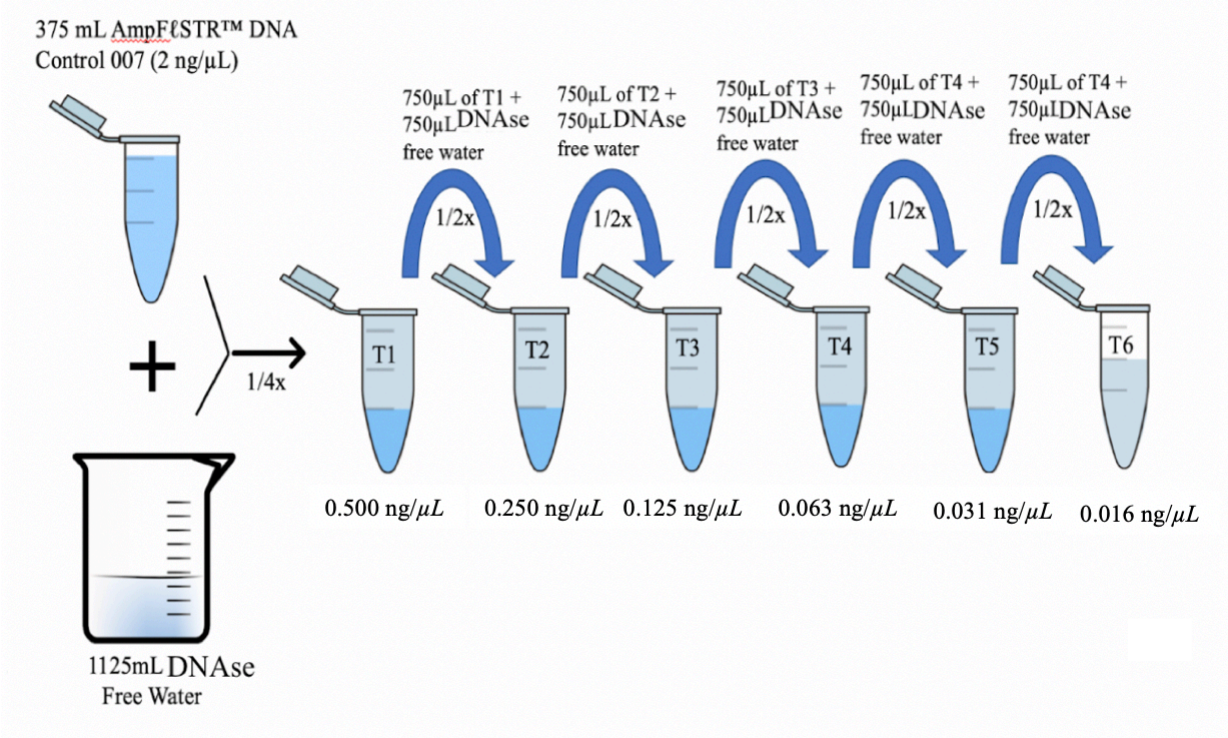
Table 2 HemaSpot™-HF samples. *The following table represents the labeling system for the hypersensitivity study HemaSpot™-HF samples. Three cartridges (labeled A, B, and C) were distributed to each of the 6 DNA concentrations. These samples were run in triplicate for better results.*

DNA Concentration (ng/μL)	Tube 1: 0.5	Tube 2: 0.25	Tube 3: 0.125	Tube 4: 0.0625	Tube 5: 0.0313	Tube 6: 0.0156
Sample Count (1 cartridge = 3 samples)	T1HF1/3A T1HF2/3A T1HF3/3A T1HF1/3B T1HF2/3B T1HF3/3B T1HF1/3C T1HF2/3C T1HF3/3C	T2HF1/3A T2HF2/3A T2HF3/3A T2HF1/3B T2HF2/3B T2HF3/3B T2HF1/3C T2HF2/3C T2HF3/3C	T3HF1/3A T3HF2/3A T3HF3/3A T3HF1/3B T3HF2/3B T3HF3/3B T3HF1/3C T3HF2/3C T3HF3/3C	T4HF1/3A T4HF2/3A T4HF3/3A T4HF1/3B T4HF2/3B T4HF3/3B T4HF1/3C T4HF2/3C T4HF3/3C	T5HF1/3A T5HF2/3A T5HF3/3A T5HF1/3B T5HF2/3B T5HF3/3B T5HF1/3C T5HF2/3C T5HF3/3C	T6HF1/3A T6HF2/3A T6HF3/3A T6HF1/3B T6HF2/3B T6HF3/3B T6HF1/3C T6HF2/3C T6HF3/3C

T=tube, HF=HemaSpot™-HF

Prepping the sensitivity study dilutions was accomplished by cross-linking six 2mL tubes and labeling them appropriately. Next, a serial dilution was conducted as follows:

Figure 6 Hypersensitivity Study Serial Dilution Process. *The following image depicts the process of the serial dilution performed for the hypersensitivity study. AmpFℓSTR™ DNA Control 007. (2 ng/µL) (Image 1, Appendix) was distributed and diluted from tube 1 to tube 6 using DNAse free water. The diluted control DNA was then deposited directly onto the surface of the collection membrane for each cartridge.*



Then, 100µL of sample was administered to each cartridge and given 24 hours to dry. The cartridges were then deconstructed with tweezers, and two 9x3mm cuttings from each cartridge were deposited into a 2.0mL test tube. A reagent blank was also prepared for each portion of the project to monitor contamination levels. Once all 55 samples were loaded, QIAGEN® QIAamp extractions were performed using a QIAGEN® DNA Investigator Kit.

QIAGEN® QIAamp DNA Investigator Kit extraction process:

Protocol involving the isolation of total DNA from sampling devices is mainly used for sperm, blood, and saliva samples. Therefore, a few steps were altered to accommodate the mediums for this study. This involved changing the ATL and AL buffer quantity to 500µL. The ATE buffer quantity was also changed to 60µL. This was repeated with the HemaSpot™-HF run, as neither study involved a cotton or Dacron swab. All other components were kept identical to that of the printed instructions. In total, the samples went through the following process:

-Added 20µL proteinase K (QIAGEN, Hilden, Germany) and 500µL ATL buffer (QIAGEN, Hilden, Germany), pulse vortex, spun down

-Incubated for 1 hour at 56°C, vortexed every 10 minutes, spun down

-Added 500µL AL buffer (QIAGEN, Hilden, Germany), pulse vortexed and spun down

-Incubated again for 10 minutes at 70°C, vortexed every 3 minutes, spun down

-Added 250µL of 100% ethanol (UNTHSC, Fort Worth, Texas), pulse vortexed and centrifuged

-Transferred 700µL of lysate to a designated MinElute column/2mL collection tube

-Centrifuged for 1 minute at 6,000 x g, discarded the flow through, replaced the 2mL collection tube

-Added 500µL of AW1 buffer (QIAGEN, Hilden, Germany), centrifuged for 1 minute at 6,000 x g, discarded the flow through, replaced the 2mL collection tube

-Added 700µL of AW2 buffer (QIAGEN, Hilden, Germany), centrifuged for 1 minute at 6,000 x g, discarded the flow through, replaced the 2mL collection tube

-Added 700µL of 100% ethanol (UNTHSC, Fort Worth, Texas), centrifuged for 1 minute at 6,000 x g, discarded the flow through, replaced the 2mL collection tube

-Centrifuged at full speed (16,500 x g) for 3 minutes to dry the membrane completely

-Replaced the QIAamp MinElute column with a 1.5mL tube and discarded the collection tube

-Incubated at room temperature with the lid open for 3 minutes

-Added 60µL of ATE buffer (QIAGEN, Hilden, Germany) to the membrane center

-Closed the lid and centrifuged at full speed for 1 minute (16,500 x g)

-Stored the 1.5mL tube in 2°C until quantification step

The next day, samples were quantified using an Applied Biosystem's Quantifiler™ Trio DNA Quantification Kit and a 7500 Real Time PCR Instrument (Life Technologies, Carlsbad, CA).

DNA Quantification using Applied Biosystem's Quantifiler™ Trio kit:

A total of 10 quantification standards were run alongside the 55 extracted HemaSpot™-HD and HemaSpot™-HF samples. These were made in duplicate from a series of dilutions:

Table 3 Standard Dilution. *The following table represents the series of dilutions prepared for the Applied Biosystem's Quantifiler™ Trio samples.* [8]

Standard	Concentration (ng/µL)	Example volumes	Dilution factor
Std. 1	50.000	10 µL [100 ng/µL stock] + 10 µL Quantifiler™ THP DNA dilution buffer	25
Std. 2	5.000	10 µL [Std. 1] + 90 µL Quantifiler™ THP DNA dilution buffer	105
Std. 3	0.500	10 µL [Std. 2] + 90 µL Quantifiler™ THP DNA dilution buffer	105
Std. 4	0.050	10 µL [Std. 3] + 90 µL Quantifiler™ THP DNA dilution buffer	105
Std. 5	0.005	10 µL [Std. 4] + 90 µL Quantifiler™ THP DNA dilution buffer	105

DNA standards were added to the quantification step because they are crucial for the accuracy of the run data. Preparation for the Applied Biosystem's Quantifiler™ Trio Master Mix went as follows:

For the Quantifiler™ Trio DNA Quantification Kit:

Table 4 Applied Biosystem's Quantifiler™ Trio Master Mix Preparation. *The following image represents the component makeup of the Applied Biosystem's Quantifiler™ Trio master mix.* [8]

Component	Volume per reaction (µL)
Quantifiler™ Trio Primer Mix	8
Quantifiler™ THP PCR Reaction Mix	10

10 standards + 55 HD or HF samples + Positive control + Negative control = 67 total wells

Primer Mix: (N) x (8µL) x (1.1µL) = (67) x (8µL) x (1.1µL) = 589.6µL

PCR Reaction Mix: (N) x (10µL) x (1.1µL) = (67) x (10µL) x (1.1µL) = 737µL

N = the total amount of wells

1.1µL was added to account for any loss via reagent transfer.

Together these two components made up the Quantifiler™ Trio Master Mix, which was pipetted in 18µL volumes into each of the 67 wells on a 96 well Quantifiler™ plate as shown below:

HemaSpot™-HD Plate:

Figure 7 Applied Biosystem's Quantifiler™ Trio HemaSpot™-HD Plate Layout. *The following figure represents the Applied Biosystem's Quantifiler™ Trio Plate for HemaSpot™-HD hypersensitivity samples.*

Quantifiler™ Trio DNA Quantification Plate

	1	2	3	4	5	6	7	8	9	10	11	12	
A	STD1 50 ng/µL	STD1 50 ng/µL	T1HD 1/3A	T1HD 2/3A	T1HD 3/3A	T1HD 1/3B	T1HD 2/3B	T1HD 3/3B	T1HD 1/3C	T1HD 2/3C	T1HD 3/3C	QIAamp RB	A
B	STD2 5 ng/µL	STD2 5 ng/µL	T2HD 1/3A	T2HD 2/3A	T2HD 3/3A	T2HD 1/3B	T2HD 2/3B	T2HD 3/3B	T2HD 1/3C	T2HD 2/3C	T2HD 3/3C		B
C	STD3 0.5 ng/µL	STD3 0.5 ng/µL	T3HD 1/3A	T3HD 2/3A	T3HD 3/3A	T3HD 1/3B	T3HD 2/3B	T3HD 3/3B	T3HD 1/3C	T3HD 2/3C	T3HD 3/3C		C
D	ST4 .05 ng/µL	STD4 .05 ng/µL	T4HD 1/3A	T4HD 2/3A	T4HD 3/3A	T4HD 1/3B	T4HD 2/3B	T4HD 3/3B	T4HD 1/3C	T4HD 2/3C	T4HD 3/3C		D
E	STD5 .005 ng/µL	STD5 .005 ng/µL	T5HD 1/3A	T5HD 2/3A	T5HD 3/3A	T5HD 1/3B	T5HD 2/3B	T5HD 3/3B	T5HD 1/3C	T5HD 2/3C	T5HD 3/3C		E
F	POS	NEG	T6HD 1/3A	T6HD 2/3A	T6HD 3/3A	T6HD 1/3B	T6HD 2/3B	T6HD 3/3B	T6HD 1/3C	T6HD 2/3C	T6HD 3/3C		F
G													G
H													H
	1	2	3	4	5	6	7	8	9	10	11	12	

HD = HemaSpot™-HD, HF = HemaSpot™-HF

HemaSpot™-HF Plate:

Figure 8 Applied Biosystem's Quantifiler™ Trio HemaSpot™-HF Plate Layout. *The following figure represents the Applied Biosystem's Quantifiler™ Trio Plate for HemaSpot™-HF hypersensitivity samples.*

Quantifiler™ Trio DNA Quantification Plate

	1	2	3	4	5	6	7	8	9	10	11	12	
A	STD1 50 ng/µL	STD1 50 ng/µL	T1HF 1/3A	T1HF 2/3A	T1HF 3/3A	T1HF 1/3B	T1HF 2/3B	T1HF 3/3B	T1HF 1/3C	T1HF 2/3C	T1HF 3/3C	QIAamp RB	A
B	STD2 5 ng/µL	STD2 5 ng/µL	T2HF 1/3A	T2HF 2/3A	T2HF 3/3A	T2HF 1/3B	T2HF 2/3B	T2HF 3/3B	T2HF 1/3C	T2HF 2/3C	T2HF 3/3C		B
C	STD3 0.5 ng/µL	STD3 0.5 ng/µL	T3HF 1/3A	T3HF 2/3A	T3HF 3/3A	T3HF 1/3B	T3HF 2/3B	T3HF 3/3B	T3HF 1/3C	T3HF 2/3C	T3HF 3/3C		C
D	ST4 .05 ng/µL	STD4 .05 ng/µL	T4HF 1/3A	T4HF 2/3A	T4HF 3/3A	T4HF 1/3B	T4HF 2/3B	T4HF 3/3B	T4HF 1/3C	T4HF 2/3C	T4HF 3/3C		D
E	STD5 .005 ng/µL	STD5 .005 ng/µL	T5HF 1/3A	T5HF 2/3A	T5HF 3/3A	T5HF 1/3B	T5HF 2/3B	T5HF 3/3B	T5HF 1/3C	T5HF 2/3C	T5HF 3/3C		E
F	POS	NEG	T6HF 1/3A	T6HF 2/3A	T6HF 3/3A	T6HF 1/3B	T6HF 2/3B	T6HF 3/3B	T6HF 1/3C	T6HF 2/3C	T6HF 3/3C		F
G													G
H													H
	1	2	3	4	5	6	7	8	9	10	11	12	

HD = HemaSpot™-HD, HF = HemaSpot™-HF

This was followed by the addition of 2µL of sample, Reagent Blank, Positive control, Negative control, and Standards into each of their corresponding wells. Once complete, the 7500 Real Time PCR Instrument (Life Technologies, Carlsbad, CA) was run under the following settings:

-Step 1: 95°C for 2 minutes

-Step 2: 95°C for 9 seconds

-Step 3: 60°C for 30 seconds

Number of cycles: 40

Polymerase Chain Reaction (PCR) set up:

Quantification results showed that the samples contained minute quantities of DNA, below the 0.067ng/µL standard concentration for normalization. Therefore, normalization of the HD/HF samples was not performed, and PCR was initiated. This was achieved with the use of an Applied Biosystem's GlobalFiler® PCR Kit and a GeneAmp™ PCR System 9700 Thermocycler. The following table and equation were used to calculate the total master mix used for PCR:

Table 5 GlobalFiler® PCR Master Mix. *The following table represents the component makeup for the GlobalFiler® PCR master mix. [8]*

Reaction component	Volume per reaction
Master Mix	7.5 µL
Primer Set	2.5 µL

55 samples + Positive control + Negative control = 57 total MicroAmp™ tubes

GF Master Mix Equation: (N) x (7.5/2µL) x (1.1µL) = (57) x (7.5/2µL) x (1.1µL) = 235.13µL

N = the total amount of wells

1.1µL was added to account for any loss via reagent transfer

Each MicroAmp™ tube received 5µL of Master Mix and 7.5µL of sample, Reagent Blank, Positive control + DNAse free water, or Negative control as appropriate. Next, the following samples were placed on a GeneAmp™ PCR System 9700 Thermocycler and set to the following 29-cycle parameters:

Table 6 PCR Run Cycle Parameters. *The following image demonstrates the PCR run cycles performed in this study. [8]*

Initial incubation step	Cycle (29 or 30 cycles)		Final extension	Final hold
	Denature	Anneal/Extend		
HOLD	CYCLE		HOLD	HOLD
95°C, 1 minute	94°C, 10 seconds	59°C, 90 seconds	60°C, 10 minutes	4°C, Up to 24 hours[1]

[1] The infinity (∞) setting allows an unlimited hold time.

Once the PCR run was complete, the samples were stored at 2°C until Capillary Electrophoresis was performed.

Capillary Electrophoresis Protocol:

Capillary Electrophoresis was initiated within the following week, and used GeneScan® 600 LIZ dye, Hi-Di Formamide (Thermo Fisher Scientific, Waltham, MA), and an Applied Biosystems 3130xL® Genetic Analyzer.

Plate setups for the HemaSpot™-HD, HemaSpot™-HF, and part 2 study samples ran as shown below:

GeneScan® 600 LIZ size standard v2.0: (N) x (0.4µL) x (1.1µL) = (61) x (0.4µL) x (1.1µL) = 26.84µL total LIZ volume into Master Mix

Hi-Di Formamide (Thermo Fisher Scientific, Waltham, MA): (N) x (9.6µL) x (1.1µL) = (61) x (9.6µL) x (1.1µL) = 644.16µL total Hi-Di volume into Master Mix

N = 55 samples + Positive control + Negative control + 4 ladders = 61 wells

1.1µL was added to account for any loss via reagent transfer.

10µL of the combined master mix (LIZ + Hi-Di) went into each well, plus 1µL of PCR product or 1µL of allelic ladder.

Total in each well after the addition of Master Mix + PCR sample = 11µL

Plate set up for the samples ran as follows:

HemaSpot™-HD Plate:

Figure 9 HemaSpot™-HD Plate Layout. *The following plate layout was used for the HemaSpot™-HD hypersensitivity samples.*

Capillary Electrophoresis Plate HD

	1	2	3	4	5	6	7	8	9	10	11	12	
A	Ladder 1	T1HD 2/3C	T2HD 3/3B	T3HD 1/3B	T4HD 3/3A	T5HD 1/3A	T5HD 3/3C	T6HD 1/3C					A
B	T1HD 1/3A	T1HD 3/3C	Ladder 2	T3HD 2/3B	T4HD 1/3B	T5HD 2/3A	T6HD 1/3A	T6HD 2/3C					B
C	T1HD 2/3A	QIAamp RB	T2HD 1/3C	T3HD 3/3B	Ladder 3	T5HD 3/3A	T6HD 2/3A	T6HD 3/3C					C
D	T1HD 3/3A	T2HD 1/3A	T2HD 2/3C	T3HD 1/3C	T4HD 2/3B	T5HD 1/3B	Ladder 4	Positive					D
E	T1HD 1/3B	T2HD 2/3A	T2HD 3/3C	T3HD 2/3C	T4HD 3/3B	T5HD 2/3B	T6HD 3/3A	Negative					E
F	T1HD 2/3B	T2HD 3/3A	T3HD 1/3A	T3HD 3/3C	T4HD 1/3C	T5HD 3/3B	T6HD 1/3B						F
G	T1HD 3/3B	T2HD 1/3B	T3HD 2/3A	T4HD 1/3A	T4HD 2/3C	T5HD 1/3C	T6HD 2/3B						G
H	T1HD 1/3C	T2HD 2/3B	T3HD 3/3A	T4HD 2/3A	T4HD 3/3C	T5HD 2/3C	T6HD 3/3B						H
	1	2	3	4	5	6	7	8	9	10	11	12	

HemaSpot™-HF and Part 2 Study Plate:

Figure 10 HemaSpot™-HF Plate Layout. *The following plate layout was used for the HemaSpot™-HF hypersensitivity samples.*

Capillary Electrophoresis Plate HF and Part 2 Study

	1	2	3	4	5	6	7	8	9	10	11	12	
A	Ladder 1	T1HF 2/3C	T2HF 3/3B	T3HF 1/3B	T4HF 3/3A	T5HF 1/3A	T5HF 3/3C	T6HF 1/3C	S1/3W	HD2/3W	HF3/3K	HF1/3B	A
B	T1HF 1/3A	T1HF 3/3C	Ladder 2	T3HF 2/3B	T4HF 1/3B	T5HF 2/3A	T6HF 1/3A	T6HF 2/3C	S2/3W	HD3/3W	HD1/3K	HF2/3B	B
C	T1HF 2/3A	QIAamp RB	T2HF 1/3C	T3HF 3/3B	Ladder 3	T5HF 3/3A	T6HF 2/3A	T6HF 3/3C	S3/3W	Part 2 RB	HD2/3K	HF3/3B	C
D	T1HF 3/3A	T2HF 1/3A	T2HF 2/3C	T3HF 1/3C	T4HF 2/3B	T5HF 1/3B	Ladder 4	Positive	HF1/3W	S1/3K	HD3/3K	HD1/3B	D
E	T1HF 1/3B	T2HF 2/3A	T2HF 3/3C	T3HF 2/3C	T4HF 3/3B	T5HF 2/3B	T6HF 3/3A	Negative	Ladder 5	S2/3K	S1/3B	HD2/3B	E
F	T1HF 2/3B	T2HF 3/3A	T3HF 1/3A	T3HF 3/3C	T4HF 1/3C	T5HF 3/3B	T6HF 1/3B		HF2/3W	S3/3K	Ladder 6	HD3/3B	F
G	T1HF 3/3B	T2HF 1/3B	T3HF 2/3A	T4HF 1/3A	T4HF 2/3C	T5HF 1/3C	T6HF 2/3B		HF3/3W	HF1/3K	S2/3B	Positive	G
H	T1HF 1/3C	T2HF 2/3B	T3HF 3/3A	T4HF 2/3A	T4HF 3/3C	T5HF 2/3C	T6HF 3/3B		HD1/3W	HF2/3K	S3/3B	Negative	H
	1	2	3	4	5	6	7	8	9	10	11	12	

Note: HF and Part 2 samples were run on the same plate to save resources

All profiles were then downloaded onto a disc drive and imported into the GeneMapper® *ID-X* Software in the UNTHSC GSBS student computer lab room 311.

Part 2: Testing HemaSpot™-HF and HemaSpot™-HD as Mediums for Trace DNA

The samples for the second study were collected in accordance with UNTHSC policies and were exempt from an IRB since no human subjects were tested. The samples were collected by the analyst and recorded appropriately. The three swabbed items included a pocket knife and steering wheel donated by Subject 1, and bullets donated by Subject 2. The substrates were chosen with the intent of covering a wide variety of objects left behind at a genuine crime scene. In order to compare the HD and HF results to that of a common crime lab cotton swab, three

Puritan® Cotton Swabs, HemaSpot™-HD, HemaSpot™-HF cartridges were used to each object, and labeled as follows:

Table 7 Trace DNA study sample labeling system. *The following table was used to label all samples in the Trace DNA study. Each medium was run in triplicate for better statistical results.*

Object Swabbed:	Wheel	Knife	Bullet
Sampling Medium:	Cotton Swab 1/3 Cotton Swab 2/3 Cotton Swab 3/3	Cotton Swab 1/3 Cotton Swab 2/3 Cotton Swab 3/3	Cotton Swab 1/3 Cotton Swab 2/3 Cotton Swab 3/3
Sampling Medium:	HF 1/3 HF 2/3 HF 3/3	HF 1/3 HF 2/3 HF 3/3	HF 1/3 HF 2/3 HF 3/3
Sampling Medium:	HD 1/3 HD 2/3 HD 3/3	HD 1/3 HD 2/3 HD 3/3	HD 1/3 HD 2/3 HD 3/3

HD = HemaSpot™-HD, HF = HemaSpot™-HF

For better statistical results, each sample was also run in triplicate. To collect the DNA, each of the 9 cartridges were moistened with DNAse free water and deconstructed to allow for the removal of the sampling paper inside. The paper was rubbed against the object in a clockwise fashion, collecting as much DNA from the surface as possible before being stored back in its respective cartridge. This was repeated for every cartridge, and the samples were stored at ambient temperature to dry overnight. Once dry, all cartridges were deconstructed once more and dissected, allotting two 9x3mm cuttings per tube. Extractions then proceeded identically to that of the hypersensitivity study, using the QIAGEN® QIAamp DNA Investigator Kit protocol of total DNA isolation. The layout for the Quantifiler™ Trio Master Mix and Quantifiler™ Trio Quantification Plate ran as follows:

Figure 11 Trace DNA study sample plate layout. *The following plate layout was used for the HemaSpot™-HF, HemaSpot™-HD, and cotton swab trace DNA samples.*

Quantifiler™ Trio DNA Quantification Plate

	1	2	3	4	5	6	7	8	9	10	11	12	
A	STD1 50 ng/µL	STD1 50 ng/µL	S1/3W	S2/3W	S3/3W	HF1/3W	HF2/3W	HF3/3W	HD1/3W	HD2/3W	HD3/3W	Part 2 RB	A
B	STD2 5 ng/µL	STD2 5 ng/µL	S1/3K	S2/3K	S3/3K	HF1/3K	HF2/3K	HF3/3K	HD1/3K	HD2/3K	HD3/3K		B
C	STD3 0.5 ng/µL	STD3 0.5 ng/µL	S1/3B	S2/3B	S3/3B	HF1/3B	HF2/3B	HF3/3B	HD1/3B	HD2/3B	HD3/3B		C
D	ST4 .05 ng/µL	STD4 .05 ng/µL											D
E	STD5 .005 ng/µL	STD5 .005 ng/µL											E
F	POS	NEG											F
G													G
H													H
	1	2	3	4	5	6	7	8	9	10	11	12	

S = Swab, W = Wheel, K = Knife, B = Bullet, HD = HemaSpot™-HD, HF = HemaSpot™-HF

After the quantification process, PCR amplification and capillary electrophoresis were run identically to that of the hypersensitivity study. To save space and resources, the trace DNA study samples were run on the same plate as the HemaSpot™-HF samples (Image 4). Once all samples were quantified, amplified, and electrophoresed, the profiles were downloaded onto a disc drive, and analyzed on GeneMapper® *ID-X* Software in the UNTHSC GSBS student computer lab room 311.

CHAPTER III

RESULTS

Part 1: Hypersensitivity Study

Once all samples were quantified, amplified, and electrophoresed, the profiles were downloaded onto a disc drive, and analyzed on GeneMapper® *ID-X* Software in the UNTHSC GSBS student computer lab room 311. In total, the hypersensitivity study amassed 122 GeneMapper® files. This broke down into 108 HemaSpot™-HD and HemaSpot™-HF samples, 2 Reagent Blanks, 2 Positive controls, 2 Negative controls, and 8 capillary electrophoresis ladders. In order for the data to be acceptable for evaluation by an analyst, all ladders, reagent blanks, and controls must meet the predetermined standards for quality. After confirming that there were no outliers, and all samples met the requirements, each profile was analyzed to determine the overall usefulness of the data. Each profile was measured for percent allelic dropout and Peak Height Ratio Imbalance.

The results for HemaSpot™-HD samples are as follows:

Table 8: HemaSpot™-HD Quant Results, Percent Dropout, and PHR Imbalances*. The following quant concentrations for small and large autosomal are displayed below. The samples were then assessed for percent allelic drop out, and peak height ratio imbalances (PHR). Of the 59 samples below, the 3 which gave the best results were highlighted.*

Sample Name	Quant Results (Small Autosomal, ng/µL)	Quant Results (Large Autosomal, ng/µL)	Percent Dropout	PHR Imbalance
HDT11/3A	0.001	0.001	91.67%	N/A
HDT12/3A	0.001	0.001	100%	N/A
HDT13/3A	0.001	0.001	100%	N/A
HDT11/3B	0.000	0.000	100%	N/A
HDT12/3B	0.001	0.001	96%	N/A
HDT13/3B	0.001	0.002	100%	N/A
HDT11/3C	0.001	0.000	95.83%	N/A
HDT12/3C	0.001	0.001	95.83%	N/A
HDT13/3C	0.001	0.000	100%	N/A
HDT21/3A	0.001	0.001	100%	N/A
HDT22/3A	0.002	0.003	83.33%	D2S1338=51.71%, D8S1179=22.96%
HDT23/3A	0.001	0.001	79.17%	N/A
HDT21/3B	0.000	0.001	100%	N/A
HDT22/3B	0.001	0.001	95.83%	N/A
HDT23/3B	0.001	0.001	100%	N/A
HDT21/3C	0.000	0.000	100%	N/A
HDT22/3C	0.001	0.000	79.17%	N/A
HDT23/3C	0.000	0.001	100%	N/A
HDT31/3A	0.001	0.001	100%	N/A
HDT32/3A	0.000	0.001	100%	N/A
HDT33/3A	0.001	0.000	100%	N/A
HDT31/3B	0.001	0.000	100%	N/A
HDT32/3B	0.000	0.000	100%	N/A
HDT33/3B	0.001	0.001	100%	N/A
HDT31/3C	0.000	0.001	100%	N/A
HDT32/3C	0.000	0.000	100%	N/A
HDT33/3C	0.000	0.000	100%	N/A
HDT41/3A	0.001	0.001	100%	N/A
HDT42/3A	0.000	0.000	100%	N/A
HDT43/3A	0.000	0.001	100%	N/A
HDT41/3B	0.002	0.002	100%	N/A
HDT42/3B	0.002	0.002	100%	N/A
HDT43/3B	0.001	0.001	100%	N/A
HDT41/3C	0.000	0.001	100%	N/A
HDT42/3C	0.000	0.000	100%	N/A

HDT43/3C	0.001	0.001	95.83%	N/A
HDT51/3A	0.001	0.000	100%	N/A
HDT52/3A	0.003	0.001	100%	N/A
HDT53/3A	0.000	0.000	100%	N/A
HDT51/3B	0.000	0.000	100%	N/A
HDT52/3B	0.000	0.000	100%	N/A
HDT53/3B	0.000	0.000	100%	N/A
HDT51/3C	0.000	0.000	100%	N/A
HDT52/3C	0.000	0.000	100%	N/A
HDT53/3C	0.000	0.000	100%	N/A
HDT61/3A	0.001	0.001	95.83%	N/A
HDT62/3A	0.002	0.001	96%	N/A
HDT63/3A	0.002	0.003	95.83%	N/A
HDT61/3B	0.000	0.001	100%	N/A
HDT62/3B	0.001	0.000	100%	N/A
HDT63/3B	0.000	0.001	100%	N/A
HDT61/3C	0.005	0.007	92%	N/A
HDT62/3C	0.000	0.000	100%	N/A
HDT63/3C	0.000	0.000	100%	N/A
HDReagentBlank	0.000	0.000	0%	N/A
Positive	0.107	0.109	0%	D3S1358=68.42%
Negative	0.001	0.001	0%	N/A
Ladder 1	N/A	N/A	0%	N/A
Ladder 2	N/A	N/A	0%	N/A
Ladder 3	N/A	N/A	0%	N/A
Ladder 4	N/A	N/A	0%	N/A

The results for HemaSpot™-HF samples are as follows:

Table 9: HemaSpot™-HF Quant Results, Percent Dropout, and PHR Imbalances. *The following quant concentrations for small and large autosomal are displayed below. The samples were then assessed for percent allelic drop out, and peak height ratio imbalances (PHR). Of the 59 samples below, the 3 which gave the best results were highlighted.*

Sample Name	Quant Results (Small Autosomal, ng/µL)	Quant Results (Large Autosomal, ng/µL)	Percent Dropout	PHR Imbalance
HFT11/3A	0.001	0.001	79.17%	D16S539=66.07%
HFT12/3A	0.002	0.001	100%	N/A
HFT13/3A	0.001	0.001	83.33%	D8S1179=63.74%
HFT11/3B	0.003	0.001	91.67%	D8S1179=39.93%
HFT12/3B	0.002	0.001	100%	N/A
HFT13/3B	0.002	0.001	95.83%	N/A
HFT11/3C	0.003	0.002	70.83%	AMEL=33.43%, D10S1248=26.53%
HFT12/3C	0.001	0.001	79.17%	D22S1045=65.54%
HFT13/3C	0.002	0.002	100%	N/A
HFT21/3A	0.000	0.001	100%	N/A
HFT22/3A	0.001	0.000	95.83%	N/A
HFT23/3A	0.001	0.001	100%	N/A
HFT21/3B	0.002	0.001	87.50%	N/A
HFT22/3B	0.001	0.001	100%	N/A
HFT23/3B	0.001	0.001	95.83%	N/A
HFT21/3C	0.001	0.001	100%	N/A
HFT22/3C	0.001	0.000	100%	N/A
HFT23/3C	0.001	0.001	95.83%	N/A
HFT31/3A	0.000	0.000	100%	N/A
HFT32/3A	0.000	0.000	100%	N/A
HFT33/3A	0.001	0.000	100%	N/A
HFT31/3B	0.000	0.000	100%	N/A
HFT32/3B	0.000	0.001	100%	N/A
HFT33/3B	0.000	0.001	100%	N/A
HFT31/3C	0.000	0.000	100%	N/A
HFT32/3C	0.000	0.000	100%	N/A
HFT33/3C	0.000	0.000	100%	N/A
HFT41/3A	0.000	0.000	100%	N/A
HFT42/3A	0.001	0.001	100%	N/A
HFT43/3A	0.000	0.000	100%	N/A
HFT41/3B	0.000	0.000	100%	N/A
HFT42/3B	0.000	0.000	100%	N/A

HFT43/3B	0.000	0.000	100%	N/A
HFT41/3C	0.000	0.000	100%	N/A
HFT42/3C	0.000	0.000	100%	N/A
HFT43/3C	0.000	0.000	100%	N/A
HFT51/3A	0.000	0.000	100%	N/A
HFT52/3A	0.000	0.000	100%	N/A
HFT53/3A	0.000	0.000	100%	N/A
HFT51/3B	0.000	0.000	100%	N/A
HFT52/3B	0.000	0.000	100%	N/A
HFT53/3B	0.000	0.000	100%	N/A
HFT51/3C	0.000	0.000	100%	N/A
HFT52/3C	0.000	0.000	100%	N/A
HFT53/3C	0.000	0.000	100%	N/A
HFT61/3A	0.000	0.000	100%	N/A
HFT62/3A	0.000	0.000	100%	N/A
HFT63/3A	0.000	0.000	100%	N/A
HFT61/3B	0.000	0.000	100%	N/A
HFT62/3B	0.000	0.000	100%	N/A
HFT63/3B	0.000	0.000	100%	N/A
HFT61/3C	0.000	0.000	100%	N/A
HFT62/3C	0.000	0.000	100%	N/A
HFT63/3C	0.000	0.000	100%	N/A
HFReagentBlank	0.000	0.000	0%	N/A
Positive	0.139	0.131	0%	D3S1358=68.42%
Negative	0.000	0.000	0%	N/A
HF Ladder 1	N/A	N/A	0%	N/A
HF Ladder 2	N/A	N/A	0%	N/A
HF Ladder 3	N/A	N/A	0%	N/A
HF Ladder 4	N/A	N/A	0%	N/A

Table 10: HemaSpot™-HD and HemaSpot™-HF Quantification Results Vs. Input DNA Quantity. *The following table represents a comparison between the input concentrations of DNA per sample, and the quantification results post-extraction for the 110 HemaSpot™-HD and HemaSpot™-HF hyper sensitivity study samples.*

Sample Name	Input DNA (ng/µL)	Quant DNA Results (Small Autosomal, ng/µL)	Quant Results (Large Autosomal, ng/µL)
HDT11/3A	0.500	0.001	0.001
HDT12/3A	0.500	0.001	0.001
HDT13/3A	0.500	0.001	0.001
HDT11/3B	0.500	0.000	0.000
HDT12/3B	0.500	0.001	0.001
HDT13/3B	0.500	0.001	0.002
HDT11/3C	0.500	0.001	0.000
HDT12/3C	0.500	0.001	0.001
HDT13/3C	0.500	0.001	0.000
HDT21/3A	0.250	0.001	0.001
HDT22/3A	0.250	0.002	0.003
HDT23/3A	0.250	0.001	0.001
HDT21/3B	0.250	0.000	0.001
HDT22/3B	0.250	0.001	0.001
HDT23/3B	0.250	0.001	0.001
HDT21/3C	0.250	0.000	0.000
HDT22/3C	0.250	0.001	0.000
HDT23/3C	0.250	0.000	0.001
HDT31/3A	0.125	0.001	0.001
HDT32/3A	0.125	0.000	0.001
HDT33/3A	0.125	0.001	0.000
HDT31/3B	0.125	0.001	0.000
HDT32/3B	0.125	0.000	0.000
HDT33/3B	0.125	0.001	0.001
HDT31/3C	0.125	0.000	0.001
HDT32/3C	0.125	0.000	0.000
HDT33/3C	0.125	0.000	0.000
HDT41/3A	0.063	0.001	0.001
HDT42/3A	0.063	0.000	0.000
HDT43/3A	0.063	0.000	0.001
HDT41/3B	0.063	0.002	0.002
HDT42/3B	0.063	0.002	0.002
HDT43/3B	0.063	0.001	0.001
HDT41/3C	0.063	0.000	0.001
HDT42/3C	0.063	0.000	0.000

HDT43/3C	0.063	0.001	0.001
HDT51/3A	0.031	0.001	0.000
HDT52/3A	0.031	0.003	0.001
HDT53/3A	0.031	0.000	0.000
HDT51/3B	0.031	0.000	0.000
HDT52/3B	0.031	0.000	0.000
HDT53/3B	0.031	0.000	0.000
HDT51/3C	0.031	0.000	0.000
HDT52/3C	0.031	0.000	0.000
HDT53/3C	0.031	0.000	0.000
HDT61/3A	0.016	0.001	0.001
HDT62/3A	0.016	0.002	0.001
HDT63/3A	0.016	0.002	0.003
HDT61/3B	0.016	0.000	0.001
HDT62/3B	0.016	0.001	0.000
HDT63/3B	0.016	0.000	0.001
HDT61/3C	0.016	0.005	0.007
HDT62/3C	0.016	0.000	0.000
HDT63/3C	0.016	0.000	0.000
HDReagentBlank	0.000	0.000	0.000
HFT11/3A	0.500	0.001	0.001
HFT12/3A	0.500	0.002	0.001
HFT13/3A	0.500	0.001	0.001
HFT11/3B	0.500	0.003	0.001
HFT12/3B	0.500	0.002	0.001
HFT13/3B	0.500	0.002	0.001
HFT11/3C	0.500	0.003	0.002
HFT12/3C	0.500	0.001	0.001
HFT13/3C	0.500	0.002	0.002
HFT21/3A	0.250	0.000	0.001
HFT22/3A	0.250	0.001	0.000
HFT23/3A	0.250	0.001	0.001
HFT21/3B	0.250	0.002	0.001
HFT22/3B	0.250	0.001	0.001
HFT23/3B	0.250	0.001	0.001
HFT21/3C	0.250	0.001	0.001
HFT22/3C	0.250	0.001	0.000
HFT23/3C	0.250	0.001	0.001
HFT31/3A	0.125	0.000	0.000
HFT32/3A	0.125	0.000	0.000
HFT33/3A	0.125	0.001	0.000
HFT31/3B	0.125	0.000	0.000
HFT32/3B	0.125	0.000	0.001
HFT33/3B	0.125	0.000	0.001
HFT31/3C	0.125	0.000	0.000

HFT32/3C	0.125	0.000	0.000
HFT33/3C	0.125	0.000	0.000
HFT41/3A	0.063	0.000	0.000
HFT42/3A	0.063	0.001	0.001
HFT43/3A	0.063	0.000	0.000
HFT41/3B	0.063	0.000	0.000
HFT42/3B	0.063	0.000	0.000
HFT43/3B	0.063	0.000	0.000
HFT41/3C	0.063	0.000	0.000
HFT42/3C	0.063	0.000	0.000
HFT43/3C	0.063	0.000	0.000
HFT51/3A	0.031	0.000	0.000
HFT52/3A	0.031	0.000	0.000
HFT53/3A	0.031	0.000	0.000
HFT51/3B	0.031	0.000	0.000
HFT52/3B	0.031	0.000	0.000
HFT53/3B	0.031	0.000	0.000
HFT51/3C	0.031	0.000	0.000
HFT52/3C	0.031	0.000	0.000
HFT53/3C	0.031	0.000	0.000
HFT61/3A	0.016	0.000	0.000
HFT62/3A	0.016	0.000	0.000
HFT63/3A	0.016	0.000	0.000
HFT61/3B	0.016	0.000	0.000
HFT62/3B	0.016	0.000	0.000
HFT63/3B	0.016	0.000	0.000
HFT61/3C	0.016	0.000	0.000
HFT62/3C	0.016	0.000	0.000
HFT63/3C	0.016	0.000	0.000
HFReagentBlank	0.000	0.000	0.000

Table 11 HemaSpot™-HD and HF Small Autosomal Average Comparison. *The bar graph below represents a comparison of averages for the input DNA vs recovered DNA (ng/µL) in regard to the HD and HF small autosomal data.*

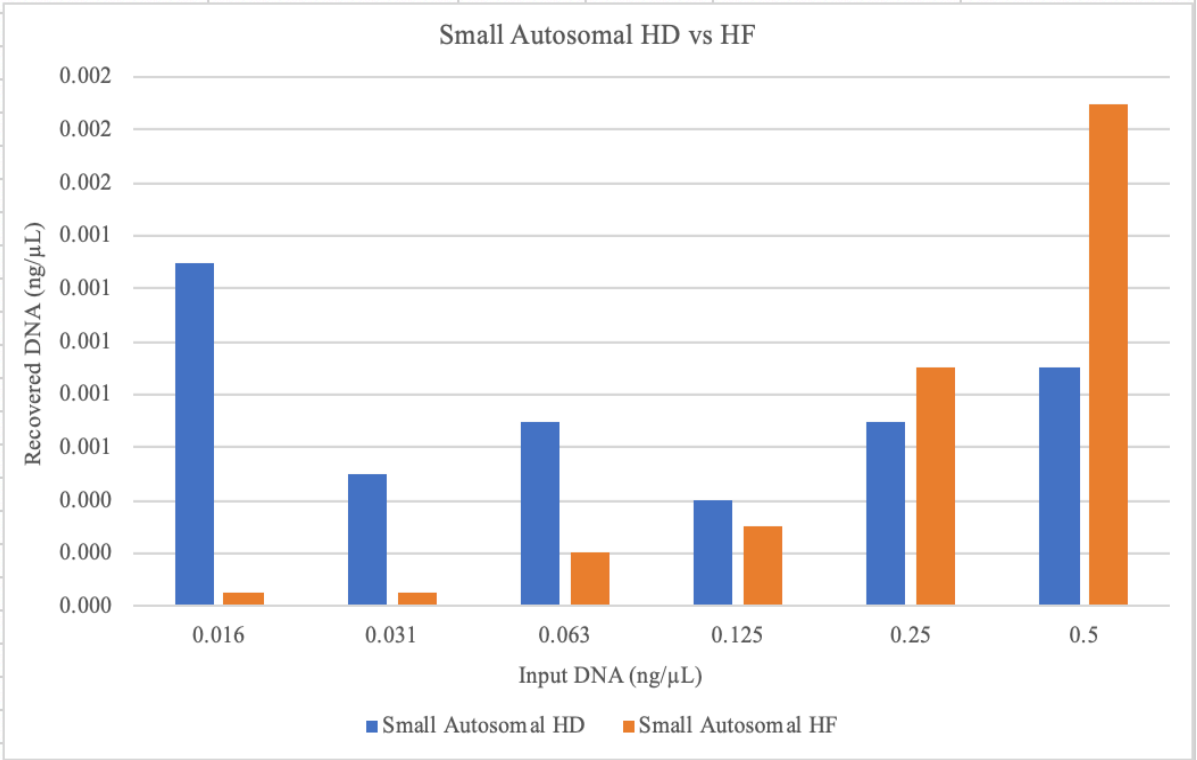


Table 12 HemaSpot™-HD and HF Large Autosomal Average Comparison. *The bar graph below represents a comparison of averages for the input DNA vs recovered DNA (ng/µL) in regard to the HD and HF large autosomal data.*

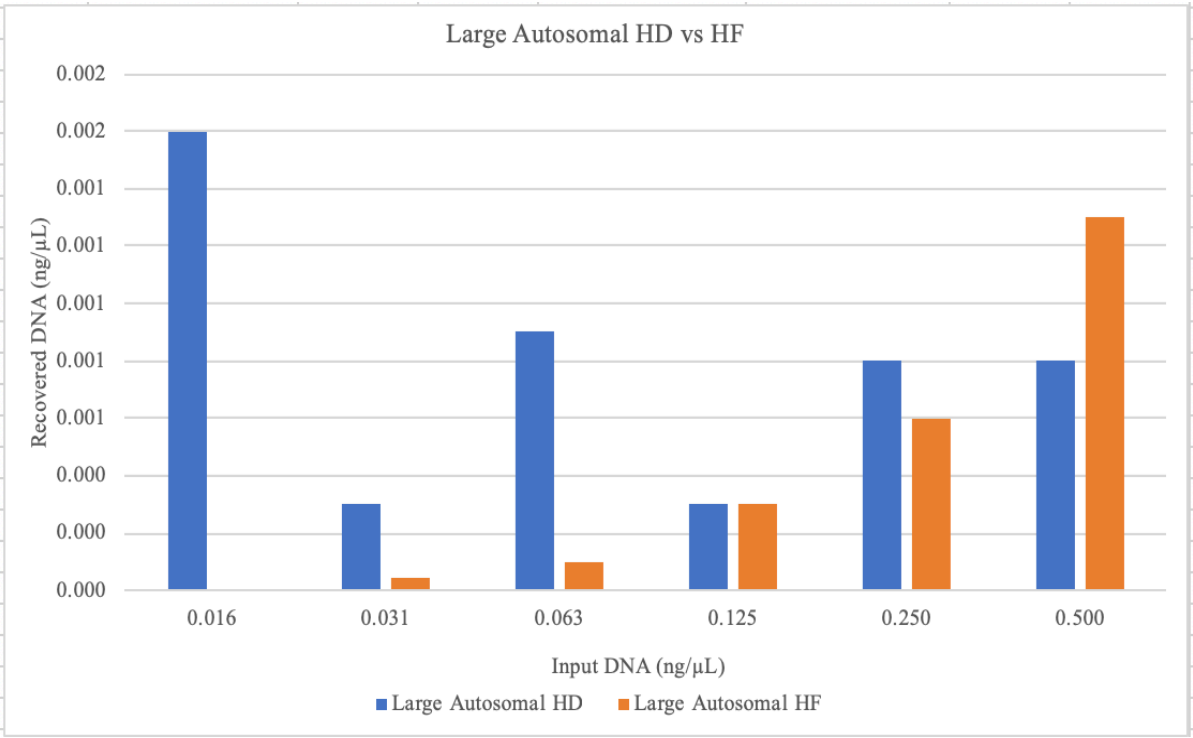


Table 13: T-Test Comparison Between HemaSpot™-HD and HF Small Autosomal Data. *A t-test was administered to each set of small and large autosomal data for both HD and HF samples. Each test was run under the same characteristics (two tails, type 3) in order to make the test stricter. The results are as follows:*

Sample Name (Applied to HD and HF)	HD Quant Results (Small Autosomal, ng/μL)	HF Quant Results (Small Autosomal, ng/μL)
T11/3A	0.001	0.001
T12/3A	0.001	0.002
T13/3A	0.001	0.001
T11/3B	0.000	0.003
T12/3B	0.001	0.002
T13/3B	0.001	0.002
T11/3C	0.001	0.003
T12/3C	0.001	0.001
T13/3C	0.001	0.002
T21/3A	0.001	0.000
T22/3A	0.002	0.001
T23/3A	0.001	0.001
T21/3B	0.000	0.002
T22/3B	0.001	0.001
T23/3B	0.001	0.001
T21/3C	0.000	0.001
T22/3C	0.001	0.001
T23/3C	0.000	0.001
T31/3A	0.001	0.000
T32/3A	0.000	0.000
T33/3A	0.001	0.001
T31/3B	0.001	0.000
T32/3B	0.000	0.000
T33/3B	0.001	0.000
T31/3C	0.000	0.000
T32/3C	0.000	0.000
T33/3C	0.000	0.000
T41/3A	0.001	0.000
T42/3A	0.000	0.001
T43/3A	0.000	0.000
T41/3B	0.002	0.000
T42/3B	0.002	0.000
T43/3B	0.001	0.000
T41/3C	0.000	0.000
T42/3C	0.000	0.000
T43/3C	0.001	0.000

T51/3A	0.001	0.000
T52/3A	0.003	0.000
T53/3A	0.000	0.000
T51/3B	0.000	0.000
T52/3B	0.000	0.000
T53/3B	0.000	0.000
T51/3C	0.000	0.000
T52/3C	0.000	0.000
T53/3C	0.000	0.000
T61/3A	0.001	0.000
T62/3A	0.002	0.000
T63/3A	0.002	0.000
T61/3B	0.000	0.000
T62/3B	0.001	0.000
T63/3B	0.000	0.000
T61/3C	0.005	0.000
T62/3C	0.000	0.000
T63/3C	0.000	0.000
Count	54.000	54.000
Average	0.001	0.001
Standard Deviation	0.001	0.001
P-Value	***0.232 or 23.3%*** ***23.3% calculated > 5% standard***	***ACCEPT H0*** ***NO STATISTICAL SAMPLE DIFFERENCES***

Table 14: T-Test Comparison Between HemaSpot™-HD and HF Large Autosomal Data. *A t-test was administered to each set of small and large autosomal data for both HD and HF samples. Each test was run under the same characteristics (two tails, type 3) in order to make the test stricter. The results are as follows:*

Sample Name (Applied to HD and HF)	HD Quant Results (Large Autosomal, ng/μL)	HF Quant Results (Large Autosomal, ng/μL)
T11/3A	0.001	0.001
T12/3A	0.001	0.001
T13/3A	0.001	0.001
T11/3B	0.000	0.001
T12/3B	0.001	0.001
T13/3B	0.002	0.001
T11/3C	0.000	0.002
T12/3C	0.001	0.001
T13/3C	0.000	0.002
T21/3A	0.001	0.001
T22/3A	0.003	0.000
T23/3A	0.001	0.001
T21/3B	0.001	0.001
T22/3B	0.001	0.001
T23/3B	0.001	0.001
T21/3C	0.000	0.001
T22/3C	0.000	0.000
T23/3C	0.001	0.001
T31/3A	0.001	0.000
T32/3A	0.001	0.000
T33/3A	0.000	0.000
T31/3B	0.000	0.000
T32/3B	0.000	0.001
T33/3B	0.001	0.001
T31/3C	0.001	0.000
T32/3C	0.000	0.000
T33/3C	0.000	0.000
T41/3A	0.001	0.000
T42/3A	0.000	0.001
T43/3A	0.001	0.000
T41/3B	0.002	0.000
T42/3B	0.002	0.000
T43/3B	0.001	0.000
T41/3C	0.001	0.000
T42/3C	0.000	0.000
T43/3C	0.001	0.000

T51/3A	0.000	0.000
T52/3A	0.001	0.000
T53/3A	0.000	0.000
T51/3B	0.000	0.000
T52/3B	0.000	0.000
T53/3B	0.000	0.000
T51/3C	0.000	0.000
T52/3C	0.000	0.000
T53/3C	0.000	0.000
T61/3A	0.001	0.000
T62/3A	0.001	0.000
T63/3A	0.003	0.000
T61/3B	0.001	0.000
T62/3B	0.000	0.000
T63/3B	0.001	0.000
T61/3C	0.007	0.000
T62/3C	0.000	0.000
T63/3C	0.000	0.000
Count	54.000	54.000
Average	0.001	0.000
Standard Deviation	0.001	0.001
P-Value	***0.015 or 1.5%*** ***1.5% calculated < 5% standard***	***REJECT H0*** ***STATISTICAL SAMPLE DIFFERENCES***

Part 2: Testing HemaSpot™-HF and HemaSpot™-HD as Mediums for Trace DNA

Much like the first study, the trace DNA files were downloaded onto a disc drive and analyzed on GeneMapper® *ID-X* Software in the UNTHSC GSBS student computer lab room 311. In total the trace DNA study amassed 32 GeneMapper® files. This broke down into 27 HemaSpot™-HD, HemaSpot™-HF, and Puritan® Cotton Swabs samples, 1 Reagent Blank, 1 Positive control, 1 Negative control, and 2 capillary electrophoresis ladders. In order for the data to be acceptable for evaluation by an analyst, all ladders, reagent blanks, and controls must meet the predetermined standards for quality. After confirming that there were no outliers, and all samples met the requirements, each profile was analyzed to determine the overall usefulness of the data. The overall goal of the trace study was to observe if it was possible to obtain a DNA profile of any kind based solely on the mediums' abilities to store and release trace levels of DNA taken off of the 3 different objects. For those that did, each profile was measured for percent allelic dropout and Peak Height Ratio Imbalance.

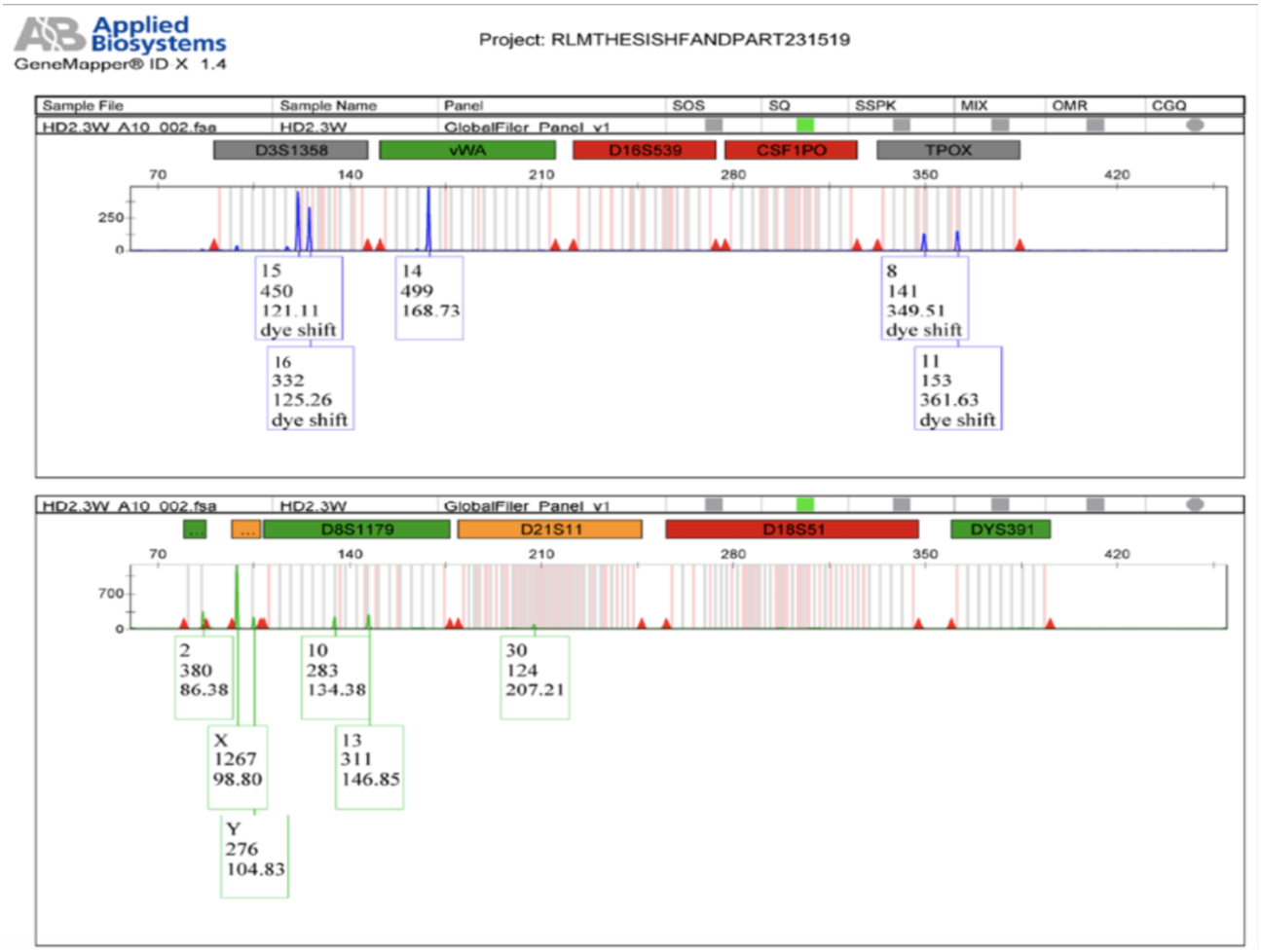
The results are as follows:

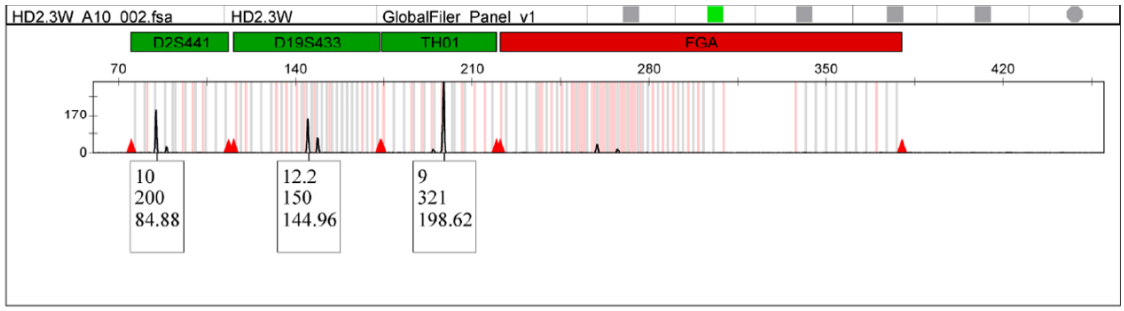
Table 15: Trace Study Quant Results, Percent Dropout, and PHR Imbalances. *The following quant concentrations for small and large autosomal are displayed below. The samples were then assessed for percent allelic drop out, and peak height ratio imbalances (PHR). Of the 32 samples below, the 3 which gave the best results were highlighted.*

Sample Name	Quant Results (Small Autosomal, ng/µL)	Quant Results (Large Autosomal, ng/µL)	Percent Dropout	PHR Imbalance
Swab1/3Wheel	0.000	0.000	95.83%	N/A
Swab2/3Wheel	0.001	0.001	100%	N/A
Swab3/3Wheel	0.000	0.000	100%	N/A
HF1/3Wheel	0.001	0.001	91.67%	N/A
HF2/3Wheel	0.000	0.000	100%	N/A
HF3/3Wheel	0.001	0.000	100%	N/A
HD1/3Wheel	0.002	0.000	100%	N/A
HD2/3Wheel	0.003	0.002	45.83%	AMEL=21.78%, D5S818=62.96%, D13S317=53.33%, D10S1248=39.95%, D12S391=27.30%
HD3/3Wheel	0.002	0.001	100%	N/A
Swab1/3Knife	0.000	0.000	100%	N/A
Swab2/3Knife	0.000	0.000	100%	N/A
Swab3/3Knife	0.000	0.001	100%	N/A
HF1/3Knife	0.000	0.000	100%	N/A
HF2/3Knife	0.000	0.000	100%	N/A
HF3/3Knife	0.000	0.000	100%	N/A
HD1/3Knife	0.001	0.001	100%	N/A
HD2/3Knife	0.001	0.000	100%	N/A
HD3/3Knife	0.000	0.000	100%	N/A
Swab1/3Bullet	0.000	0.000	100%	N/A
Swab2/3Bullet	0.000	0.000	100%	N/A
Swab3/3Bullet	0.000	0.000	100%	N/A
HF1/3Bullet	0.001	0.000	100%	N/A
HF2/3Bullet	0.001	0.001	100%	N/A
HF3/3Bullet	0.000	0.000	100%	N/A
HD1/3Bullet	0.001	0.001	95.83%	N/A
HD2/3Bullet	0.001	0.001	95.83%	N/A
HD3/3Bullet	0.001	0.001	100%	N/A
TraceRgt.Blank	0.000	0.000	0%	N/A
Positive	0.106	0.128	0%	D3S1358=68.42%
Negative	0.000	0.000	0%	N/A
Trace Ladder 1	N/A	N/A	0%	N/A
Trace Ladder 2	N/A	N/A	0%	N/A

Of all profiles assessed, the most complete profile, remarkably, came from the trace DNA HemaSpot™-HD steering wheel sample. It's 45.83% allelic dropout rate was less than that of any other sample by far. However, it also had the most peak height imbalances of any other observable profile, including AMEL=21.78%, D5S818=62.96%, D13S317=53.33%, D10S1248=39.95%, and D12S391=27.30%. The profile itself is presented below:

Figure 12. Trace Sample for HemaSpot™-HD tube 2 of 3 steering wheel swab. *The following image represents the Trace Sample for HemaSpot™-HD tube 2 of 3 steering wheel swab from the Trace DNA study. This was the most complete profile in the study and demonstrates the HD's potential. Minor unrelated instrumental dye shifting was identified and corrected.*





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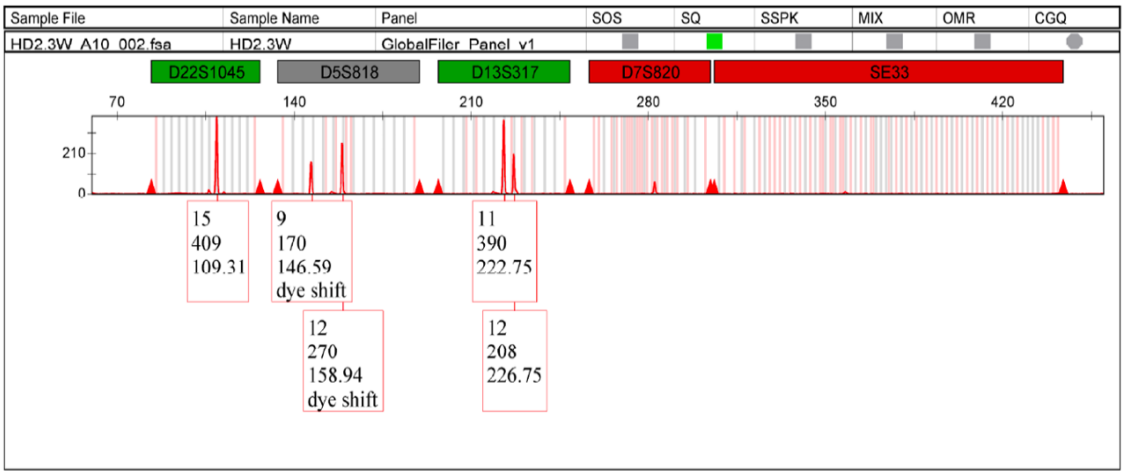
Applied Biosystems
GeneMapper® ID X 1.4

Project: RLMTHESISHFANDPART231519



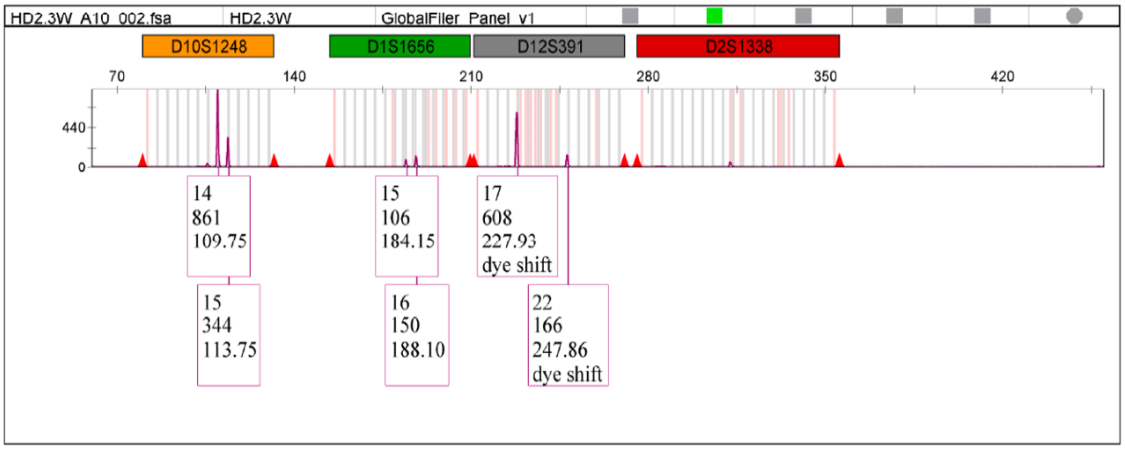


Table 16: T-Test Comparison Between Trace Wheel HemaSpot™-HD and HF Small Autosomal Data. *A t-test was administered to each set of small and large autosomal data for both HD and HF samples. Each test was run under the same characteristics (two tails, type 3) in order to make the test stricter. The results are as follows:*

	COMPARING HD TO HF	
Sample Name	HD Quant Results (Small Autosomal, ng/μL)	HF Quant Results (Small Autosomal, ng/μL)
1/3 Wheel	0.002	0.001
2/3 Wheel	0.003	0.000
3/3 Wheel	0.002	0.000
	p-value 0.025 <0.05 = REJECT H0, DEPENDENT	
Sample Name	HD Quant Results (Large Autosomal, ng/μL)	HF Quant Results (Large Autosomal, ng/μL)
1/3 Wheel	0.000	0.001
2/3 Wheel	0.002	0.000
3/3 Wheel	0.001	0.000
	p-value 0.431 >0.05 = ACCEPT H0, INDEPENDENT	

Table 17: T-Test Comparison Between Trace Knife HemaSpot™-HD and HF Small Autosomal Data. *A t-test was administered to each set of small and large autosomal data for both HD and HF samples. Each test was run under the same characteristics (two tails, type 3) in order to make the test stricter. The results are as follows:*

	COMPARING HD TO HF	
Sample Name	HD Quant Results (Small Autosomal, ng/µL)	HF Quant Results (Small Autosomal, ng/µL)
1/3Knife	0.001	0.000
2/3Knife	0.000	0.000
3/3Knife	0.000	0.000
	p-value	
	0.593	
	>0.05 = ACCEPT H0, INDEPENDENT	
Sample Name	HD Quant Results (Large Autosomal, ng/µL)	HF Quant Results (Large Autosomal, ng/µL)
1/3Knife	0.001	0.000
2/3Knife	0.000	0.000
3/3Knife	0.000	0.000
	p-value	
	0.264	
	>0.05 = ACCEPT H0, INDEPENDENT	

Table 18: T-Test Comparison Between Trace Bullet HemaSpot™-HD and HF Small Autosomal Data. *A t-test was administered to each set of small and large autosomal data for both HD and HF samples. Each test was run under the same characteristics (two tails, type 3) in order to make the test stricter. The results are as follows:*

	COMPARING HD TO HF	
Sample Name	HD Quant Results (Small Autosomal, ng/µL)	HF Quant Results (Small Autosomal, ng/µL)
1/3Bullet	0.001	0.001
2/3Bullet	0.001	0.001
3/3Bullet	0.001	0.000
	p-value 0.180 >0.05 = ACCEPT H0, INDEPENDENT	
Sample Name	HD Quant Results (Large Autosomal, ng/µL)	HF Quant Results (Large Autosomal, ng/µL)
1/3Bullet	0.001	0.000
2/3Bullet	0.001	0.001
3/3Bullet	0.001	0.000
	p-value 0.203 >0.05 = ACCEPT H0, INDEPENDENT	

CHAPTER IV

DISCUSSION AND CONCLUSIONS

The aims of this study were:

-To observe the HemaSpot™-HD and HF's extreme qualitative and quantitative features from a series of control DNA dilutions

-To formulate and execute a proof of concept study which focused on the ability of the HemaSpot™ to collect touch DNA from mock crime scene evidence

The Hyper Sensitivity Study achieved the first aim by gathering data from two sets of extractions, (HD and HF), and evaluating their qualitative and quantitative features. This was demonstrated by a series of serial control DNA dilutions, followed by QIAamp® DNA extractions, Quantifiler Trio® analysis, Polymerase Chain Reactions, and Capillary Electrophoresis evaluations. Next, the generated quantification data was tabulated and presented as tables 8, 9, and 10. A comparison of small autosomal vs large autosomal averages was also displayed in graphs 11, and 12. In order to maximize statistical results, all samples were run in triplicate. The results were then evaluated for statistical relationships via t-tests displayed as Tables 13 and 14. Surprisingly, there was noticeable variation between the two set's small autosomal and large autosomal data. For both Tables 13 and 14, a two-tail characteristic was chosen due to the uncertainty of the data's direction. In other words, it was unknown if either of

the data sets would differ by moving the mean in one direction. Type 3 t-test characteristics were also used for both analyses because the data originated from different groups. In addition, the variants were assumed to be unequal to make the test stricter. The final p-values were then generated in Excel. These t-tests calculated the averages, variants, and standard deviations of the data, and determined if the sets really were different. In a typical biological test, anything less than a p-value of 5% or 0.05 is often a cause for null hypothesis rejection. In contrast, if the p-value is greater than 5% or 0.05, the null hypothesis is often accepted. For Table 13, a p-value of 23.2% or 0.232 was generated. Since this result was much larger than 0.05 or 5%, the null hypothesis (H0) was accepted. This hypothesis declared that there were no statistical differences between the HD and HF small autosomal data sets. Surprisingly however, the p-value generated for Table 14 gave an opposing result with a value of 1.5% or 0.015. Since this number was much smaller than 0.05, the null hypothesis (H0) was rejected. This led to the conclusion that the large autosomal HD and HF data sets had noticeable statistical differences. The main theory for the cause of this phenomenon revolves around the base pair sizing. During the quantification process the analyzed small autosomal fragments were roughly 80 base pairs long. In contrast, the large autosomal fragments were roughly 215 base pairs long. Because the size and quantity of the control DNA was relatively minute to begin with, chances are small autosomal had an easier time working with the samples the quantification process.

The trace DNA study accomplished the second aim in a series of steps. First, a proof of concept outline was formulated in order to address the study's requirements. Next, mock crime scene evidence was gathered and swabbed against a collection of HemaSpot™-HD, HF, and Puritan Cotton applicators. Afterwards, the collection devices were deposited into individual tubes, and processed identically to that of the hyper sensitivity study. In order to maximize

statistical results, all samples were run in triplicate. The resulting data was then displayed in Table 15. From this information, statistical evaluations were limited, however all data was still assessed for possible relationships and trends. This was accomplished by noting electropherogram characteristics, peak height ratio imbalances, and individual Quant Trio™ values for each sample. One unique sample in particular came from the HemaSpot™-HD steering wheel swab. This sample not only contained the most allelic presence by far, but also had the highest level of peak height imbalances. Additionally, two more unique data sets were discovered in the HemaSpot™-HD bullet swab samples. The reason for this drastic difference in DNA recovery most likely comes from inhibition. To specify, it is possible that the components in the bullet primer mix inhibited the DNA extraction process, as heavy metals are known to be common DNA inhibitors. Unlike the first example, these samples contained roughly 95.8% allelic dropout, and no recorded peak height imbalances.

Unlike the first study, the trace DNA study was not expected to follow any linear trends. Therefore, the amount of DNA in each sample tube relied heavily on the device's ability to pick up whatever cellular materials it came into contact with. In other words, each result was sample dependent, and profile generation was based mostly on sample quality rather than cartridge type.

In addition to evaluating the trace DNA sample quantitative and qualitative features, a series of short t-tests were run to compare the HD and HF quantitative results to the wheel, swab, and bullet data. This was displayed in tables 16, 17, and 18. Out of all 6 tests, only one failed to accept the null hypothesis of sample independence. This comparison of the HD to HF small autosomal wheel p-value was just shy of the 0.05 standard for sample independence at value of 0.03. However, this characteristic was believed to be a case of sample variation due to the table's limited size.

Limitations: Quantification

From the first round of quantification, the presence of low-level DNA was noticeable, much more than previously expected. In order to maximize the success of a GlobalFiler® PCR reaction, samples must be normalized to an acceptable level as to not overload the electrophoresis and GeneMapper® analysis. Due to the minute quantities of DNA found within the samples, this step was not performed. This low DNA level could be due to many factors. First and foremost, the paper inside of the cartridges was intended for blood sampling and medical research purposes. In a healthy individual, the body contains roughly 4-7x106 leukocytes per blood milliliter, deviating at about 30 to 40μL/mL of blood depending on the person who donates. [9] Therefore, the overall quantity of DNA obtainable from an average 100μL blood sample far outweighs that of a trace DNA sample, mainly consisting of loose skin cells. That being said, the paper itself might not be capable of releasing such miniscule amounts of sample efficient enough for genetic analysis.

Secondly, the sheer size of this project might have been cause for questioning. To clarify, throughout the quantification, amplification, and electrophoresis processes, some of the required master mix components are light sensitive. In turn, the amount of time it takes to load 5 samples vs 55 samples can greatly alter the effectiveness of the master mix. Although all steps in the preparation process were followed verbatim, the longer load times may have partially degraded the master mix, reducing its ability to operate.

Thirdly, perhaps the size of the sample cuttings themselves simply were not big enough for the study to be effective. As stated previously, two 9x3mm cuttings were deposited into every sample tube. This was inspired by the common 5x5mm cutting size of a typical cloth sample for similar UNTHSC extraction processes such as Organic, Differential, and Chelex.

Limitations: Capillary Electrophoresis

The results from electrophoresis as viewed on the GeneMapper® *ID-X* Software slightly deviated from the expected results. Specifically, in the case of hypersensitivity HemaSpot™-HD, which had a few alleles present in the T6A and T6C samples. This sudden presence of observable alleles could be explained by allelic drop-in, a common occurrence when working with extremely low levels of DNA.

Limitations: Partial Profiles

The most prevalent feature shared by the majority of samples in these studies was the high level of partial allelic dropout. In order for an allele to be counted as present, it must meet certain criteria depending on its heterozygotic or homozygotic qualities. These alleles must contain both peaks (heterozygous) at a height of no less than 50 RFU (Analytical Threshold), or ideally a height of 200 RFU (Stochastic Threshold, homozygous). [11] In most cases, heterozygous alleles from the samples contained only one of their two peaks, and therefore contributed to the percent dropout score.

Conclusions

Out of all of the samples analyzed in this study (excluding ladders, controls and reagent blanks), not one produced a complete genetic profile. However, as the basis of this study focused on trace DNA, this was not unexpected. Based on the hyper sensitivity and trace DNA statistics generated from Tables 13, 14, 16, 17, and 18, most results indicated that both the HemaSpot™-HD and HemaSpot™-HF samples performed similarly within the series of dilutions. The reasons for the high occurrence of allelic dropout are believed to be from sample dependent variation,

primer inhibition (bullet trace samples), and an inability of the membrane to successfully release trapped DNA into the QIAamp extraction mixture. Perhaps a solution to this predicament lies in the physical qualities of the sample itself and will require studies with more tissue-based samples.

The Quantifiler Trio kit used in this study contained multiple-copy target loci. Their design was to improve sample detection sensitivity. The test was fine-tuned and looked at 3 different target loci specific to humans. These include Small Autosomal, Large Autosomal, and Y-chromosome loci. Each target contains numerous copies distributed on varying autosomal chromosomes or the Y-chromosome. For this study, Small and Large Autosomal data was compared against each other in order to determine the relationships between the sample concentrations. One observation made, was that there seemed to be a slight difference between the two categories. The cause of this phenomenon was most likely due to the already miniscule DNA levels within the samples having an easier time interacting with that of the 80bp (Small Autosomal) targets as opposed to the 215bp (Large Autosomal) targets. In a non-trace study, these two autosomal categories would most likely share near identical results unless the sample was degraded.

For the hypersensitivity study, the majority of the data was too small to generate any kind of reliable statistics. However, it was still possible to analyze samples based on their electropherogram and PHR qualities to gather useful information about the quality of the samples. These hypersensitive samples all seemed to show that they were more reflective of the lower threshold limits for the Quantifiler kit. Because of this, a majority of the profiles contained high/complete allelic dropout, and minor PHR imbalances.

For the trace study, sample sizing was too small to give any sort of novel information based on the t-tests alone. However, after observing the electropherograms and comparing the profile PHRs to allelic dropout ratios, a few conclusions were drawn. First, most trace profiles were too minute to give any kind of translatable result. Second, for those that did generate data, a majority of their loci contained allelic dropout or simply did not meet the stochastic thresholds for allele calling. Third, sample success seemed to depend more on quality of the sample itself rather than the cartridge it was stored on. In conclusion, the results showed that both cartridges gave data that was more sample dependent than collection device dependent. In addition, the swabs both performed similarly and promisingly since the condition of the sample was more limiting than anything else.

Future Studies

For future studies, a comparison of collection methods would be ideal. In the trace section of this study, cartridges were deconstructed to retrieve the sampling paper inside. This paper was then moistened with DNAse free water and rubbed against the mock crime scene evidence for collection. Next, the cartridge was carefully reconstructed so the samples could dry safely until extraction was initiated. In common cases, a moist cotton swab is also used to collect a sample, which is then placed into a plastic bag or similar storage container. This difference in collection and storage might have some profound effect on the data, and a study to compare the two collection methods should be demonstrated.

Based on the results and observations from this study, it is recommended that the manufacturer should design a special collection device for use in the field. A crime scene friendly collection device might have features similar to that of a cotton swab, but with the added

benefit of a protective shell to store the sample without the need for plastic bags. The design for the current HemaSpot™ has similar features, including the plastic shell and desiccant for quick drying. However, unlike the current HemaSpot™, a more compact and smaller application surface would help to increase DNA yield, as the current size of the sampling paper is much too large for trace analysis. These qualities redesigned into an all-in-one sampling tool for crime scene collection would make the product extremely appealing to crime scene investigators. In addition, eliminating the need to deconstruct the cartridge to get to the sampling paper will not only save time, but could greatly reduce opportunities for contamination.

The analysis of cast-off cellular material in trace DNA testing has unforeseen potential in forensics. However, the ability to collect and examine such evidence lies heavily in the microscopic traits of the given object. Conceptually, a porous surface should be able to hold more cellular material than that of a non-porous surface. This also plays a critical role when conducting a trace DNA research study. Future testing should be performed with the HemaSpot™ to test its ability to collect touch DNA from a broader range of contact surfaces and sample sizes. Potential items should include but not be limited to: glass, cloth, wood, paint, plastic, and foam. The quantitative results from Table 15 also align with this concept. In particular, the most complete DNA profile originated from a steering wheel swab, which had a more porous surface than that of the pocket knife and bullet casings. Likewise, environmental factors play a critical role in the successful recovery of cellular material from a crime scene and should be addressed during collection.

In similar future studies, some additional changes like substituting TE (Tris-EDTA) buffer for DNAse free water might give higher quality results. TE buffer is commonly used to solubilize DNA and keep samples from degrading. since it has stabilizing traits. In addition, the

use of carrier RNA mixed in to the AL buffer of the QIAamp extraction kit could serve to increase smaller sample DNA yields. Using samples containing diluted blood or other cellular material might also have a profound effect on the sample's yield, as the original design of the HemaSpot was manufactured to store human tissue samples.

Another study to assess the HemaSpot™'s forensic capabilities includes the analysis of trace cellular samples from both direct and indirect transfers. In a direct transfer, the object in question should come directly into contact with the source of the DNA. This includes touching, licking, sneezing, speaking, and coughing. Indirect transfer methods should also be tested by the HemaSpot™, including variations of secondary transfer such as multiple handshakes before touching a door handle. This design was inspired by Fonneløp et al., who's study investigated the initial transport of DNA from plastic, wood, and metal substrates. The team also studied secondary and tertiary transfer from individuals using nitrile-gloves to interact with a series of objects before sample collection and analysis.

The forensic potential for the HemaSpot™-HD and HemaSpot™-HF sampling kits travels far beyond the study of trace DNA. Not only have the devices themselves proven their ability to store and release DNA, but the patented designs serve as a monumental benefit to facilities which cannot accommodate large scale cold storage samples. According to Spot On Sciences staff, the HemaSpot™-HD has had success with not only blood, but nasopharyngeal wash as well as diarrhea fluids, and may even work well with vaginal wash fluids for future studies. In addition, the in-production phone application provides another large-scale benefit to the HemaSpot™ kits, as the field of forensics works to stay up to date with rapidly advancing technology. Future studies on these kits would be extremely beneficial and deserve to be

analyzed to their fullest potential in the forensic field. However, until that time comes, the true superiority between the HemaSpot™-HD and HemaSpot™-HF will remain uncertain.

APPENDIX

Figure 1: AmpFℓSTR™ DNA Control 007. (2 ng/µL) Catalog Number: 100028107 Pub No. MAN0017401
The following chart depicts the genetic profile of the control DNA used in the hypersensitivity study serial dilutions and extractions. It aided in differentiating control DNA from contamination DNA. [10]

Contents and storage

Contents	Amount	Storage conditions
DNA Control 007 Contains 2.0 ng/µL of human male genomic DNA in 0.05% sodium azide and buffer. The profile of this DNA when using the procedures described in the *GlobalFiler™ Express PCR Amplification Kit User Guide* is: D3S1358 15, 16; vWA 14, 16; D16S539 9, 10; CSF1PO 11, 12; TPOX 8; Y indel 2; Amelogenin X, Y; D8S1179 12, 13; D21S11 28, 31; D18S51 12, 15; DYS391 11; D2S441 14, 15; D19S433 14, 15; TH01 7, 9.3; FGA 24, 26; D22S1045 11, 16; D5S818 11; D13S317 11; D7S820 7, 12; SE33 17, 25.2; D10S1248 12, 15; D1S1656 13, 16; D12S391 18, 19; D2S1338 20, 23.	10 tubes, 0.1 mL/tube	–25°C to –15°C on receipt. 2°C to 8°C after first use up to the expiration date stated on the kit.

Table 1: Subject 1's GlobalFiler® GeneMapper® ID-X DNA Profile. This information was used as a source comparison to the profiles rendered from the Trace DNA steering wheel and knife samples. Any called alleles outside of this profile were counted as contamination.

Profile:	Subject 1
Donation:	Steering Wheel & Knife
Marker:	Called Alleles:
D3S1358	15,16
vWA	14,14
D16S539	11,12
CSF1PO	11,12
TPOX	8,11
Y-Indel	2,2
AMELOGENIN	X,Y
D8S1179	10,13
D21S11	28,30
D18S51	16,17
DYS391	10,10
D2S441	10,11
D19S433	13,14
TH01	9,9
FGA	22,24
D22S1045	15,15
D5S818	9,12
D13S317	11,12
D7S820	10,11
SE33	17,28.2
D10S1248	14,15
D1S1656	15,16
D12S391	17,22
D2S1338	19,24

Table 2: Subject 2's GlobalFiler® GeneMapper® ID-X DNA Profile. This information was used as a source comparison to the profiles rendered from the Trace DNA bullet samples. Any called alleles outside of this profile were counted as contamination.

Profile:	Subject 2
Donation:	Bullets
Marker:	Called Alleles:
D3S1358	15,16
vWA	18,19
D16S539	11,13
CSF1PO	10,12
TPOX	8,8
Y-Indel	2,2
AMELOGENIN	X,Y
D8S1179	8,13
D21S11	28,29
D18S51	11,17
DYS391	11,11
D2S441	10,15
D19S433	14,15.2
TH01	6,6
FGA	21,24
D22S1045	15,16
D5S818	12,13
D13S317	9,11
D7S820	10,12
SE33	20,20
D10S1248	14,14
D1S1656	12,14
D12S391	17.3,22
D2S1338	20,20

Figure 2: Applied Biosystem's Quantifiler™ Trio Data for HemaSpot™-HD. The following image depicts the standard curve for the HemaSpot™-HD samples of the hypersensitivity study. A correlation coefficient value (R^2) of at least 0.99 is required for further analysis. As shown, all criteria were met, and PCR was initiated.

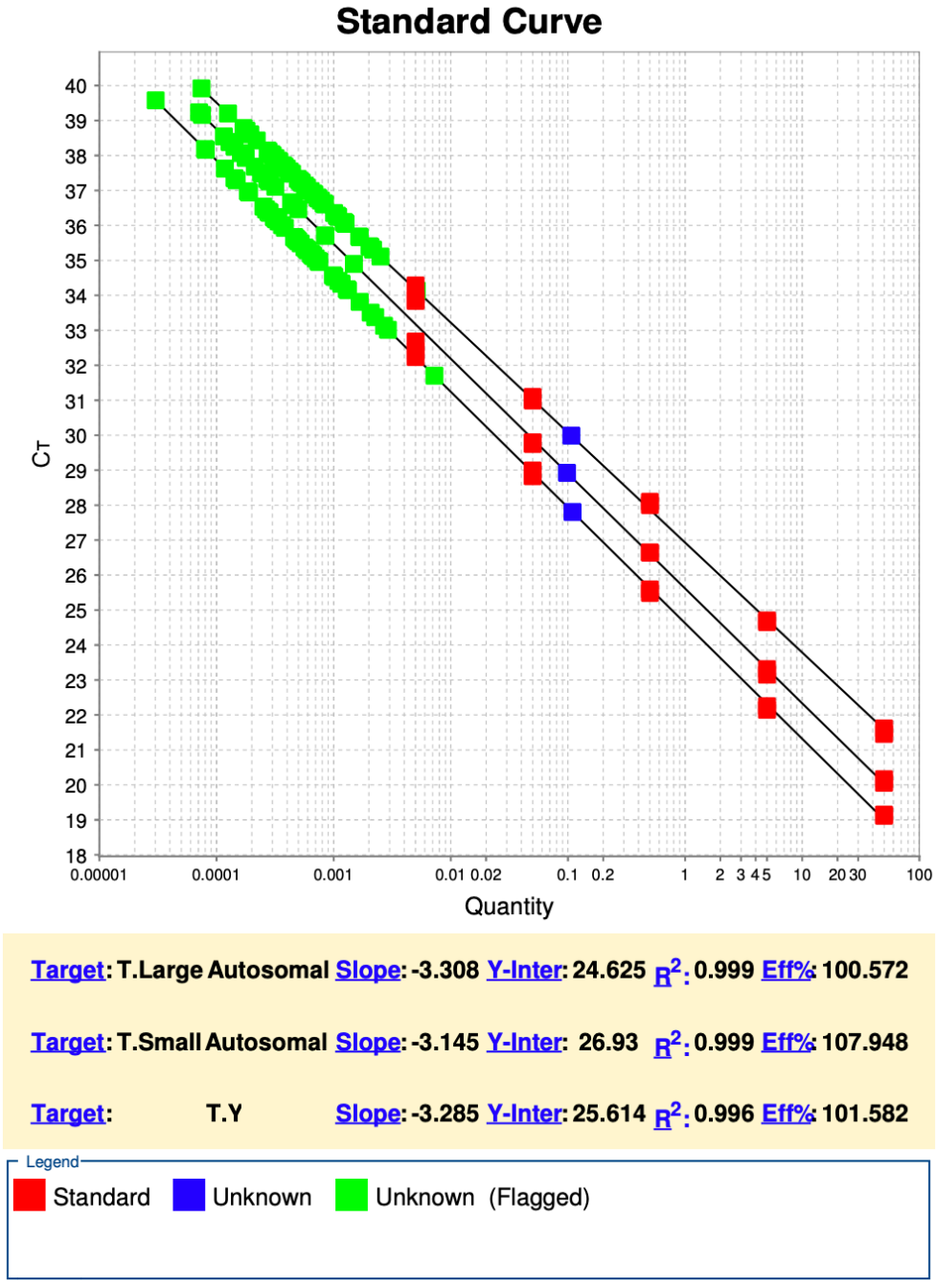


Table 3: Applied Biosystem's Quantifiler™ Trio Data for HemaSpot™-HD *Quantity in ng/µL*

Well	Sample Name	Target Name	Quantity
A1	Trio Standard 1	T.IPC	
A1	Trio Standard 1	T.Large Autosomal	50
A1	Trio Standard 1	T.Small Autosomal	50
A1	Trio Standard 1	T.Y	50
A2	Trio Standard 1	T.IPC	
A2	Trio Standard 1	T.Large Autosomal	50
A2	Trio Standard 1	T.Small Autosomal	50
A2	Trio Standard 1	T.Y	50
A3	HDT11/3A	T.IPC	
A3	HDT11/3A	T.Large Autosomal	0.000740596
A3	HDT11/3A	T.Small Autosomal	0.001243832
A3	HDT11/3A	T.Y	0.000848519
A4	HDT12/3A	T.IPC	
A4	HDT12/3A	T.Large Autosomal	0.000702381
A4	HDT12/3A	T.Small Autosomal	0.001091106
A4	HDT12/3A	T.Y	0.001486187
A5	HDT13/3A	T.IPC	
A5	HDT13/3A	T.Large Autosomal	0.000636531
A5	HDT13/3A	T.Small Autosomal	0.000778293
A5	HDT13/3A	T.Y	0.000842908
A6	HDT11/3B	T.IPC	
A6	HDT11/3B	T.Large Autosomal	0.000383144
A6	HDT11/3B	T.Small Autosomal	0.000421617
A6	HDT11/3B	T.Y	0.000115817
A7	HDT12/3B	T.IPC	
A7	HDT12/3B	T.Large Autosomal	0.001325389
A7	HDT12/3B	T.Small Autosomal	0.001220385
A7	HDT12/3B	T.Y	0.000161161
A8	HDT13/3B	T.IPC	
A8	HDT13/3B	T.Large Autosomal	0.001666958
A8	HDT13/3B	T.Small Autosomal	0.001222537
A8	HDT13/3B	T.Y	
A9	HDT11/3C	T.IPC	
A9	HDT11/3C	T.Large Autosomal	0.000250674
A9	HDT11/3C	T.Small Autosomal	0.00067272
A9	HDT11/3C	T.Y	0.000450394
A10	HDT12/3C	T.IPC	
A10	HDT12/3C	T.Large Autosomal	0.000983873
A10	HDT12/3C	T.Small Autosomal	0.000742539

A10	HDT12/3C	T.Y	0.000458661
A11	HDT13/3C	T.IPC	
A11	HDT13/3C	T.Large Autosomal	0.000337001
A11	HDT13/3C	T.Small Autosomal	0.000535849
A11	HDT13/3C	T.Y	0.000433609
B1	Trio Standard 2	T.IPC	
B1	Trio Standard 2	T.Large Autosomal	5
B1	Trio Standard 2	T.Small Autosomal	5
B1	Trio Standard 2	T.Y	5
B2	Trio Standard 2	T.IPC	
B2	Trio Standard 2	T.Large Autosomal	5
B2	Trio Standard 2	T.Small Autosomal	5
B2	Trio Standard 2	T.Y	5
B3	HDT21/3A	T.IPC	
B3	HDT21/3A	T.Large Autosomal	0.00059933
B3	HDT21/3A	T.Small Autosomal	0.00052915
B3	HDT21/3A	T.Y	0.000142049
B4	HDT22/3A	T.IPC	
B4	HDT22/3A	T.Large Autosomal	0.002691466
B4	HDT22/3A	T.Small Autosomal	0.002055892
B4	HDT22/3A	T.Y	0.000177396
B5	HDT23/3A	T.IPC	
B5	HDT23/3A	T.Large Autosomal	0.000652421
B5	HDT23/3A	T.Small Autosomal	0.001006911
B5	HDT23/3A	T.Y	0.000278651
B6	HDT21/3B	T.IPC	
B6	HDT21/3B	T.Large Autosomal	0.000754191
B6	HDT21/3B	T.Small Autosomal	0.000381703
B6	HDT21/3B	T.Y	
B7	HDT22/3B	T.IPC	
B7	HDT22/3B	T.Large Autosomal	0.000751738
B7	HDT22/3B	T.Small Autosomal	0.000492737
B7	HDT22/3B	T.Y	0.000239707
B8	HDT23/3B	T.IPC	
B8	HDT23/3B	T.Large Autosomal	0.000521882
B8	HDT23/3B	T.Small Autosomal	0.000512612
B8	HDT23/3B	T.Y	
B9	HDT21/3C	T.IPC	
B9	HDT21/3C	T.Large Autosomal	0.000184803
B9	HDT21/3C	T.Small Autosomal	0.00034321

B9	HDT21/3C	T.Y	
B10	HDT22/3C	T.IPC	
B10	HDT22/3C	T.Large Autosomal	0.000308467
B10	HDT22/3C	T.Small Autosomal	0.001065619
B10	HDT22/3C	T.Y	0.000498245
B11	HDT23/3C	T.IPC	
B11	HDT23/3C	T.Large Autosomal	0.000461869
B11	HDT23/3C	T.Small Autosomal	0.000220089
B11	HDT23/3C	T.Y	7.49657E-05
C1	Trio Standard 3	T.IPC	
C1	Trio Standard 3	T.Large Autosomal	0.5
C1	Trio Standard 3	T.Small Autosomal	0.5
C1	Trio Standard 3	T.Y	0.5
C2	Trio Standard 3	T.IPC	
C2	Trio Standard 3	T.Large Autosomal	0.5
C2	Trio Standard 3	T.Small Autosomal	0.5
C2	Trio Standard 3	T.Y	0.5
C3	HDT31/3A	T.IPC	
C3	HDT31/3A	T.Large Autosomal	0.000564214
C3	HDT31/3A	T.Small Autosomal	0.000825537
C3	HDT31/3A	T.Y	
C4	HDT32/3A	T.IPC	
C4	HDT32/3A	T.Large Autosomal	0.000499497
C4	HDT32/3A	T.Small Autosomal	0.000301235
C4	HDT32/3A	T.Y	
C5	HDT33/3A	T.IPC	
C5	HDT33/3A	T.Large Autosomal	0.000283331
C5	HDT33/3A	T.Small Autosomal	0.0005683
C5	HDT33/3A	T.Y	
C6	HDT31/3B	T.IPC	
C6	HDT31/3B	T.Large Autosomal	0.000189066
C6	HDT31/3B	T.Small Autosomal	0.000676288
C6	HDT31/3B	T.Y	0.000270585
C7	HDT32/3B	T.IPC	
C7	HDT32/3B	T.Large Autosomal	0.000362818
C7	HDT32/3B	T.Small Autosomal	0.000397956
C7	HDT32/3B	T.Y	0.000285583
C8	HDT33/3B	T.IPC	
C8	HDT33/3B	T.Large Autosomal	0.000660856
C8	HDT33/3B	T.Small Autosomal	0.000684505

C8	HDT33/3B	T.Y	
C9	HDT31/3C	T.IPC	
C9	HDT31/3C	T.Large Autosomal	0.000461173
C9	HDT31/3C	T.Small Autosomal	0.000337868
C9	HDT31/3C	T.Y	
C10	HDT32/3C	T.IPC	
C10	HDT32/3C	T.Large Autosomal	
C10	HDT32/3C	T.Small Autosomal	0.000125247
C10	HDT32/3C	T.Y	
C11	HDT33/3C	T.IPC	
C11	HDT33/3C	T.Large Autosomal	3.0163E-05
C11	HDT33/3C	T.Small Autosomal	
C11	HDT33/3C	T.Y	0.00028194
D1	Trio Standard 4	T.IPC	
D1	Trio Standard 4	T.Large Autosomal	0.050000001
D1	Trio Standard 4	T.Small Autosomal	0.050000001
D1	Trio Standard 4	T.Y	0.050000001
D2	Trio Standard 4	T.IPC	
D2	Trio Standard 4	T.Large Autosomal	0.050000001
D2	Trio Standard 4	T.Small Autosomal	0.050000001
D2	Trio Standard 4	T.Y	0.050000001
D3	HDT41/3A	T.IPC	
D3	HDT41/3A	T.Large Autosomal	0.000585037
D3	HDT41/3A	T.Small Autosomal	0.000730078
D3	HDT41/3A	T.Y	
D4	HDT42/3A	T.IPC	
D4	HDT42/3A	T.Large Autosomal	0.000264871
D4	HDT42/3A	T.Small Autosomal	0.000194055
D4	HDT42/3A	T.Y	7.10918E-05
D5	HDT43/3A	T.IPC	
D5	HDT43/3A	T.Large Autosomal	0.000485065
D5	HDT43/3A	T.Small Autosomal	0.000180066
D5	HDT43/3A	T.Y	0.000317543
D6	HDT41/3B	T.IPC	
D6	HDT41/3B	T.Large Autosomal	0.002064943
D6	HDT41/3B	T.Small Autosomal	0.001666567
D6	HDT41/3B	T.Y	
D7	HDT42/3B	T.IPC	
D7	HDT42/3B	T.Large Autosomal	0.002260489
D7	HDT42/3B	T.Small Autosomal	0.002166399

D7	HDT42/3B	T.Y	
D8	HDT43/3B	T.IPC	
D8	HDT43/3B	T.Large Autosomal	0.000670424
D8	HDT43/3B	T.Small Autosomal	0.00082378
D8	HDT43/3B	T.Y	
D9	HDT41/3C	T.IPC	
D9	HDT41/3C	T.Large Autosomal	0.00074445
D9	HDT41/3C	T.Small Autosomal	0.00027267
D9	HDT41/3C	T.Y	
D10	HDT42/3C	T.IPC	
D10	HDT42/3C	T.Large Autosomal	8.08341E-05
D10	HDT42/3C	T.Small Autosomal	
D10	HDT42/3C	T.Y	
D11	HDT43/3C	T.IPC	
D11	HDT43/3C	T.Large Autosomal	0.00101456
D11	HDT43/3C	T.Small Autosomal	0.000636696
D11	HDT43/3C	T.Y	
E1	Trio Standard 5	T.IPC	
E1	Trio Standard 5	T.Large Autosomal	0.005
E1	Trio Standard 5	T.Small Autosomal	0.005
E1	Trio Standard 5	T.Y	0.005
E2	Trio Standard 5	T.IPC	
E2	Trio Standard 5	T.Large Autosomal	0.005
E2	Trio Standard 5	T.Small Autosomal	0.005
E2	Trio Standard 5	T.Y	0.005
E3	HDT51/3A	T.IPC	
E3	HDT51/3A	T.Large Autosomal	0.000321614
E3	HDT51/3A	T.Small Autosomal	0.000843551
E3	HDT51/3A	T.Y	
E4	HDT52/3A	T.IPC	
E4	HDT52/3A	T.Large Autosomal	0.001098776
E4	HDT52/3A	T.Small Autosomal	0.00250664
E4	HDT52/3A	T.Y	0.000243062
E5	HDT53/3A	T.IPC	
E5	HDT53/3A	T.Large Autosomal	0.000277521
E5	HDT53/3A	T.Small Autosomal	0.000371353
E5	HDT53/3A	T.Y	
E6	HDT51/3B	T.IPC	
E6	HDT51/3B	T.Large Autosomal	0.000142098
E6	HDT51/3B	T.Small Autosomal	

E6	HDT51/3B	T.Y	
E7	HDT52/3B	T.IPC	
E7	HDT52/3B	T.Large Autosomal	0.00031763
E7	HDT52/3B	T.Small Autosomal	
E7	HDT52/3B	T.Y	
E8	HDT53/3B	T.IPC	
E8	HDT53/3B	T.Large Autosomal	0.000279739
E8	HDT53/3B	T.Small Autosomal	0.000170057
E8	HDT53/3B	T.Y	0.000211823
E9	HDT51/3C	T.IPC	
E9	HDT51/3C	T.Large Autosomal	0.000117917
E9	HDT51/3C	T.Small Autosomal	0.000277617
E9	HDT51/3C	T.Y	
E10	HDT52/3C	T.IPC	
E10	HDT52/3C	T.Large Autosomal	0.000148526
E10	HDT52/3C	T.Small Autosomal	0.000440938
E10	HDT52/3C	T.Y	
E11	HDT53/3C	T.IPC	
E11	HDT53/3C	T.Large Autosomal	7.96684E-05
E11	HDT53/3C	T.Small Autosomal	0.000293225
E11	HDT53/3C	T.Y	
F1	POS	T.IPC	
F1	POS	T.Large Autosomal	0.109335981
F1	POS	T.Small Autosomal	0.106675453
F1	POS	T.Y	0.098080114
F2	NTC	T.IPC	
F2	NTC	T.Large Autosomal	
F2	NTC	T.Small Autosomal	
F2	NTC	T.Y	
F3	HDT61/3A	T.IPC	
F3	HDT61/3A	T.Large Autosomal	0.001165393
F3	HDT61/3A	T.Small Autosomal	0.001262912
F3	HDT61/3A	T.Y	
F4	HDT62/3A	T.IPC	
F4	HDT62/3A	T.Large Autosomal	0.001296126
F4	HDT62/3A	T.Small Autosomal	0.001647602
F4	HDT62/3A	T.Y	
F5	HDT63/3A	T.IPC	
F5	HDT63/3A	T.Large Autosomal	0.002905103
F5	HDT63/3A	T.Small Autosomal	0.002016304

F5	HDT63/3A	T.Y	
F6	HDT61/3B	T.IPC	
F6	HDT61/3B	T.Large Autosomal	0.000476076
F6	HDT61/3B	T.Small Autosomal	0.000339886
F6	HDT61/3B	T.Y	0.000129695
F7	HDT62/3B	T.IPC	
F7	HDT62/3B	T.Large Autosomal	
F7	HDT62/3B	T.Small Autosomal	0.000589349
F7	HDT62/3B	T.Y	
F8	HDT63/3B	T.IPC	
F8	HDT63/3B	T.Large Autosomal	0.00116029
F8	HDT63/3B	T.Small Autosomal	7.42464E-05
F8	HDT63/3B	T.Y	
F9	HDT61/3C	T.IPC	
F9	HDT61/3C	T.Large Autosomal	0.007244119
F9	HDT61/3C	T.Small Autosomal	0.005123559
F9	HDT61/3C	T.Y	0.000129295
F10	HDT62/3C	T.IPC	
F10	HDT62/3C	T.Large Autosomal	
F10	HDT62/3C	T.Small Autosomal	
F10	HDT62/3C	T.Y	
F11	HDT63/3C	T.IPC	
F11	HDT63/3C	T.Large Autosomal	0.00030823
F11	HDT63/3C	T.Small Autosomal	0.000319795
F11	HDT63/3C	T.Y	

Figure 3: Applied Biosystem's Quantifiler™ Trio Data for HemaSpot™-HF. The following image depicts the standard curve for the HemaSpot™-HF samples of the hypersensitivity study. A correlation coefficient value (R^2) of at least 0.99 is required for further analysis. As shown, all criteria were met, and PCR was initiated.

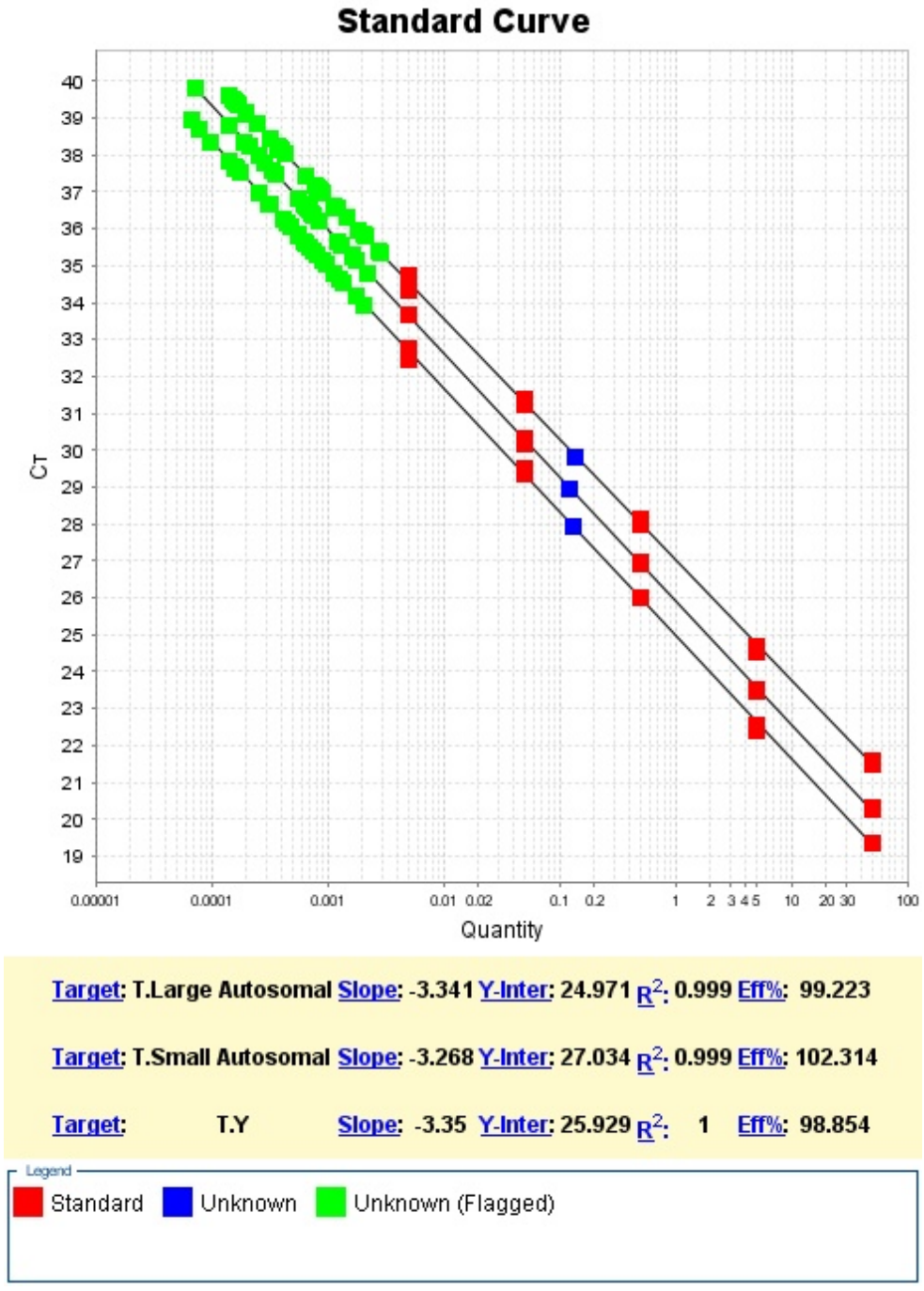


Table 4: Applied Biosystem's Quantifiler™ Trio Data for HemaSpot™-HF *Quantity in ng/µL*

Well	Sample Name	Target Name	Quantity
A1	Trio Standard 1	T.IPC	
A1	Trio Standard 1	T.Large Autosomal	50
A1	Trio Standard 1	T.Small Autosomal	50
A1	Trio Standard 1	T.Y	50
A2	Trio Standard 1	T.IPC	
A2	Trio Standard 1	T.Large Autosomal	50
A2	Trio Standard 1	T.Small Autosomal	50
A2	Trio Standard 1	T.Y	50
A3	HFT11.3A	T.IPC	
A3	HFT11.3A	T.Large Autosomal	0.001136588
A3	HFT11.3A	T.Small Autosomal	0.001200811
A3	HFT11.3A	T.Y	0.001623702
A4	HFT12.3A	T.IPC	
A4	HFT12.3A	T.Large Autosomal	0.001380192
A4	HFT12.3A	T.Small Autosomal	0.002024024
A4	HFT12.3A	T.Y	0.000643397
A5	HFT13.3A	T.IPC	
A5	HFT13.3A	T.Large Autosomal	0.001377908
A5	HFT13.3A	T.Small Autosomal	0.001226699
A5	HFT13.3A	T.Y	0.000731171
A6	HFT11.3B	T.IPC	
A6	HFT11.3B	T.Large Autosomal	0.000964438
A6	HFT11.3B	T.Small Autosomal	0.002784677
A6	HFT11.3B	T.Y	0.002251043
A7	HFT12.3B	T.IPC	
A7	HFT12.3B	T.Large Autosomal	0.00075143
A7	HFT12.3B	T.Small Autosomal	0.001833491
A7	HFT12.3B	T.Y	0.001792956
A8	HFT13.3B	T.IPC	
A8	HFT13.3B	T.Large Autosomal	0.000902303
A8	HFT13.3B	T.Small Autosomal	0.002021327
A8	HFT13.3B	T.Y	0.001336516
A9	HFT11.3C	T.IPC	
A9	HFT11.3C	T.Large Autosomal	0.002063104
A9	HFT11.3C	T.Small Autosomal	0.002896376
A9	HFT11.3C	T.Y	0.001244735
A10	HFT12.3C	T.IPC	
A10	HFT12.3C	T.Large Autosomal	0.000968847
A10	HFT12.3C	T.Small Autosomal	0.001447101

A10	HFT12.3C	T.Y	0.000852071
A11	HFT13.3C	T.IPC	
A11	HFT13.3C	T.Large Autosomal	0.00175439
A11	HFT13.3C	T.Small Autosomal	0.002102197
A11	HFT13.3C	T.Y	0.001668819
A12	HFRB1.2QIAamp	T.IPC	
A12	HFRB1.2QIAamp	T.Large Autosomal	
A12	HFRB1.2QIAamp	T.Small Autosomal	
A12	HFRB1.2QIAamp	T.Y	
B1	Trio Standard 2	T.IPC	
B1	Trio Standard 2	T.Large Autosomal	5
B1	Trio Standard 2	T.Small Autosomal	5
B1	Trio Standard 2	T.Y	5
B2	Trio Standard 2	T.IPC	
B2	Trio Standard 2	T.Large Autosomal	5
B2	Trio Standard 2	T.Small Autosomal	5
B2	Trio Standard 2	T.Y	5
B3	HFT21.3A	T.IPC	
B3	HFT21.3A	T.Large Autosomal	0.000656728
B3	HFT21.3A	T.Small Autosomal	0.000432521
B3	HFT21.3A	T.Y	0.000194274
B4	HFT22.3A	T.IPC	
B4	HFT22.3A	T.Large Autosomal	0.000315062
B4	HFT22.3A	T.Small Autosomal	0.000858886
B4	HFT22.3A	T.Y	0.000554364
B5	HFT23.3A	T.IPC	
B5	HFT23.3A	T.Large Autosomal	0.000475724
B5	HFT23.3A	T.Small Autosomal	0.000784484
B5	HFT23.3A	T.Y	0.000553956
B6	HFT21.3B	T.IPC	
B6	HFT21.3B	T.Large Autosomal	0.001259938
B6	HFT21.3B	T.Small Autosomal	0.001865958
B6	HFT21.3B	T.Y	0.000255785
B7	HFT22.3B	T.IPC	
B7	HFT22.3B	T.Large Autosomal	0.000564086
B7	HFT22.3B	T.Small Autosomal	0.000906144
B7	HFT22.3B	T.Y	0.000358934
B8	HFT23.3B	T.IPC	
B8	HFT23.3B	T.Large Autosomal	0.000809898
B8	HFT23.3B	T.Small Autosomal	0.001213391

B8	HFT23.3B	T.Y	0.000142391
B9	HFT21.3C	T.IPC	
B9	HFT21.3C	T.Large Autosomal	0.000477826
B9	HFT21.3C	T.Small Autosomal	0.000804798
B9	HFT21.3C	T.Y	0.000764458
B10	HFT22.3C	T.IPC	
B10	HFT22.3C	T.Large Autosomal	0.000319424
B10	HFT22.3C	T.Small Autosomal	0.00088689
B10	HFT22.3C	T.Y	0.000663204
B11	HFT23.3C	T.IPC	
B11	HFT23.3C	T.Large Autosomal	0.000554809
B11	HFT23.3C	T.Small Autosomal	0.000652522
B11	HFT23.3C	T.Y	0.000617787
B12			
C1	Trio Standard 3	T.IPC	
C1	Trio Standard 3	T.Large Autosomal	0.5
C1	Trio Standard 3	T.Small Autosomal	0.5
C1	Trio Standard 3	T.Y	0.5
C2	Trio Standard 3	T.IPC	
C2	Trio Standard 3	T.Large Autosomal	0.5
C2	Trio Standard 3	T.Small Autosomal	0.5
C2	Trio Standard 3	T.Y	0.5
C3	HFT31.3A	T.IPC	
C3	HFT31.3A	T.Large Autosomal	6.65066E-05
C3	HFT31.3A	T.Small Autosomal	0.000321228
C3	HFT31.3A	T.Y	
C4	HFT32.3A	T.IPC	
C4	HFT32.3A	T.Large Autosomal	0.000140799
C4	HFT32.3A	T.Small Autosomal	0.000387666
C4	HFT32.3A	T.Y	
C5	HFT33.3A	T.IPC	
C5	HFT33.3A	T.Large Autosomal	0.000413001
C5	HFT33.3A	T.Small Autosomal	0.00078756
C5	HFT33.3A	T.Y	7.24859E-05
C6	HFT31.3B	T.IPC	
C6	HFT31.3B	T.Large Autosomal	0.000256875
C6	HFT31.3B	T.Small Autosomal	0.000385076
C6	HFT31.3B	T.Y	0.000290484
C7	HFT32.3B	T.IPC	
C7	HFT32.3B	T.Large Autosomal	0.000694593

C7	HFT32.3B	T.Small Autosomal	0.000143163
C7	HFT32.3B	T.Y	
C8	HFT33.3B	T.IPC	
C8	HFT33.3B	T.Large Autosomal	0.000452375
C8	HFT33.3B	T.Small Autosomal	0.000369423
C8	HFT33.3B	T.Y	0.000689972
C9	HFT31.3C	T.IPC	
C9	HFT31.3C	T.Large Autosomal	7.84994E-05
C9	HFT31.3C	T.Small Autosomal	0.000194709
C9	HFT31.3C	T.Y	
C10	HFT32.3C	T.IPC	
C10	HFT32.3C	T.Large Autosomal	
C10	HFT32.3C	T.Small Autosomal	0.000154273
C10	HFT32.3C	T.Y	
C11	HFT33.3C	T.IPC	
C11	HFT33.3C	T.Large Autosomal	0.000155976
C11	HFT33.3C	T.Small Autosomal	
C11	HFT33.3C	T.Y	0.000333895
C12			
D1	Trio Standard 4	T.IPC	
D1	Trio Standard 4	T.Large Autosomal	0.050000001
D1	Trio Standard 4	T.Small Autosomal	0.050000001
D1	Trio Standard 4	T.Y	0.050000001
D2	Trio Standard 4	T.IPC	
D2	Trio Standard 4	T.Large Autosomal	0.050000001
D2	Trio Standard 4	T.Small Autosomal	0.050000001
D2	Trio Standard 4	T.Y	0.050000001
D3	HFT41.3A	T.IPC	
D3	HFT41.3A	T.Large Autosomal	0.000163149
D3	HFT41.3A	T.Small Autosomal	
D3	HFT41.3A	T.Y	
D4	HFT42.3A	T.IPC	
D4	HFT42.3A	T.Large Autosomal	0.000635743
D4	HFT42.3A	T.Small Autosomal	0.001149059
D4	HFT42.3A	T.Y	
D5	HFT43.3A	T.IPC	
D5	HFT43.3A	T.Large Autosomal	
D5	HFT43.3A	T.Small Autosomal	
D5	HFT43.3A	T.Y	
D6	HFT41.3B	T.IPC	

D6	HFT41.3B	T.Large Autosomal	
D6	HFT41.3B	T.Small Autosomal	
D6	HFT41.3B	T.Y	
D7	HFT42.3B	T.IPC	
D7	HFT42.3B	T.Large Autosomal	0.000175246
D7	HFT42.3B	T.Small Autosomal	
D7	HFT42.3B	T.Y	
D8	HFT43.3B	T.IPC	
D8	HFT43.3B	T.Large Autosomal	
D8	HFT43.3B	T.Small Autosomal	
D8	HFT43.3B	T.Y	
D9	HFT41.3C	T.IPC	
D9	HFT41.3C	T.Large Autosomal	
D9	HFT41.3C	T.Small Autosomal	0.000170048
D9	HFT41.3C	T.Y	0.000213038
D10	HFT42.3C	T.IPC	
D10	HFT42.3C	T.Large Autosomal	
D10	HFT42.3C	T.Small Autosomal	
D10	HFT42.3C	T.Y	
D11	HFT43.3C	T.IPC	
D11	HFT43.3C	T.Large Autosomal	
D11	HFT43.3C	T.Small Autosomal	0.000402507
D11	HFT43.3C	T.Y	
D12	HFRB2.2QIAamp	T.IPC	
D12	HFRB2.2QIAamp	T.Large Autosomal	
D12	HFRB2.2QIAamp	T.Small Autosomal	
D12	HFRB2.2QIAamp	T.Y	
E1	Trio Standard 5	T.IPC	
E1	Trio Standard 5	T.Large Autosomal	0.005
E1	Trio Standard 5	T.Small Autosomal	0.005
E1	Trio Standard 5	T.Y	0.005
E2	Trio Standard 5	T.IPC	
E2	Trio Standard 5	T.Large Autosomal	0.005
E2	Trio Standard 5	T.Small Autosomal	0.005
E2	Trio Standard 5	T.Y	0.005
E3	HFT51.3A	T.IPC	
E3	HFT51.3A	T.Large Autosomal	
E3	HFT51.3A	T.Small Autosomal	
E3	HFT51.3A	T.Y	
E4	HFT52.3A	T.IPC	

E4	HFT52.3A	T.Large Autosomal	0.000161295
E4	HFT52.3A	T.Small Autosomal	0.000245082
E4	HFT52.3A	T.Y	
E5	HFT53.3A	T.IPC	
E5	HFT53.3A	T.Large Autosomal	
E5	HFT53.3A	T.Small Autosomal	
E5	HFT53.3A	T.Y	
E6	HFT51.3B	T.IPC	
E6	HFT51.3B	T.Large Autosomal	
E6	HFT51.3B	T.Small Autosomal	
E6	HFT51.3B	T.Y	
E7	HFT52.3B	T.IPC	
E7	HFT52.3B	T.Large Autosomal	
E7	HFT52.3B	T.Small Autosomal	
E7	HFT52.3B	T.Y	
E8	HFT53.3B	T.IPC	
E8	HFT53.3B	T.Large Autosomal	9.85005E-05
E8	HFT53.3B	T.Small Autosomal	
E8	HFT53.3B	T.Y	
E9	HFT51.3C	T.IPC	
E9	HFT51.3C	T.Large Autosomal	0.00016492
E9	HFT51.3C	T.Small Autosomal	0.000199095
E9	HFT51.3C	T.Y	
E10	HFT52.3C	T.IPC	
E10	HFT52.3C	T.Large Autosomal	
E10	HFT52.3C	T.Small Autosomal	
E10	HFT52.3C	T.Y	
E11	HFT53.3C	T.IPC	
E11	HFT53.3C	T.Large Autosomal	
E11	HFT53.3C	T.Small Autosomal	
E11	HFT53.3C	T.Y	
E12			
F1	Pos	T.IPC	
F1	Pos	T.Large Autosomal	0.131346822
F1	Pos	T.Small Autosomal	0.138984561
F1	Pos	T.Y	0.124696329
F2	NTC	T.IPC	
F2	NTC	T.Large Autosomal	
F2	NTC	T.Small Autosomal	
F2	NTC	T.Y	

F3	HFT61.3A	T.IPC	
F3	HFT61.3A	T.Large Autosomal	
F3	HFT61.3A	T.Small Autosomal	0.000168452
F3	HFT61.3A	T.Y	
F4	HFT62.3A	T.IPC	
F4	HFT62.3A	T.Large Autosomal	
F4	HFT62.3A	T.Small Autosomal	
F4	HFT62.3A	T.Y	
F5	HFT63.3A	T.IPC	
F5	HFT63.3A	T.Large Autosomal	
F5	HFT63.3A	T.Small Autosomal	
F5	HFT63.3A	T.Y	
F6	HFT61.3B	T.IPC	
F6	HFT61.3B	T.Large Autosomal	
F6	HFT61.3B	T.Small Autosomal	0.000164083
F6	HFT61.3B	T.Y	
F7	HFT62.3B	T.IPC	
F7	HFT62.3B	T.Large Autosomal	
F7	HFT62.3B	T.Small Autosomal	0.000157765
F7	HFT62.3B	T.Y	
F8	HFT63.3B	T.IPC	
F8	HFT63.3B	T.Large Autosomal	
F8	HFT63.3B	T.Small Autosomal	
F8	HFT63.3B	T.Y	
F9	HFT61.3C	T.IPC	
F9	HFT61.3C	T.Large Autosomal	
F9	HFT61.3C	T.Small Autosomal	
F9	HFT61.3C	T.Y	
F10	HFT62.3C	T.IPC	
F10	HFT62.3C	T.Large Autosomal	
F10	HFT62.3C	T.Small Autosomal	
F10	HFT62.3C	T.Y	
F11	HFT63.3C	T.IPC	
F11	HFT63.3C	T.Large Autosomal	
F11	HFT63.3C	T.Small Autosomal	
F11	HFT63.3C	T.Y	
F12			

Figure 4: Applied Biosystem's Quantifiler™ Trio Data for Trace Study. The following image depicts the standard curve for the Trace Study samples. A correlation coefficient value (R^2) of at least 0.99 is required for further analysis. As shown, all criteria were met, and PCR was initiated.

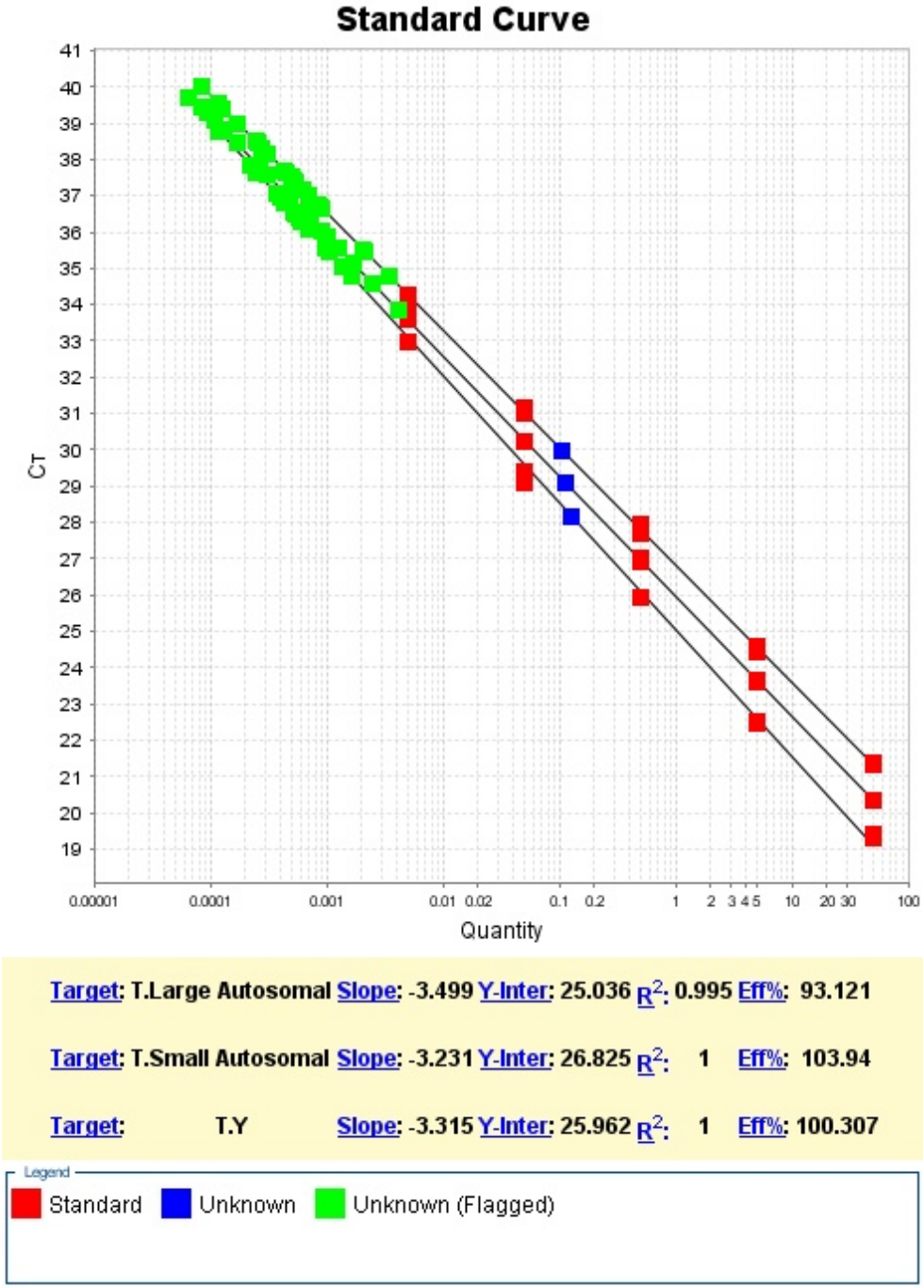


Table 5: Applied Biosystem's Quantifiler™ Trio Data for Trace Study *Quantity in ng/μL*

Well	Sample Name	Target Name	Quantity
A1	Trio Standard 1	T.IPC	
A1	Trio Standard 1	T.Large Autosomal	50
A1	Trio Standard 1	T.Small Autosomal	50
A1	Trio Standard 1	T.Y	50
A2	Trio Standard 1	T.IPC	
A2	Trio Standard 1	T.Large Autosomal	50
A2	Trio Standard 1	T.Small Autosomal	50
A2	Trio Standard 1	T.Y	50
A3	Swab1.3Wheel	T.IPC	
A3	Swab1.3Wheel	T.Large Autosomal	0.000116791
A3	Swab1.3Wheel	T.Small Autosomal	0.000254681
A3	Swab1.3Wheel	T.Y	0.000913862
A4	Swab2.3Wheel	T.IPC	
A4	Swab2.3Wheel	T.Large Autosomal	0.000534276
A4	Swab2.3Wheel	T.Small Autosomal	0.000635375
A4	Swab2.3Wheel	T.Y	0.000310339
A5	Swab3.3Wheel	T.IPC	
A5	Swab3.3Wheel	T.Large Autosomal	0.000396045
A5	Swab3.3Wheel	T.Small Autosomal	0.000172032
A5	Swab3.3Wheel	T.Y	
A6	HF1.3Wheel	T.IPC	
A6	HF1.3Wheel	T.Large Autosomal	0.001357953
A6	HF1.3Wheel	T.Small Autosomal	0.000691989
A6	HF1.3Wheel	T.Y	0.0017235
A7	HF2.3Wheel	T.IPC	
A7	HF2.3Wheel	T.Large Autosomal	
A7	HF2.3Wheel	T.Small Autosomal	
A7	HF2.3Wheel	T.Y	
A8	HF3.3Wheel	T.IPC	
A8	HF3.3Wheel	T.Large Autosomal	
A8	HF3.3Wheel	T.Small Autosomal	0.000494053
A8	HF3.3Wheel	T.Y	
A9	HD1.3Wheel	T.IPC	
A9	HD1.3Wheel	T.Large Autosomal	0.00024854
A9	HD1.3Wheel	T.Small Autosomal	0.002107144
A9	HD1.3Wheel	T.Y	0.00250611
A10	HD2.3Wheel	T.IPC	
A10	HD2.3Wheel	T.Large Autosomal	0.001653886
A10	HD2.3Wheel	T.Small Autosomal	0.003400147

A10	HD2.3Wheel	T.Y	0.004200705
A11	HD3.3Wheel	T.IPC	
A11	HD3.3Wheel	T.Large Autosomal	0.001057394
A11	HD3.3Wheel	T.Small Autosomal	0.002041709
A11	HD3.3Wheel	T.Y	0.00100617
A12	Part2RB	T.IPC	
A12	Part2RB	T.Large Autosomal	
A12	Part2RB	T.Small Autosomal	
A12	Part2RB	T.Y	
B1	Trio Standard 2	T.IPC	
B1	Trio Standard 2	T.Large Autosomal	5
B1	Trio Standard 2	T.Small Autosomal	5
B1	Trio Standard 2	T.Y	5
B2	Trio Standard 2	T.IPC	
B2	Trio Standard 2	T.Large Autosomal	5
B2	Trio Standard 2	T.Small Autosomal	5
B2	Trio Standard 2	T.Y	5
B3	Swab1.3Knife	T.IPC	
B3	Swab1.3Knife	T.Large Autosomal	
B3	Swab1.3Knife	T.Small Autosomal	
B3	Swab1.3Knife	T.Y	
B4	Swab2.3Knife	T.IPC	
B4	Swab2.3Knife	T.Large Autosomal	
B4	Swab2.3Knife	T.Small Autosomal	0.000119823
B4	Swab2.3Knife	T.Y	0.000130754
B5	Swab3.3Knife	T.IPC	
B5	Swab3.3Knife	T.Large Autosomal	0.000572533
B5	Swab3.3Knife	T.Small Autosomal	0.000243998
B5	Swab3.3Knife	T.Y	9.47606E-05
B6	HF1.3Knife	T.IPC	
B6	HF1.3Knife	T.Large Autosomal	
B6	HF1.3Knife	T.Small Autosomal	0.000436653
B6	HF1.3Knife	T.Y	0.000471568
B7	HF2.3Knife	T.IPC	
B7	HF2.3Knife	T.Large Autosomal	0.00021756
B7	HF2.3Knife	T.Small Autosomal	0.000307963
B7	HF2.3Knife	T.Y	
B8	HF3.3Knife	T.IPC	
B8	HF3.3Knife	T.Large Autosomal	
B8	HF3.3Knife	T.Small Autosomal	

B8	HF3.3Knife	T.Y	
B9	HD1.3Knife	T.IPC	
B9	HD1.3Knife	T.Large Autosomal	0.000594726
B9	HD1.3Knife	T.Small Autosomal	0.000536533
B9	HD1.3Knife	T.Y	0.00047517
B10	HD2.3Knife	T.IPC	
B10	HD2.3Knife	T.Large Autosomal	0.000434877
B10	HD2.3Knife	T.Small Autosomal	0.000455816
B10	HD2.3Knife	T.Y	0.000171463
B11	HD3.3Knife	T.IPC	
B11	HD3.3Knife	T.Large Autosomal	
B11	HD3.3Knife	T.Small Autosomal	8.3847E-05
B11	HD3.3Knife	T.Y	0.000271513
B12			
C1	Trio Standard 3	T.IPC	
C1	Trio Standard 3	T.Large Autosomal	0.5
C1	Trio Standard 3	T.Small Autosomal	0.5
C1	Trio Standard 3	T.Y	0.5
C2	Trio Standard 3	T.IPC	
C2	Trio Standard 3	T.Large Autosomal	0.5
C2	Trio Standard 3	T.Small Autosomal	0.5
C2	Trio Standard 3	T.Y	0.5
C3	Swab1.3Bullet	T.IPC	
C3	Swab1.3Bullet	T.Large Autosomal	
C3	Swab1.3Bullet	T.Small Autosomal	0.000116931
C3	Swab1.3Bullet	T.Y	0.000108217
C4	Swab2.3Bullet	T.IPC	
C4	Swab2.3Bullet	T.Large Autosomal	0.000369235
C4	Swab2.3Bullet	T.Small Autosomal	
C4	Swab2.3Bullet	T.Y	
C5	Swab3.3Bullet	T.IPC	
C5	Swab3.3Bullet	T.Large Autosomal	
C5	Swab3.3Bullet	T.Small Autosomal	0.00012683
C5	Swab3.3Bullet	T.Y	0.00044262
C6	HF1.3Bullet	T.IPC	
C6	HF1.3Bullet	T.Large Autosomal	0.000221496
C6	HF1.3Bullet	T.Small Autosomal	0.000517808
C6	HF1.3Bullet	T.Y	8.52205E-05
C7	HF2.3Bullet	T.IPC	
C7	HF2.3Bullet	T.Large Autosomal	0.000700742

C7	HF2.3Bullet	T.Small Autosomal	0.000853951
C7	HF2.3Bullet	T.Y	0.000652918
C8	HF3.3Bullet	T.IPC	
C8	HF3.3Bullet	T.Large Autosomal	6.42449E-05
C8	HF3.3Bullet	T.Small Autosomal	0.000279534
C8	HF3.3Bullet	T.Y	0.000722997
C9	HD1.3Bullet	T.IPC	
C9	HD1.3Bullet	T.Large Autosomal	0.000991099
C9	HD1.3Bullet	T.Small Autosomal	0.000901344
C9	HD1.3Bullet	T.Y	0.000714907
C10	HD2.3Bullet	T.IPC	
C10	HD2.3Bullet	T.Large Autosomal	0.000511718
C10	HD2.3Bullet	T.Small Autosomal	0.00087667
C10	HD2.3Bullet	T.Y	0.001286827
C11	HD3.3Bullet	T.IPC	
C11	HD3.3Bullet	T.Large Autosomal	0.000600243
C11	HD3.3Bullet	T.Small Autosomal	0.00088612
C11	HD3.3Bullet	T.Y	0.001689915
C12			
D1	Trio Standard 4	T.IPC	
D1	Trio Standard 4	T.Large Autosomal	0.050000001
D1	Trio Standard 4	T.Small Autosomal	0.050000001
D1	Trio Standard 4	T.Y	0.050000001
D2	Trio Standard 4	T.IPC	
D2	Trio Standard 4	T.Large Autosomal	0.050000001
D2	Trio Standard 4	T.Small Autosomal	0.050000001
D2	Trio Standard 4	T.Y	0.050000001
D3			
D4			
D5			
D6			
D7			
D8			
D9			
D10			
D11			
D12			
E1	Trio Standard 5	T.IPC	
E1	Trio Standard 5	T.Large Autosomal	0.005
E1	Trio Standard 5	T.Small Autosomal	0.005

E1	Trio Standard 5	T.Y	0.005
E2	Trio Standard 5	T.IPC	
E2	Trio Standard 5	T.Large Autosomal	0.005
E2	Trio Standard 5	T.Small Autosomal	0.005
E2	Trio Standard 5	T.Y	0.005
E3			
E4			
E5			
E6			
E7			
E8			
E9			
E10			
E11			
E12			
F1	Pos	T.IPC	
F1	Pos	T.Large Autosomal	0.12759088
F1	Pos	T.Small Autosomal	0.106099904
F1	Pos	T.Y	0.113950916
F2	NTC	T.IPC	
F2	NTC	T.Large Autosomal	
F2	NTC	T.Small Autosomal	
F2	NTC	T.Y	

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