Civil War Field Hospital: A Bloody Surgical Table's Link to the Past and its Impact on Future DNA Analysis

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Abstract

During the Battle of Sailor's Creek, the Hillsman House was used as a field hospital to treat hundreds of soldiers. In 2018, Jones and McClintock traveled to the historical site and generated human DNA profiles from bloodstains found on the floor of the house, thus demonstrating the ability to profile aged bloodstains. During their study, marks observed on the floor were presumed to be from the surgical table, however, the original table was no longer at the house.

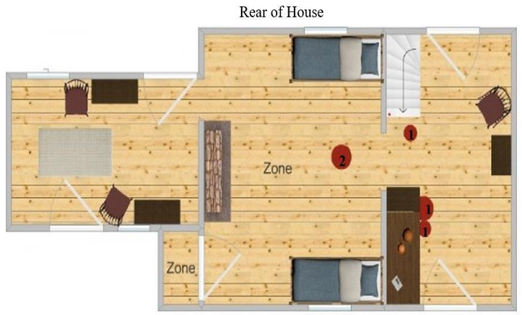

Figure 1. Hillsman House 2d Reconstruction
Reconstruction of Hillsman house from Jones and McClintock Research. Dimensions of marks on floor where the surgical table was presumed to be align with dimensions of recovered table.

Since then, the original wooden surgical table was acquired, and presumed bloodstains were observed and available for collection and analysis. In this study, stains from the table were visualized using an alternate light source (ALS) and collected via swabbing and scraping. The samples were subjected to the presumptive tests Phenolphthalein and Leucomalachite Green (LCG) to determine if blood was present in these samples. Then, the Qiagen DNeasy® Blood & Tissue Kit and Qiagen Investigator® Lyse & Spin Basket Kit were used as DNA extraction methods and later compared. Finally, the extracted DNA was quantified using the NanoDrop200. All presumptive tests yielded negative results, but after extraction, each sample yielded a quantity of DNA. Scraping samples had higher yields than swabs and the DNeasy extraction had higher yields than the Investigator kit in every sample. This study corroborates the findings of Jones and McClintock (2018) in that DNA can be successfully extracted from 160-year-old bloodstains. Additionally, this research shows that higher concentrations of DNA are yielded from scrapings over swabbing collections. Lastly, the Investigator® Lyse & Spin Kit is ineffective in the extraction of DNA from wood shavings and underperforms when compared to the DNeasy® Blood & Tissue Kit.

Introduction and Research Question

The Qiagen DNeasy® Blood & Tissue Kit is used in most forensic science labs as the preferred option for DNA extraction. Forensic science laboratories subject their procedures and materials to extensive testing to determine if it can be validated for use with evidence in criminal cases. However, heftier materials such as wood can be difficult to conduct extraction from due to their bulkiness and chemical hindrances within the material. Another product from the same manufacturer, the Investigator® Lyse & Spin Basket Kit, is advertised to be designed specifically for the lyse and extraction of DNA from solid substrates (3). If this kit yields results as is claimed, it may be a beneficial addition for laboratories to streamline the extraction procedure for bulkier solids. Conversely, if the kit underperforms or if it does not streamline the extraction process it may not be worth a laboratory's time to examine for future use. From our literature review, no studies have compared the basket's yield to the standard DNeasy® protocol. Therefore, this research hopes to answer which kit and procedure is more successful, and thus which should be used in forensic science labs.

DNA sample collection is an integral part of both lab and law enforcement procedures, as a better collection could yield better evidence in a trial. Many studies have examined different types of collection methods (1) (2). In this study, the difference between scrapping and swabbing collection methods will be compared, specifically in conjunction with their later yield after DNA extraction.

Figure 2. Surgical Table Expanded Bottom View
View of the surgical table from the bottom, where a majority of blood stains were located. The table has two panels on the side that expand and legs that drop down to support it.

Figure 3. Surgical Table With Evidence Markers
Potential blood stains were marked and given an evidence label. Marks near the labels can be seen as drops, smears, and drip patterns.

Samples	DNeasy® Blood & Tissue Kit Swab Sample (ng/µL)	Investigator® Lyse & Spin Basket Kit Swab Sample (ng/µL)
L1	5.8	2.7
L2 (glow)	3.3	2.4
L4	4.3	1.6
L8	5.3	2.3
T1	4	2.7
T11	2.8	1.8
T14	5.9	1.7
T17	1.6	1.7
U5	4.9	3.3
U7	3.4	1.2
U14	6.2	1.5
U15	6	5.1
U17	2.9	0.8
U21	5	1
U22	3.5	0.9
U23	2.4	2.9
U30 (glow)	3.9	2.9
U34	2.6	1.8

Table 1. Extraction yields from swabbed samples. Samples with (glow) next to the sample number glowed under ALS, which is unusual for blood samples.

Samples	DNeasy® Blood & Tissue Kit Scrapping Sample (ng/µL)	Investigator® Lyse&Spin Basket Kit Scrapping Sample (ng/µL)
L1	187.9	16.7
L2 (glow)	11.8	-
L4	497.6	202.5
L8	314	62.5
T11	136.7	2.4
T14	89.1	11.4
U5	60.4	33.4
U14	71.6	9
U15	86.1	15.5
U19 (glow)	6.4	-
U21	16	5.5
U22	39.2	3.7
U23	49.6	14.2
U30 (glow)	169.8	-

Table 2. Extraction yields from scrapped samples. Samples with (glow) next to the sample number glowed under ALS, which is unusual for blood samples. Samples with no data were not extracted do to limited sample.

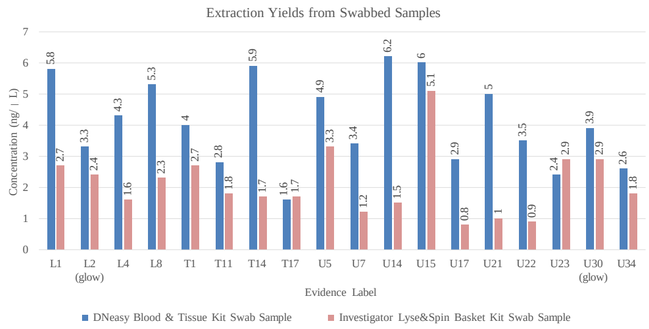

Figure 5. Extraction Yield Comparison Swabbed Samples. Comparison between extraction kits based on DNA yields for swabbed samples. Blue columns represent the DNeasy kit yields, pink columns represent Investigator kit yields.

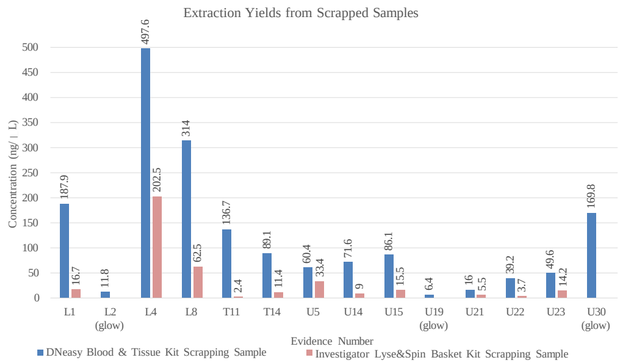

Figure 6. Extraction Yield Comparison Scrapped Samples Comparison between extraction kits based on DNA yields for scrapped samples. Blue columns represent the DNeasy kit yields, pink columns Investigator kit yields.

References

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Methodology

1) Stains Visualized and Marked on All Sides of the Surgical Table
   - Stains visualized using ALS
   - Stains visualized in normal conditions
2) Stains Collected from Numerous Locations on the Surgical Table
   - Samples collected from the table by swabbing
   - Samples collected from the table by scraping
3) Presumptive Tests Performed on Swab Samples
   - Phenolphthalein presumptive test
   - Leucomalachite Green presumptive test
4) DNA Isolated and Purified from Swab and Scraping Samples
   - Qiagen DNeasy® Blood & Tissue Kit
   - Qiagen Investigator® Lyse & Spin Basket Kit
5) DNA Quantified from Extracted Samples Using the ThermoScientific Nanodrop 2000
   - Measured in ng/µL for the samples listed in Table 1&2

Results and Conclusion

Results:
The presumptive testing done with both Phenolphthalein and LCG yielded negative results. These results were not unexpected, and do not disqualify the samples as being from a blood origin. Due to the age and degradation of the sample, it is likely that the heme protein in blood cells, which is what causes the colorimetric reaction in these presumptive tests, had degraded too much to react with the reagents in the test. However, these samples did come from a bodily origin, as they did yield DNA through extraction.

There were significantly higher yields for DNA extracted from the DNeasy® Blood & Tissue Kit compared to the Investigator® Lyse & Spin Basket Kit. The Investigator® Lyse and Spin Basket also had additional issues during extraction due to the sample material not allowing flow through the column when centrifuged. A modified procedure that deviated from original manufacture protocol was developed to potentially fix these issues, to no avail. This modified procedure also caused the extraction to take significantly longer than the DNeasy® extractions.

When comparing the sample collection methods and their yields, scrapped samples had a significantly higher DNA yield than swabbed samples.

Conclusions
We have shown that it may not be wise for forensic science labs to use or test the Investigator® Lyse & Spin Basket as a replacement for the commonly used DNeasy® Blood & Tissue Kit. The Investigator® Lyse & Spin Basket produces lower DNA yields, is not able to easily handle large solid materials, and takes significantly longer than the standard methods with the DNeasy® Blood & Tissue Kit. This study corroborates the findings of Jones and McClintock (2018) showing that DNA can be successfully extracted from 160-year-old dried blood from a wooden substrate that has been used and attempted to be cleaned. Furthermore, even when presumptive tests give negative results in degraded samples, DNA can still be extracted. Lastly, dried blood stains on wooden substrates are best collected through scrapping than swabbing, as it gives a higher DNA yield.

Future Work

1) Amplify and generate genetic profiles from the extracted DNA.
2) Test other marked stains from the table that were not used for extraction in this study.
3) Conduct additional testing on the glowing samples to determine what phenomenon caused them to react differently to ALS.
4) Conduct further testing with the Investigator® Lyse & Spin Basket to determine if it has a lower yield for materials other than wood.