DNA Degradation Rate in Embalmed Tissue Over Time

Alyssa Venditti[1*], Hélène N. LeBlanc[1], PhD, Guy Sovak[2], PhD, Stacey Sainte-Marie[1], MSc
Ontario Tech University[1], Oshawa, ON
Canadian Memorial Chiropractic College[2], Toronto, ON

Research Workflow

1. DNA Degradation: A Result of the Embalming Process
2. Justification and Significance for Project
3. Project Goals
4. Research Collaboration Agreement
5. Acquiring Permissions
6. Sample Types and Sampling Methods
7. DNA Kit Determination
8. Addressing the Knowledge Gaps
9. Conclusion and Future Directions

1. DNA Degradation: A Result of the Embalming Process

- Embalming is used in the preservation of a deceased body, as it slows down putrefaction
- Embalming chemicals contribute to DNA degradation
- Rate of DNA degradation in embalmed tissues is unknown
- Retrieval of DNA after death is still crucial

2. Justification and Significance for Project

- Current publications revealed that DNA yield, level of DNA degradation, and Short Tandem Repeat (STR) success varies between cadavers and tissue types[(1-3)]
- Knowledge gaps discovered in current publications:
  - **Minimal sample diversity**
  - **Lack of repetition at sample sites**
  - **Deficient variation in collection time points**
  - **Absence of pre-embalming control samples**
- **Significance: This project is significant as it has a large focus on closing the existing knowledge gaps, which in turn will benefit applications in a vast range of scientific fields including medico-legal investigations, chiropractic studies, and genetic analysis.**

3. Project Goals

- **Establish a research collaboration between participating institutions that all address multiple disciplines including forensic science, chiropractic research, and pathology**
- **Develop and implement a research plan to further understand DNA degradation over time in embalmed tissues, to close the knowledge gaps presented**

4. Research Collaboration Agreement

- Research collaboration was established between Ontario Tech University and The Canadian Memorial Chiropractic College (CMCC)
- **Collaboration benefits: Shared common interest in information obtained from embalmed tissue research, which can benefit: medico-legal investigations, chiropractic studies, and genetic analysis**
- CMCC: Under authority of the Anatomy Act of Ontario, Canada

5. Acquiring Approval and Internal Project Funding

- Approval requested and acquired from:
  - **The Office of the Chief Coroner**
  - **CMCC Research Ethics Board (REB)**
  - **Ontario Tech University Research Ethics Board (REB)**
- Any project involving a human cadaveric donor, must be approved by the Office of the Chief Coroner to be compliant with ethical guidelines as per **Ontario's Anatomy Act, R.S.O. 1990, c. A.21**[(4)]
  - This request also included the specifications of sample transportation
- REB approval was necessary to be compliant with **The Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2)**,[(5)] which provides ethical guidelines in regard to all research with human participant involvement regardless of methodology
  - This remains applicable to this project as DNA analysis is being conducted on the cadaveric samples
- Internal project funding acquired for necessary project materials

6. Sample Types and Sampling Methods

- Sampling trial conducted at CMCC to identify the most ideal sample types and best approach sampling methods
- To address **minimal sample diversity** and **lack of repetition at sample sites,** a sample size of 176 samples was determined **(Figure 1)**
- 10 sampling time points were selected to encompass both pre- and post- embalming time points in an effort to address **deficient variation in collection time points** and **absence of pre-embalming control samples (Figure 2)**

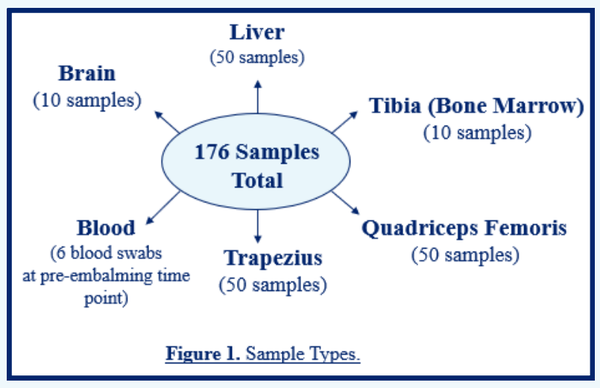

Figure 1. Sample Types.

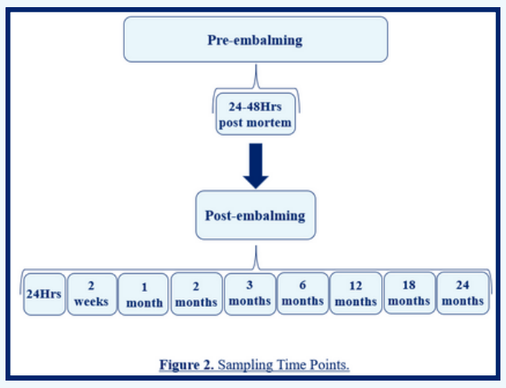

Figure 2. Sampling Time Points.

7. DNA Kit Determination

Table 1. DNA Kits Selected and The Reasoning Behind the Selection.

Name of Kit	Type of Kit	Reason for Kit Choice
QIAGEN DNeasy Blood & Tissue (Model: 69506)	Extraction	Has ability to perform extraction on the different sample types including **brain, liver, quadriceps femoris, and trapezius**
QIAamp DNA Blood Mini (Model: 51104)	Extraction	Has ability to perform extraction on the specified sample type of **bone marrow**
Thermofisher Quantifiler™ Duo DNA Quantification (Model: 4387746)	Quantification	Reliable, used by many laboratories, and has the ability to perform quantification on human DNA
Thermofisher AmpFLSTR™ Identifiler™ PCR Amplification (Model: 4427368)	Amplification/ STR Analysis	Reliable, used by many laboratories, and is designed to work with Ontario Tech University's Applied Biosystems SeqStudio genetic analyzer

8. Addressing the Knowledge Gaps

- Measures to be implemented:
  - **↑ Sample diversity (Figure 1)**
  - **↑ Repetitions of samples (Figure 1)**
  - **Establish multiple collection time points (Figure 2)**
  - **Collect control samples pre-embalming (Figure 2)**

9. Conclusion and Future Directions

- Three-step approach to close the gaps:
  - Sampling of diverse tissue types from ideal sampling locations, using the most appropriate sampling methods
  - Fast and reliable DNA analysis including analysis of STRs
  - Determination of a DNA viability time range
- **Future application:** Bone marrow viability for genotyping has been shown in publications, thus, a future application for this research could be with mass disaster identification[(2)] as often times, only bones and bone marrow may remain

References

1. Wheeler A, Czado N, Gangitano D, Turnbough M, Hughes-Stamm S. Comparison of DNA yield and STR success rates from different tissues in embalmed bodies. International Journal of Legal Medicine 2017;131(1):61–6. https://doi.org/10.1007/s00414-016-1405-5.
2. Calacal GC, Apaga DLT, Salvador JM, Jimenez JAD, Lagat LJ, Villacorta RPF, et al. Comparing different post-mortem human samples as DNA sources for downstream genotyping and identification. Forensic Science International: Genetics 2015;19:212–20. https://doi.org/10.1016/j.fsigen.2015.07.017.
3. Gielda L, Rigg S. Extraction of amplifiable DNA from embalmed human cadaver tissue. BMC Research Notes 2017;10(1). https://doi.org/10.1186/s13104-017-3066-y.
4. Anatomy Act, R.S.O. 1990, c. A.21. https://www.ontario.ca/laws/statute/90a21#:~:text=Bodies%20for%20anatomical%20dissection&text=(1)%20Subject%20to%20the%20Coroners%20Act%2C%20the%20local%20inspector,the%20purpose%20of%20anatomical%20dissection.
5. Panel on Research Ethics. TCPS 2: Core (Course on Research Ethics). https://tcps2core.ca/welcome.