LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

LLNL-TR-659969

Analysis of cellular and extracellular DNA in fingerprints

J. M. Button

September 9, 2014

Disclaimer

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

Analysis of cellular and extracellular DNA in fingerprints

Julie Button

Office of Science, Science Undergraduate Laboratory Internship (SULI)

West Virginia University

Lawrence Livermore National Laboratory

Livermore, CA

September 5, 2014

Prepared in partial fulfillment of the requirements of the Office of Science, Department of Energy's Science Undergraduate Laboratory Internship under the direction of M. Shusteff in the Materials Engineering Division at Lawrence Livermore National Laboratory.

TABLE OF CONTENTS

ABSTRACT

It has been previously shown that DNA can be recovered from latent fingerprints left on various surfaces [R. A. H. van Oorschot and M. K. Jones, Nature 387, 767 (1997)]. However, the source of the DNA, extracellular versus cellular origin, is difficult to determine. If the DNA is cellular, it is believed to belong to skin cells while extracellular DNA is believed to originate from body fluids such as sweat [D. J. Daly *et. al*, Forensic Sci. Int. Genet. 6, 41-46 (2012); V. V. Vlassov *et. al*, BioEssays 29, 654-667 (2007)]. The origin of the DNA in fingerprints has implications for processing and interpretation of forensic evidence. The determination of the origin of DNA in fingerprints is further complicated by the fact that the DNA in fingerprints tends to be at a very low quantity [R. A. H. van Oorschot and M. K. Jones, Nature 387, 767 (1997)]. This study examined fingerprints from five volunteers left on sterilized glass slides and plastic pens. Three fingerprints were left on each glass slide (thumb, index, and middle fingers) while the pens were held as if one was writing with them. The DNA was collected from the objects using the wet swabbing technique (TE buffer). Following collection, the cellular and extracellular components of each sample were separated using centrifugation and an acoustofluidics system. Centrifugation is still the primary separation technique utilized in forensics laboratories, while acoustic focusing uses sound waves to focus large particles (cells) into low pressure nodes, separating them from the rest of the sample matrix. After separation, all samples were quantified using real-time quantitative PCR (qPCR). The overall trend is that there is more DNA in the extracellular fractions than cellular fractions for both centrifugation and acoustofluidic processing. Additionally, more DNA was generally collected from the pen samples than the samples left on glass slides.

INTRODUCTION

Research has demonstrated that DNA can be obtained from latent fingerprints, which could aid in forensics investigations.[1] The quantity of DNA is typically low,[1] which requires analysts to use very efficient methods of collection and processing, as well as be very cognizant of sources of contamination. Although it has been proven that DNA can be collected from fingerprints, it has not been shown what the source of that DNA is. There are two possible sources for the DNA in fingerprints. First, if the DNA is cellular in origin, it is likely from sloughed off epithelial cells deposited when an individual touches a surface.[2] Second, if the DNA is extracellular in origin it is likely from cell free DNA contained in body fluids.[3] It has been shown that there is cell free DNA in many of our body fluids,[3] and the body fluids that the hands come in contact with the most are sweat[4] and saliva, making them good candidates for this type of DNA.[5] The goal of this research project was to determine the source of the DNA in fingerprints using two different processing methods. Centrifugation was used as a baseline separation method for comparison, as it is a method still commonly utilized in forensics laboratories. Additionally, this research project tested whether an acoustofluidics system can be used to separate the cellular and extracellular DNA in fingerprint samples. The acoustofluidics system works by using an ultrasound standing wave to separate different sized particles. Each chip (**Fig. 1**) has its own characteristic resonant frequency where it will focus particles the best. At the optimal frequency, the sound wave will focus large particles (cells) into low pressure nodes, shifting them out of the rest of the sample matrix.[6] This means that the cells (cellular DNA) will be separated from the rest of the sample, which includes the extracellular DNA (**Fig. 2**).[7] Once the DNA has been separated, it can be quantified using quantitative PCR (qPCR).

MATERIALS AND METHODS

Preparation of objects

Glass Slides (VWR Microslides) and pens (Papermate® Black Ballpoint Med. Pt.) were cleaned using 200µL of 70% ethanol and a KimWipe and allowed to air dry.

Sample Collection

Samples were collected from five volunteers. From each volunteer, six samples were collected along with a buccal swab. These six samples included three glass slides and three pens. Each glass slide had three fingerprints deposited on it from the volunteer (thumb, index, and middle fingers). These fingers were chosen since the index and middle fingers were previously shown to deposit the most DNA on an object[8], and the thumb often touches objects when they are handled. Additionally, for two of the slides the dominant hand was used, while for the third slide, the non-dominant hand was used. In the literature, there has been a determination that there is a difference in shedding between the dominant and non-dominant hand, so there may be a distinction in the results.[9] For the pens, each sample was collected by having the volunteer remove the cap, hold it in their hand like they are writing with it, and then replacing the cap. This process was repeated three times for each pen sample collected. The buccal swabs were collected in order to have confirmation of the origin of each sample if genotyping can be completed in the future.

Sample Processing

Once the samples were collected from the volunteers, the DNA was collected using sterile polyester swabs (Puritan) and TE buffer (TEKnova). Swabs were used for collection as they are the most commonly used tool for recovering DNA from non-porous surfaces.[10,11] The following procedure was used to collect the DNA from the objects and transfer them into the TE buffer:

a. Fill each 1.5 mL Eppendorf tube with 200 µL of TE buffer
b. One at a time:
    i. Open a swab and wet it will 100 µL of TE buffer.
    ii. Roll the swab over the part of the glass slide/pen where DNA was deposited.
    iii. Put the swab in the tube, cut it to the correct size, and swish it for 20 seconds with the tweezers.
    iv. Vortex the tube for 5 seconds.
    v. Hold the swab over the tube while dispensing 140 µL of TE buffer over it to remove DNA. Push the swab against the side of the tube to recover as much DNA/TE as possible after the rinse. Discard the swab.
    vi. Separate sample into 3 fractions:
        1. 175 µL for acoustofluidics (AF)
        2. 50 µL for centrifugation (C)
        3. Remainder will remain in original tube for unprocessed cells (U)
    vii. Close tubes and separate according to process (AF, C, and U).
c. After the glass slides have dried, place them in labeled plastic bags for storage.

The previous steps were used for all glass slide and pen samples. Buccal swabs were processed with the following procedure:

a. Add 1 mL of TE buffer to the 1.5 mL Eppendorf tube containing the swab.
b. Place the tube on the heat block at 70°C for 30 minutes.
c. After incubation, remove the swab and place it in waste.

Separation of Cellular and Extracellular DNA

Centrifugation

Following sample processing, the centrifugation samples were centrifuged on a 5415R centrifuge (Eppendorf). Since this step could not be tested before sample collection, the samples of different volunteers were centrifuged at different speeds and times. Below are the speeds and times used for the samples of each volunteer:

711: 400xg for 10 minutes followed by two cycles of 800xg for 10 minutes
314: 800xg for 10 minutes
79: Two cycles of 800xg for 10 minutes
51: 800xg for 10 minutes
11: 800xg for 10 minutes

The differences in centrifugation speeds and times allowed for optimization of the centrifugation procedure while the samples were being processed. Despite the differences in centrifugation

speeds and times, we are confident that the centrifugation procedure used for each volunteer was sufficient to pellet all cells from solution. There was consistently a very visible pellet in the buccal swab tubes, and there was no significant change in pellet size between the last two cycles when multiple cycles were run. Following centrifugation, each sample was split into a cellular and extracellular fraction according to the following procedure:

a. Remove 40 µL of the supernatant and place it in the respective tube (.2a) as the extracellular DNA component. All remaining supernatant should be removed and placed in waste.
b. Add 40 µL of TE to the pellet in the first tube and vortex the tube to re-suspend the pellet. This tube will be the cellular DNA component and the label should be adjusted accordingly (.2b).

These samples were then stored at 4°C until quantification.

Acoustofluidics

Separation occurred on chip AFv5.5 A(5) at a frequency of 1.69MHz, and a voltage of 16.4 Vpp driving the piezo transducer. The samples for separation on the acoustofluidics system were processed over two days. Each day, the system was first cleaned and decontaminated with 10% bleach, 70% ethanol, and DI water. Then a blank (TE buffer) was run through the system before any samples were analyzed. Samples were then processed in order of priority with a decontamination cycle after each sample. Blanks were also run before and after breaks, and at the end of each day to check for contamination.

Quantification

All samples were quantified using real-time quantitative PCR (qPCR) in triplicate. Table 1 includes the volumes of reagents used and the company that supplied each reagent. For amplification of the DNA, a two-step Platinum Taq protocol was used, which was developed by the Richmond Police Department (CA):[12]

- Initial hold at 95°C for 10 minutes

- 45 cycles of 95°C for 15 s. and 60°C for 60 s. with a plate read at the end of the 60°C cycle
- Hold at 4°C

A standard curve consisting of two or three concentrations of Human Genomic DNA (HGD, ABI) was run with each plate for quantification purposes.

RESULTS

Quantitation

A summary of the results from the quantitation of the fingerprint samples is found in Table 2. The values in each square are the average concentrations of DNA based on the quantification results while the colors represent the number of samples that had at least PCR well come up positive during qPCR. For each of the fingerprint samples collected, five fractions were obtained. Unprocessed samples were used to quantify the baseline starting quantity of DNA present in the original collection and for comparison of results. The fractions processed through centrifugation and acoustofluidics each produced an extracellular and a cellular fraction. For each PCR plate run, positive controls were run to create a standard curve run under the same conditions as the samples. This made it possible for the CT values to be converted to DNA quantities, and then onto concentrations using the pipette generated volumes for each sample. Each of the unprocessed samples had results from at least one sample. As for the processed samples, the extracellular fractions generally yielded more DNA than the cellular fractions for both centrifugation and acoustofluidics. Additionally, the pens usually yielded more DNA than the glass slides.

The data was also analyzed to determine if some of the trends described in the literature could be seen in this study. Figure 3 shows the variability in the amount of DNA (average copies of DNA) recovered from the pens and glass slides for each volunteer. This result has been well

documented in the literature, as the amount of DNA left on a surface depends on the individual as well as a number of environmental factors.

Glass Slides

Figure 4 summarizes the results from the glass slides based on the average concentrations for each fraction. This bar graph shows the percentage of the DNA recovered and processed in each fraction per method. The percentages were calculated by dividing the average concentration of each fraction by the sum of the two fractions for a processing method. The extracellular fractions for both processing methods contained more DNA than the cellular fractions.

Pens

Figure 5 summarizes the results from the pens based on average concentrations for each fraction. The bars on the graph correspond to the same fractions as the bar graph above. These results were slightly more variable than those for the glass slides. Except for the centrifugation fractions from volunteer 1, the extracellular fractions still contained more DNA than the cellular fractions. However, the percentages of the cellular fractions generally increased for the pens compared to the glass slide samples.

DISCUSSION

The clearest overall result from this study was that the extracellular fractions contained more DNA than the cellular fractions for both processing methods. This was true for samples from both the pens and the glass slides. Additionally, it is extensively documented in the literature that some people are better shedders of DNA than others, which could be due to multiple factors.[10] This trend was evident in the results, with volunteer three being the best shedder and volunteer two being the poorest shedder.

During the study, some issues came to light that will require further examination in order to make more definitive conclusions for this study. Firstly, some of the blank (TE buffer) samples run in between sets of samples during acoustofluidics processing produced cycle threshold (CT) values during quantitation by qPCR. This may mean that even with the decontamination procedure between samples, there may be some carryover of DNA from one sample to the next. This carryover was limited to the small particle outlet of the acoustofluidics chip, which is where the extracellular fraction of each sample moved through. Since the extracellular fractions had the highest DNA concentrations, the possibility of contamination brings into question the quantitation of the extracellular fraction results from this method. The most reliable samples from the acoustofluidics processing are the samples run between blanks 3 and 4, as both of these blanks came up negative for all wells during quantification. When just looking at these samples, the extracellular fractions still yielded more DNA than the cellular fractions, which indicates that the overall conclusion of this study is valid. However, the DNA yields may be slightly skewed due to carry over in other samples. The best way to determine how the contamination affects the samples is to genotype all samples and the blanks. If genotyping results are obtained from the blanks, the source of the contamination can be determined based on the profile obtained.

Secondly, during processing of samples using centrifugation it was noticed that a pellet outline was still visible after re-suspension in TE buffer by vortexing. This may indicate that not all of the cells were re-suspended by the method used; however, this cannot be determined without further studies. None-the-less, it is unlikely that a sufficient number of cells adhered to the bottom of the tubes that if all the cells were in solution, the significant DNA yield differences between the extracellular and cellular fractions would be overcome. Similarly, the results for the

cellular fractions from acoustofluidics processing are likely low quantity due to the fact that the end fractions are so diluted that the DNA concentration is below the limit of detection for the qPCR protocol. A certain minimal volume is required for proper operation of the acoustofluidics system, and although these volumes are small, the volume increases during the process further dilutes an already very dilute sample. Again, this theory could explain why so few cellular fractions for this method were able to be quantified, but still does not invalidate the overall conclusions of the study.

CONCLUSION AND FUTURE WORK

Overall, the trend that is visible in the results is that there is more extracellular than cellular DNA in fingerprints. This would suggest that the extracellular DNA from bodily fluids such as sweat makes up more of the DNA in fingerprints than cellular DNA from skin cells. However, the samples will need to undergo further testing to provide more quantitative results. Further work includes genotyping of the samples already collected to determine the quality of the results, and collection and testing of more samples in order to have a larger sample set.

ACKNOWLEDGEMENTS

I would like to thank Maxim Shusteff, Alison Burklund, Sally Hall, Elizabeth Wheeler, Joanne Osburn, Aubree Hinckley, and Stephanie Malfatti for all of their help on this project. This work was supported in part by the U.S. Department of Energy, Office of Science, Office of Workforce Development for Teachers and Scientists (WDTS) under the Science Undergraduate Laboratory Internships Program (SULI). Research with human subjects has been reviewed and approved by the LLNL Institutional Review Board Protocol #14-005. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. LLNL-TR-659969

REFERENCES

[1]Roland A. H. van Oorschot and Maxwell K. Jones, "DNA fingerprints from fingerprints," Nature 387, 767 (1997).

[2]Dyan J. Daly, Charlotte Murphy and Sean D. McDermott, "The transfer of touch DNA from hands to glass, fabric and wood," Forensic Sci. Int. Genet. 6, 41-46 (2012).

[3]Valentin V. Vlassov, Pavel P. Laktionov and Elena Y. Rykova, "Extracellular nucleic acids," BioEssays 29, 654-667 (2007).

[4]Ignacio Quinones and Barbara Daniel, "Cell free DNA as a component of forensic evidence recovered from touched surfaces," Forensic Sci. Int. Genet. 6, 26-30 (2012).

[5]Ray A. Wickenheiser, "Trace DNA: A review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact," J Forensic Sci 47(3), 442-450 (2002).

[6]Erika J. Fong, Amanda C. Johnston, Timothy Notton, Seung-Yong Jung, Klint A. Rose, Leor S. Weinberger and Maxim Shusteff, "Acoustic focusing with engineered node locations for high-performance microfluidic particle separation," Analyst 139, 1192-1200 (2014).

[7]Maxim Shusteff, "Fast, flexible, automated sample preparation to meet national biosecurity challenges," Engineering Innovations, (2011).

[8]Sarah M. Thomasma and David R. Foran, "The influence of swabbing solutions on DNA recovery from touch samples," J Forensic Sci 58(2), 465-469 (2013).

[9]Matthew Phipps and Susan Petricevic, "The Tendency of individuals to transfer DNA to handled items," Forensic Sci. Int. 168, 162-168 (2007).

[10]Georgina Meakin and Allan Jamieson, "DNA transfer: Review and implications for casework," Forensic Sci. Int. Genet. 7, 434-443 (2013).

[11]Roland A. H. van Oorschot, Kaye N. Ballantyne and R. John Mitchel, "Forensic trace DNA: a review," Investigative Genetics 1:14, 1-17 (2010).

[12]Katie L. Swango, Mark D. Timken, Mavis Date Chong and Martin R Buoncristiani, "A quantitative PCR assay for the assessment of DNA degradation in forensic samples," Forensic Sci. Int. 158, 14-26 (2006).

TABLES

Reagent	Volume (µL)
PCR-grade water	11.5
10X PCR Buffer (-$MgCl_2$, Invitrogen)	2.5
2 µg/µL BSA (Invitrogen)	3
50 mM $MgCl_2$ (Invitrogen)	1
10 mM dNTP (Invitrogen)	0.5
nuCSF Primer (F/R) 10µM (IDT)	1
nuCST Probe-6Fam 10µM (IDT)	0.25
Platinum Taq Invitrogen)	0.25
Sample	5
Total	25

Table 1: Reagent volumes for qPCR master mix and the companies who supplied the reagents.

		Volunteer				
	Fraction	**711**	**314**	**79**	**51**	**11**
Glass Slides	Unprocessed	2.64	0.44	12.25	1.16	0.21
	Centrifuged-Extracellular	101.92	18.60	341.96	8.93	4.72
	Centrifuged-Cellular	17.33		159.37		
	Acoustofluidics-Extracellular	518.53	59.97	884.32	133.13	149.02
	Acoustofluidics-Cellular				6.75	
Pens	Unprocessed	3.90	0.26	21.19	1.02	1.79
	Centrifuged-Extracellular	140.26	67.51	740.28	42.77	67.35
	Centrifuged-Cellular	175.42		120.45	12.81	13.34
	Acoustofluidics-Extracellular	618.87	109.92	1206.98	121.52	451.74
	Acoustofluidics-Cellular	15.51				

Table 2: Summary of quantification results from fingerprint samples on the glass slides and pens. The values in each square are the average DNA concentrations (pg/µL) of the three samples for each volunteer and type of object. The color of the square refers to how many of the samples had at least one positive PCR well; green=3, yellow=2, orange=1, and red=0 samples.

FIGURES

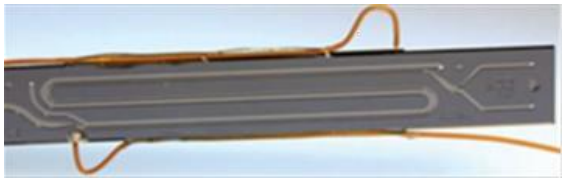

Figure 1: Acousofluidics chip for separation.[6]

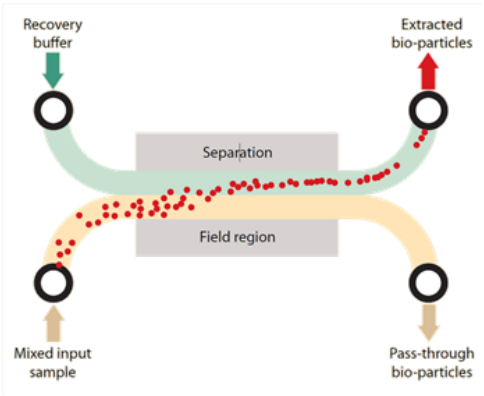

Figure 2: Separation of cellular DNA (green outlet) from extracellular DNA (yellow outlet).[7]

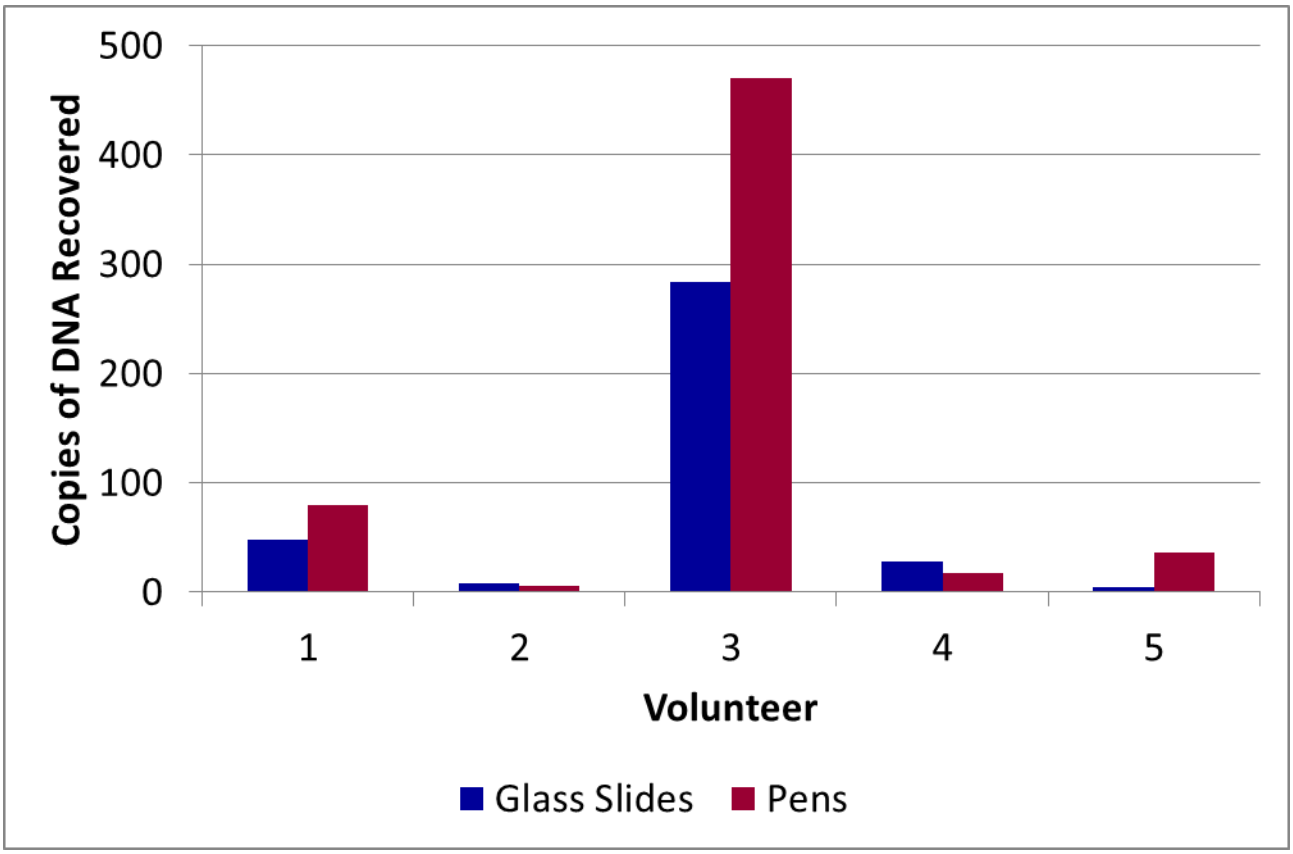

Figure 3: Average copies of DNA recovered per volunteer and object. Copies of DNA were determined by dividing total DNA recovered in the unprocessed samples by 3.3 pg. (haploid cellular DNA quantity).

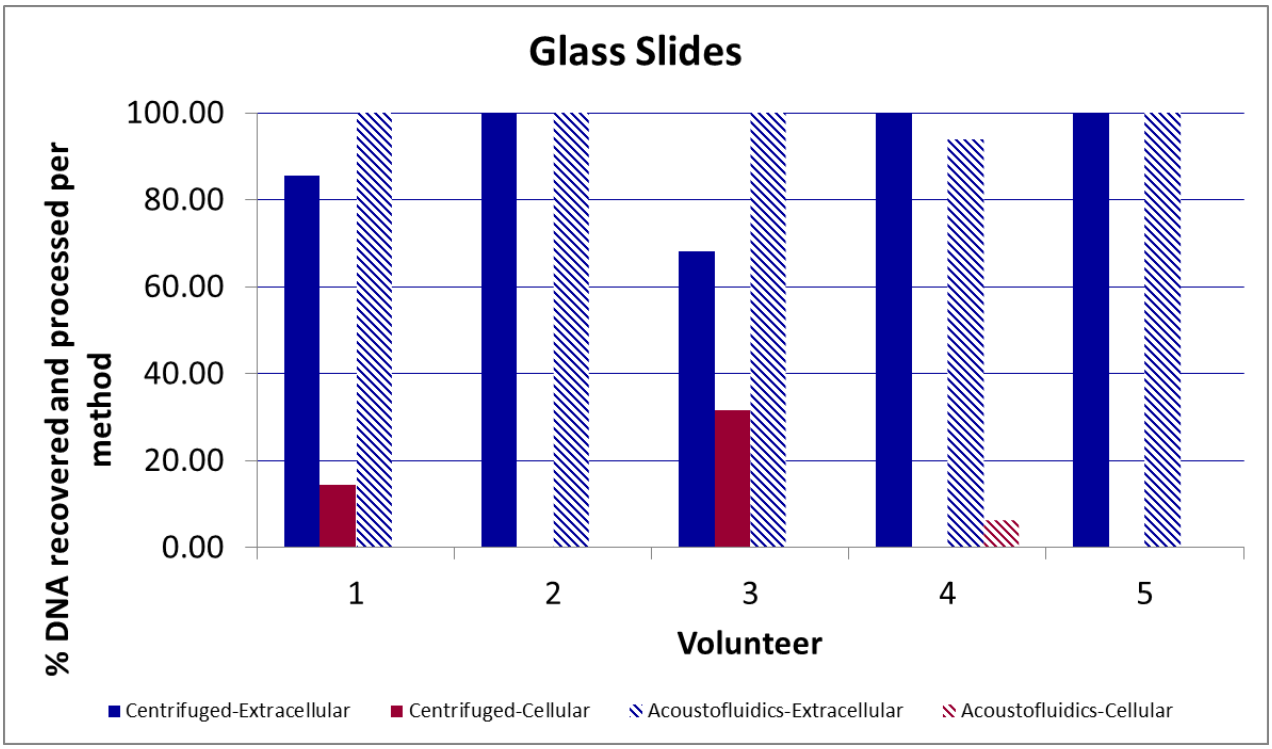

Figure 4: Summary of processing results for glass slides. Bars represent percentage of DNA recovered and processed per method for each volunteer.

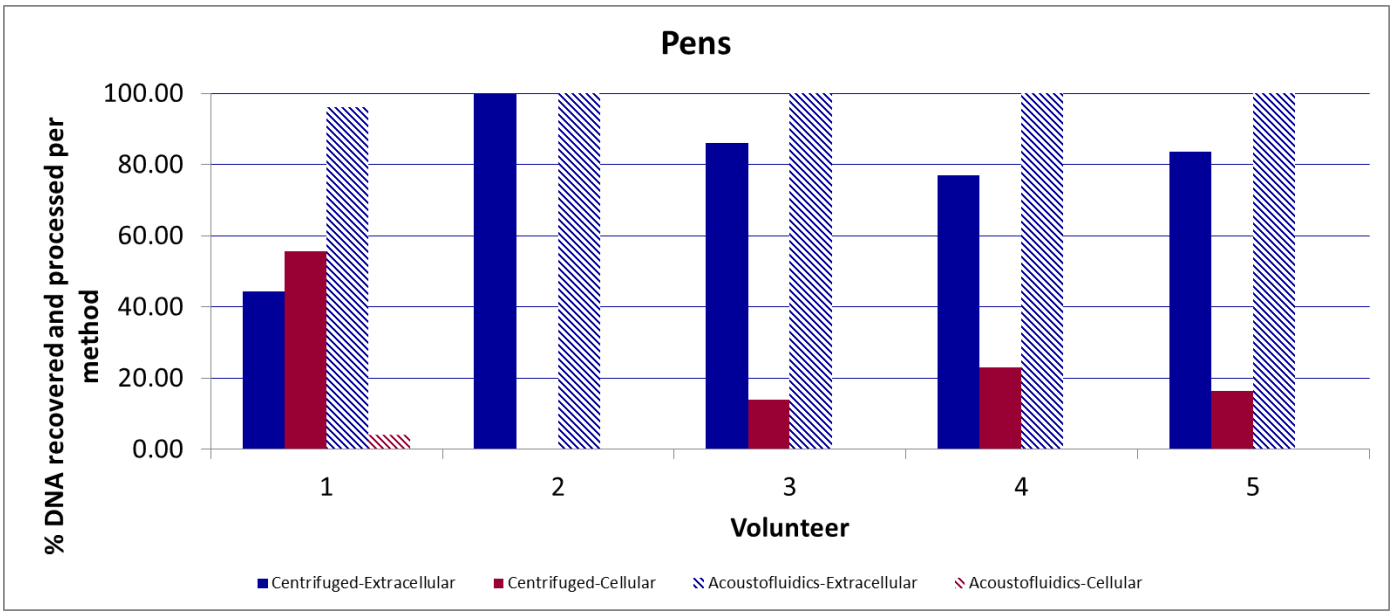

Figure 5: Summary of processing results for pens. Bars represent percentage of DNA recovered and processed per method for each volunteer.