Genotyping Strategies for Tissues Fixed with Various Embalming Fluids for Human Identification, Databasing, and Traceability

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INTRODUCTION

Embalmed tissues can serve as a source of genetic material for human identification [1]. However, chemical damage, single- and double-stranded breaks are introduced during embalming, leading to fragmentation of the DNA [2]. Within anatomical willed body programs and skeletal collections, whole bodies and their disassociated limbs, bones, and organs are currently tracked using RFID tags, individual barcodes or labels, written records, and photography [3]. However, if these tracking mechanisms fail, DNA recovered from the formalin-fixed tissues could provide an additional layer of quality assurance.

This project investigated the success of STR-typing from various soft tissue and bone samples that were fixed with commercial and in-house embalming solutions over time. Although several studies have examined DNA analysis of formaldehyde-damaged samples [2, 4], currently, this study is the first to assess the feasibility of DNA typing methods for specimen tracking and identification within a body donor program.

MATERIALS & METHODS

Sample Selection- Phase 1

- Five cadavers donated to The University of Queensland's Gross Anatomy Facility (GAF) Body Donor Program were used.
- Tissue samples were immersed in four different solutions with varying formaldehyde concentrations (**1%, 2.5%, 3.8%, 9.5%**).
- Tissues (skin, muscle, bone, heart, and kidney) were sampled at **time zero**, **one week**, and **three months** after fixation.

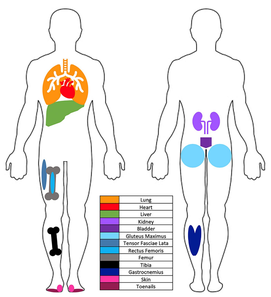

Fig. 1. Color - Coded Body Map The 57 different tissues collected from 11 cadavers in Phase 2 are displayed. (Source: Adapted from Clipart Library) [5].

RESULTS & DISCUSSION

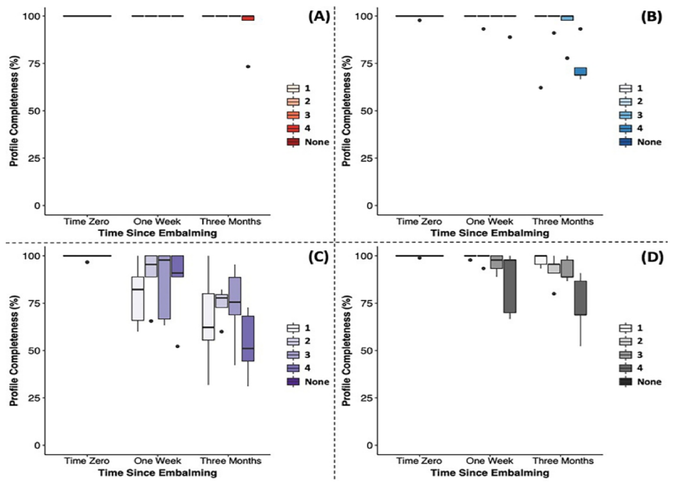

Fig. 2. Comparison of profile completeness (%) across four tissue types fixed with varying concentrations of formaldehyde over time.

Phase 1 (Fig. 2)

- Samples collected after three months of embalming yielded significantly less DNA, higher DI values, and lower STR success than samples embalmed for one week.
- STR success from kidney and bone samples, and some heart samples, significantly decreased over time compared to muscle and skin, which generated complete STR profiles for almost all samples.
- Lower DNA concentrations were obtained from samples fixed with Solution 4 (9.5% formaldehyde), and lower STR success rates became more prevalent the longer the tissues were stored in the solution.

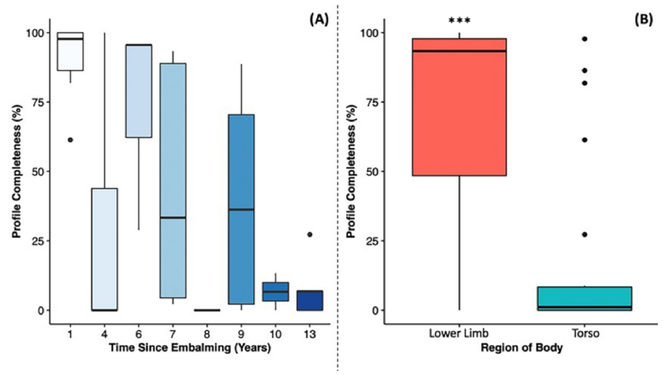

Fig. 3. (A) Comparison of profile completeness (%) over time (years) and (B) between the regions of the body.

Tissue Type		Total no. of Samples	Samples with no DNA
Skin		6	0
Bone		6	0
Nail		5	0
Muscle	Gastroc	6	1
	Glut M	6	1
	Tensor	2	0
	Rec Fem	4	3
Internal Organs	Liver	6	3
	Heart	6	4
	Kidney	5	3
	Lung	3	1
	Bladder	2	0
TOTAL		57	16

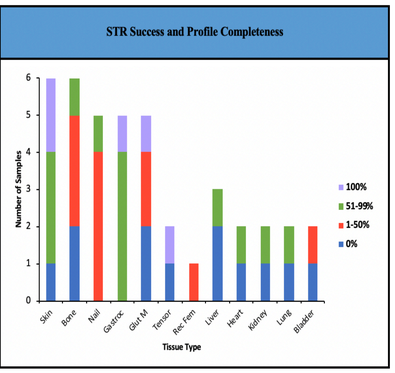

Fig. 4. Breakdown of the STR success of samples based on tissue type

Phase 2 (Fig. 3 and 4)

- The highest DNA concentrations and STR success rates were obtained by the samples in the earliest time point (one year).
- A general trend of decreasing STR success from formalin-fixed tissues the longer they were stored was observed and samples exhibited allelic dropout even in the smallest loci.
- Due to significant differences between the regions of the body (lower limb vs. torso) observed in this study, it could be suggested that sampling tissues from lower limbs may result in higher concentrations of DNA.

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Sample Selection- Phase 2

- An additional 57 tissue samples from 11 cadavers stored from one to 13 years in the GAF collection were examined (Fig. 1).

DNA Extraction and Quantification

- The QIAamp® DNA FFPE Tissue Kit (QIAGEN) was used to extract embalmed soft tissues, QIAamp® DNA Investigator Kit (QIAGEN) for non-embalmed soft tissues, and PrepFiler® BTA™ Forensic DNA Kit (ThermoFisher Scientific) for bone.
- DNA was quantified using the Investigator® QuantiPlex® Pro RGQ qPCR Kit (QIAGEN).

DNA Amplification and Analysis

- STR amplification was performed using Investigator® 24plex QS (QIAGEN) (input: 0.5 ng).
- INDEL typing was completed for samples that yielded <90% STR calls using the Investigator® DIPplex Kit (QIAGEN) (input target: 0.5 ng).
- Fragment analysis was performed on the 3500 Genetic Analyzer (ThermoFisher Scientific).
- DNA profiles were generated using GeneMarker® HID Software v2.7.1 (SoftGenetics).

CONCLUSIONS

- After one-year post-embalming, DNA was severely damaged, degraded, and often in low amounts.
- Sampling from skin and muscle tissues embalmed with ~2.5 - 5% formaldehyde solutions appear to be the best strategy for identification, while also maintaining the preservation of the tissues.
- Sampling from the lower limbs resulted in higher concentration of DNA and more complete STR profiles than from the torso region.
- Other HID approaches such as INDEL typing or SNPs via next generation sequencing may generate more successful results and warrants further investigation.

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