BODE TECHNOLOGY
June 2024

DNA Analysis of Chemically Treated Human Remains

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STAFS

SH

MEMBER THE TEXAS STATE UNIVERSITY SYSTEM

Funding

This project was partially funded by the Forensic Science Foundation Lucas Grant.

The opinions, finding, conclusions, and/or recommendations expressed in this presentation are those of the authors.

Content Warning

The following presentation contain graphic material.
Viewer discretion is advised.

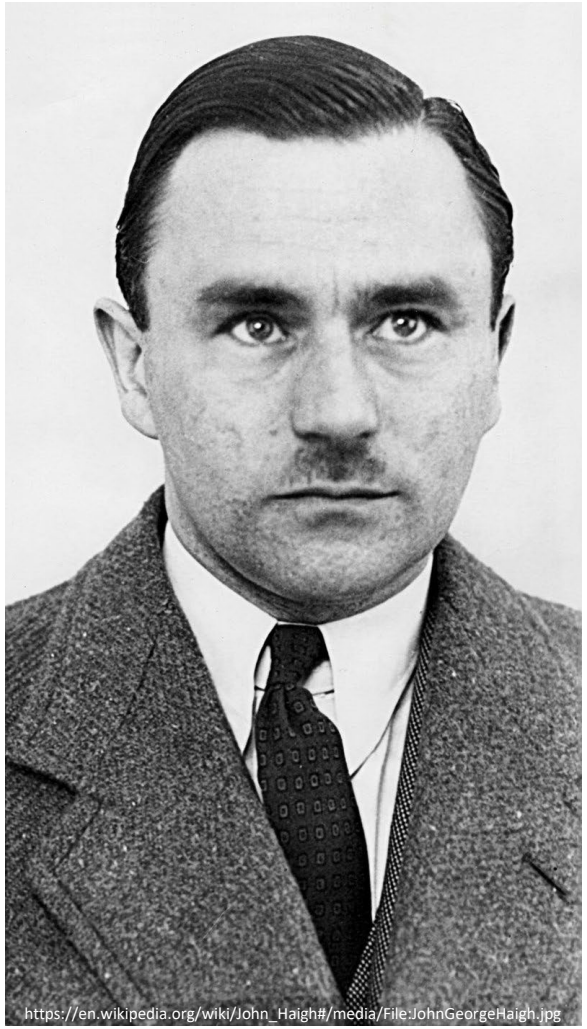
Photographs of slides containing images of human remains are **NOT** permitted.


WHY?

Acid Bath Murderer

- John George Haigh – the "Acid Bath Murderer"[1]
- Serial killer in England during 1940s[1]
- Killed at least 6 individuals and dissolved their remains in ***sulfuric acid***[1]

"I have destroyed her with acid. You will find the sludge that remains...**Every trace has gone**. How can you prove murder if there is no body?"[1]


https://en.wikipedia.org/wiki/John_Haigh#/media/File:JohnGeorgeHaigh.jpg

1. Holden DHS, Simpson DK. The Acid-Bath Murder(s): Rex v. John George Haigh. The Police Journal 1950;23(3):190–202. https://doi.org/10.1177/0032258X5002300306.

El Pozolero

- Santiago Meza Lopez – "El Pozolero" or "The Stewmaker"[2,3]
- Claims to have dissolved over 300 people for a Tijuana Drug cartel using ***lye***[3]

https://www.nbcnews.com/id/wbna28868152

2. Replogle J. Hope Dwindles For DNA Evidence At Tijuana Body Dump. KPBS. 2014. https://www.kpbs.org/news/2014/sep/03/hope-dwindles-dna-evidence-tijuana-body-dump/.
3. Booth W. "Stewmaker" stirs horror in Mexico. NBC News. 2009. https://www.nbcnews.com/id/wbna28868152.

Breaking Bad Inspired Killer

- Jason Hart murdered his girlfriend before placing her in a tote of "acid"[4]
  - Suspected to be a mix of water and ***drain cleaner***[4]
- Roommate said he was obsessed with the TV show *Breaking Bad*[4]
  - Investigators found the episode where Walter White teaches Jesse how to dissolve a body[4]

https://www.ibtimes.com/breaking-bad-chemistry-truth-behind-blue-meth-walter-whites-process-1411068

4. Ryan P. Real-life crimes echo "Breaking Bad." USA Today. 2013. https://www.usatoday.com/story/life/tv/2013/09/19/breaking-bad-real-life-crimes/2782635/ (accessed July 12, 2021).

Prevalence

- This practice spans decades
- Includes everyone from drug cartel members to TV show fanatics

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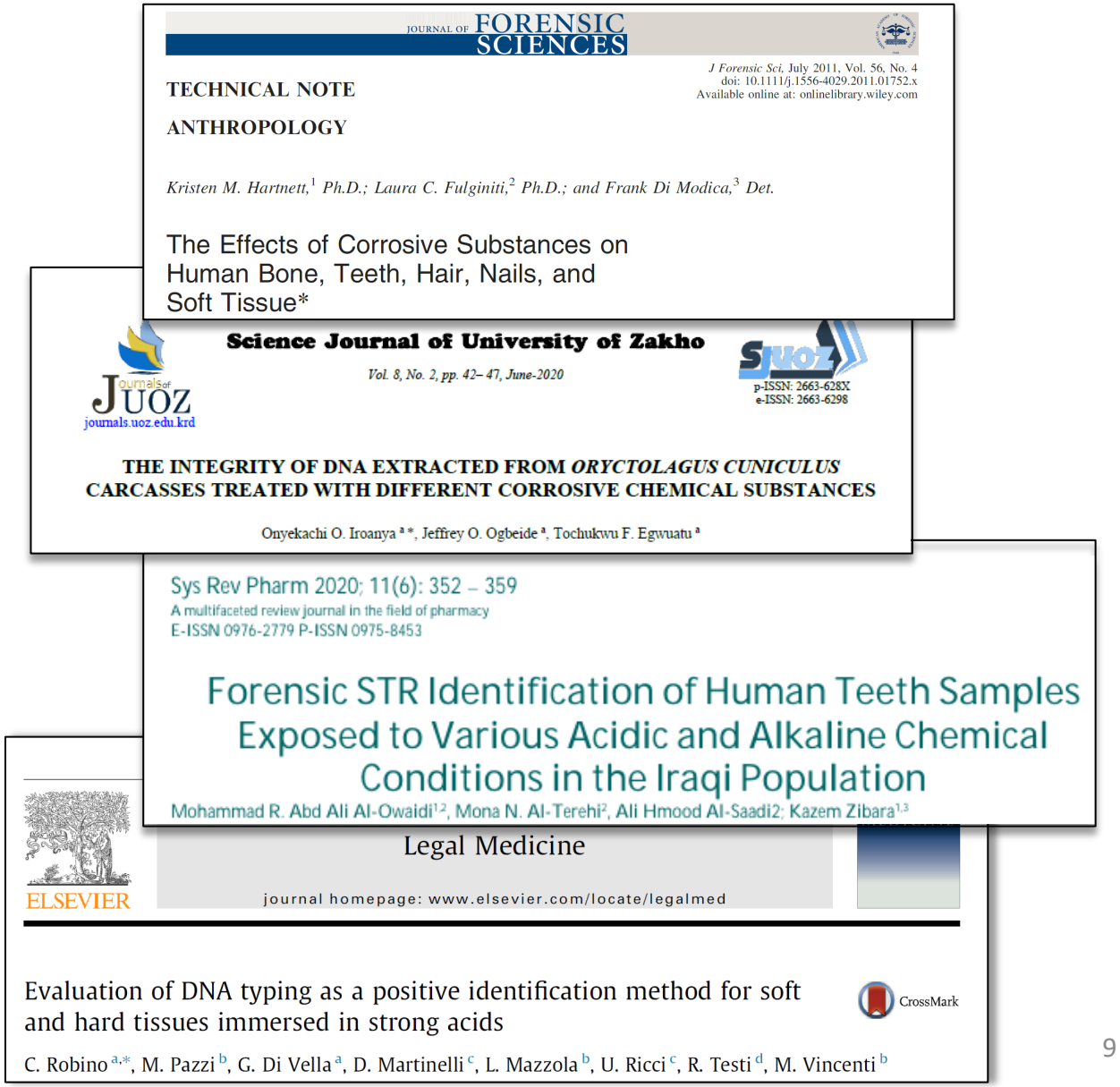
- Little to no research discussing DNA recovery from these challenging samples
  - Lack of knowledge in field

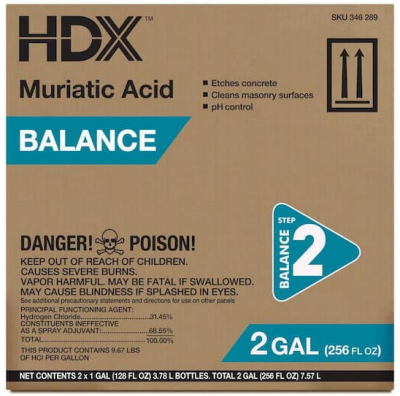
Limitations of Current Studies

1. Most work focuses on morphological changes of isolated fragments of tissue
   - Do not address DNA
2. Use animal proxies
3. Only examine isolated teeth
4. Many use laboratory grade chemicals
   - Not readily accessible to the public



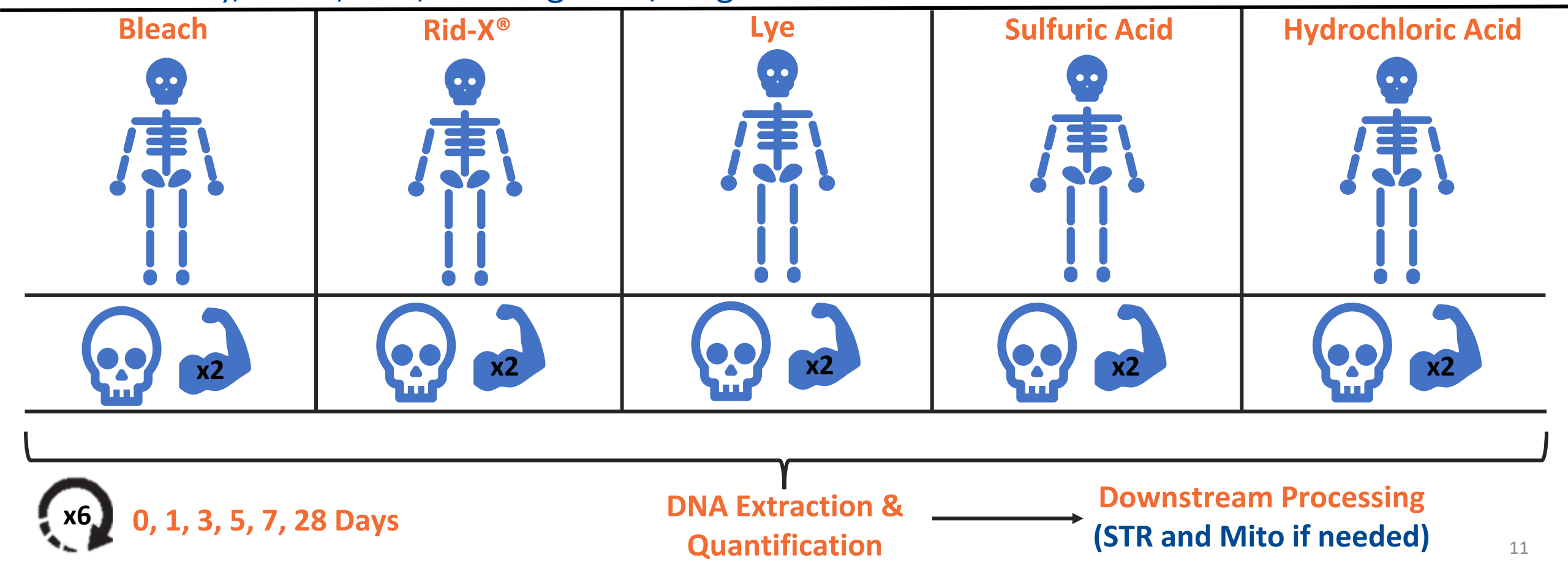
Chemicals

Product Name	Active Ingredient	Conc.	pH
HDX Germicidal Bleach	Sodium Hypochlorite	8.25%	> 12.5
Rid-X	Enzymes and Bacteria (proprietary) Glycerol	N/A 30 – 60%	6.3 – 8.8
Instant Power Crystal Lye Drain Opener	Sodium Hydroxide	1:1 mixture 25 M	13.0 – 14.0
ZEP Sulfuric Acid Drain Opener	Sulfuric Acid	90-100%	<1
HDX Muriatic Acid	Hydrogen Chloride	25-35%	<1



Sampling Design

- 5 cadavers: 1 for each chemical
- Intact large segments of human remains introduced to chemical – head and forearms
- Sampled at 6 timepoints: Bone (priority – radius & ulna), Tissue (priority – skeletal muscle), teeth, hair, and fingers w/ fingernails



Materials & Methods

- **Sample Preparation and DNA Extraction:**
  - Bone/Teeth –
    - 250 mg bone powder (powdered using a freezer mill – SPEX 6775)
    - Adaptation of Loreille et al. Total Demineralization[5]
    - Purification using MinElute® PCR Purification (QIAGEN)
  - Tissue, Fingernails, and Hair –
    - 10 mg / 2 cm
    - EZ1&2® DNA Investigator® Kit (QIAGEN)
      - 15-hour incubation
      - Purification on EZ2® Connect Fx (QIAGEN)
- **DNA Quantification:** Quantiplex Pro (QIAGEN) on the ABI 7500 (Thermo Fisher Scientific)
- **STR Typing:** Investigator 24plex QS (QIAGEN)
- **Mitochondrial DNA Analysis:** [Small Target] ≤ 2 pg/µL
  - HVI & HVII region – mini primers
  - BigDye® Direct Cycle Sequencing Kit (Thermo Fisher Scientific)
  - BigDye® Xterminator Purification Kit (Thermo Fisher Scientific)

5. Loreille OM, Diegoli TM, Irwin JA, Coble MD, Parsons TJ. High efficiency DNA extraction from bone by total demineralization. Forensic Sci Int Genet 2007;1(2):191–5. https://doi.org/10.1016/j.fsigen.2007.02.006.

STAFS Facility

- Cadavers were provided by the Southeast Texas Applied Forensic Science (STAFS) Facility
  - Willed body donor program
- All fieldwork was performed outside at STAFS

Sam Houston State University

SOUTHEAST TEXAS APPLIED FORENSIC SCIENCE FACILITY

STAFS Facility

Sheree Hughes, PhD

Director of STAFS

STAFS Graduate Assistant

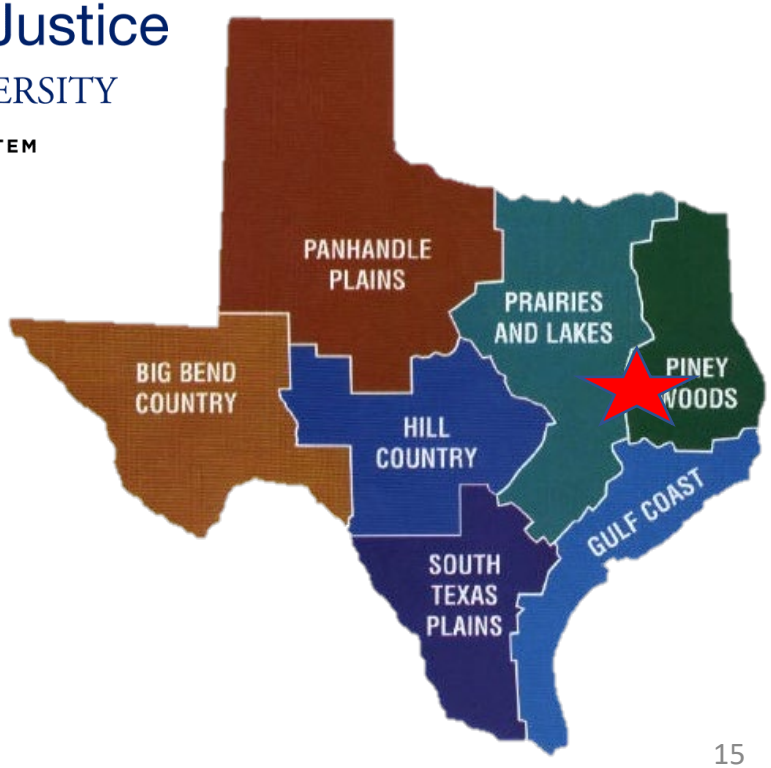
STAFS Facility: Overview

- Established 2009
- Sam Houston State University
  - Institute of Forensic Research, Training and Innovation
  - College of Criminal Justice

INSTITUTE FOR FORENSIC
RESEARCH TRAINING AND INNOVATION

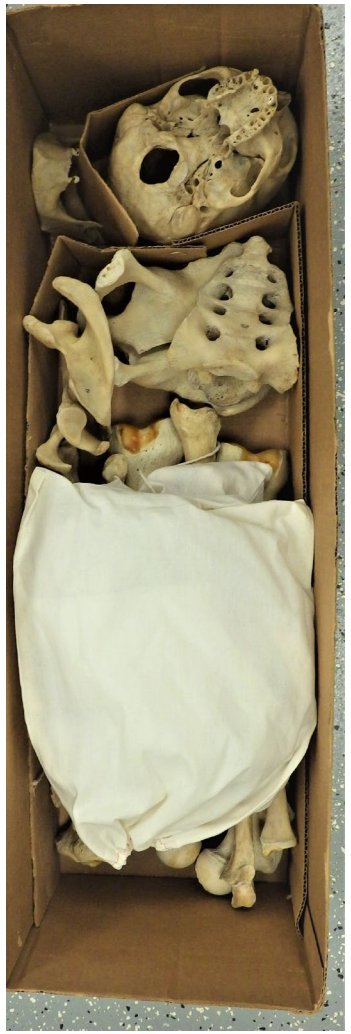
College of Criminal Justice
SAM HOUSTON STATE UNIVERSITY
MEMBER THE TEXAS STATE UNIVERSITY SYSTEM

- Humid subtropical climate
  - Piney Woods
- Living and Next-of-kin
  - All willed donations comply with The Texas Funeral Services Commission
- More than 760 donations to date
- DNA reference samples taken from all donations (post 2020)



STAFS Facility: Inside

- Skeletal Collection – Over 530 in collection



STAFS Facility: Inside

- Skeletal Collection – Over 530 in collection
- BSL-2 Prep Lab: Wet Lab
  - Body intake, maceration, cold storage, dissection, X-ray

STAFS Facility: Inside

- Skeletal Collection – Over 530 in collection
- BSL-2 Prep Lab: Wet Lab
  - Body intake, maceration, cold storage, dissection, X-ray
- Main Lab: Dry Lab
  - Drying racks, bone labelling, packing, inventory, research

STAFS Facility: Outdoor Facilities

- 2 acres with maximum security fencing
- Sectioned into 3 areas for different environments and projects
- Security cameras: 24/7 monitoring by campus PD

STAFS Facility: Trainings

- Field Application Search & Recovery

STAFS Facility: Trainings

- Field Application Search & Recovery
- Fire & Explosive Investigations

STAFS Facility: Summer Courses

Forensic Anthropology & Surface Recovery of Human Remains

Human Decomposition, Taphonomy & Postmortem Interval

Forensic Investigation of Physical Trace Evidence

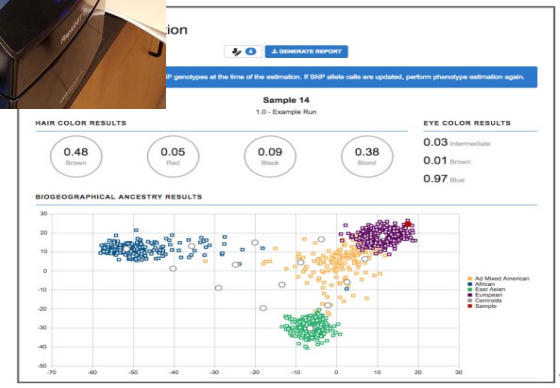
2024 Summer Short Courses

Forensic Approaches to Mass Fatalities: Challenges & Solutions

Advanced Approaches for Human Identification & Forensic Intelligence

STAFS Facility: Research

- Human Decomposition
- Forensic Anthropology
- Forensic Chemistry
- Forensic Entomology and Microbiology
- Forensic Epigenomics, Proteomics, Metabolomics, and Lipidomics
- Forensic DNA
  - DVI
  - Bone Prep & Extraction
  - Rapid DNA
  - FIGG
  - Ancestry/Phenotype Predictions
  - Challenging Bone Samples
    - Chemical Damage



https://verogen.com/wp-content/uploads/2018/08/ForenSeq-Univ-Analysis-SW-Guide-VD2018007-A.pdf

STAFS Facility: Method Development and Validation

- Industry/commercial/agency
  - Beta testing
  - ThermoFisher Scientific
  - Verogen
  - InnoGenomics
  - NASA
- Law enforcement agencies / crime labs
  - FBI
  - NY State Police
  - Signature Science
  - Harris County Institute of Forensic Sciences
  - Broward Sheriff's Office Crime Lab
  - Palm Beach County Sheriff's Office

Chemical Damage Sampling

Measure & Sample

Sampling Setup

PPE

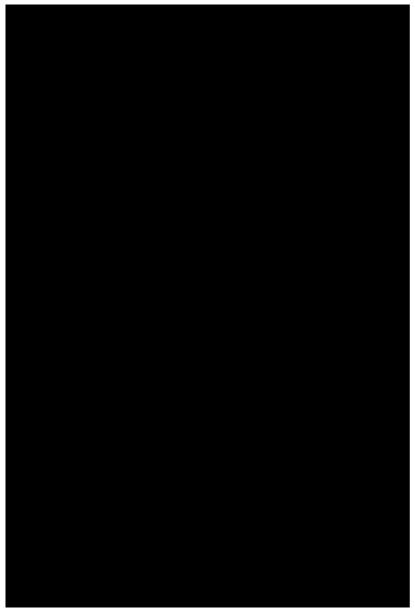
Bleach

- **Active Ingredient:** sodium hypochlorite
- **Concentration:** 8.25%
- **pH:** > 12.5

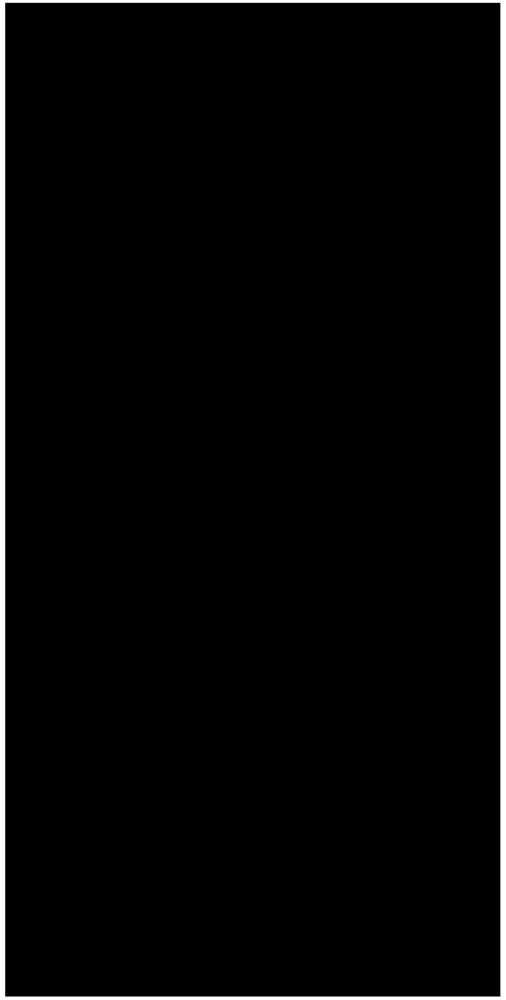
https://images.thdstatic.com/productImages/700a5145-d4b4-4cbb-8be3-c04b406325f7/svn/hdx-bleach-23268949644-64_1000.jpg

T = 0 Days	T = 1 Days	T = 3 Days	T = 5 Days	T = 7 Days	T = 28 Days
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

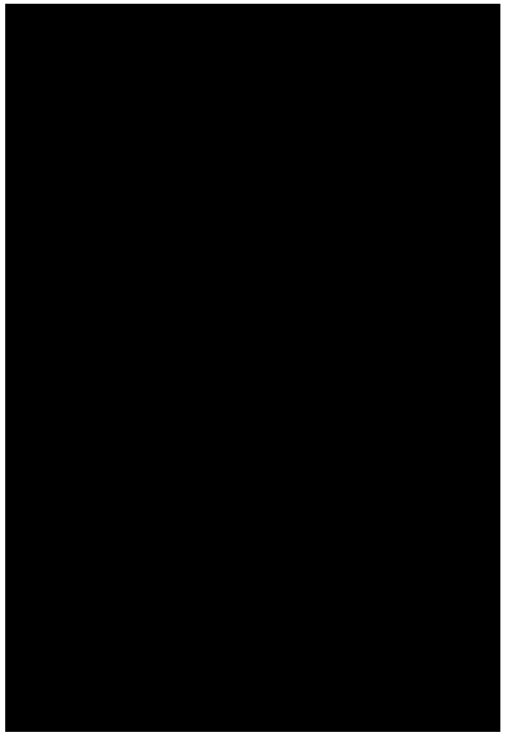
Bleach Observations



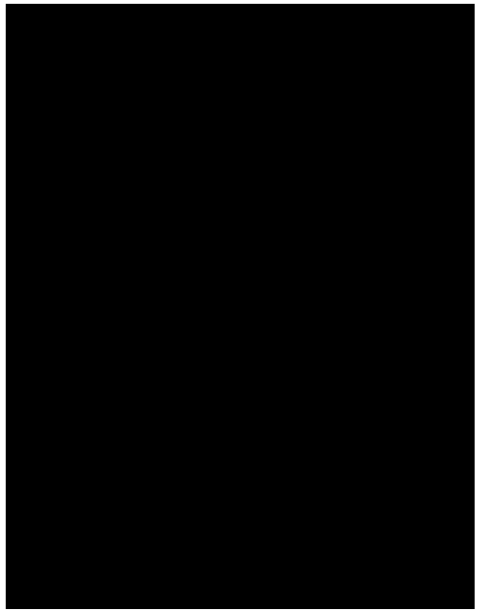
Bone exposure after one day



Fingernails became soft/gummy, but edges protected by tissue



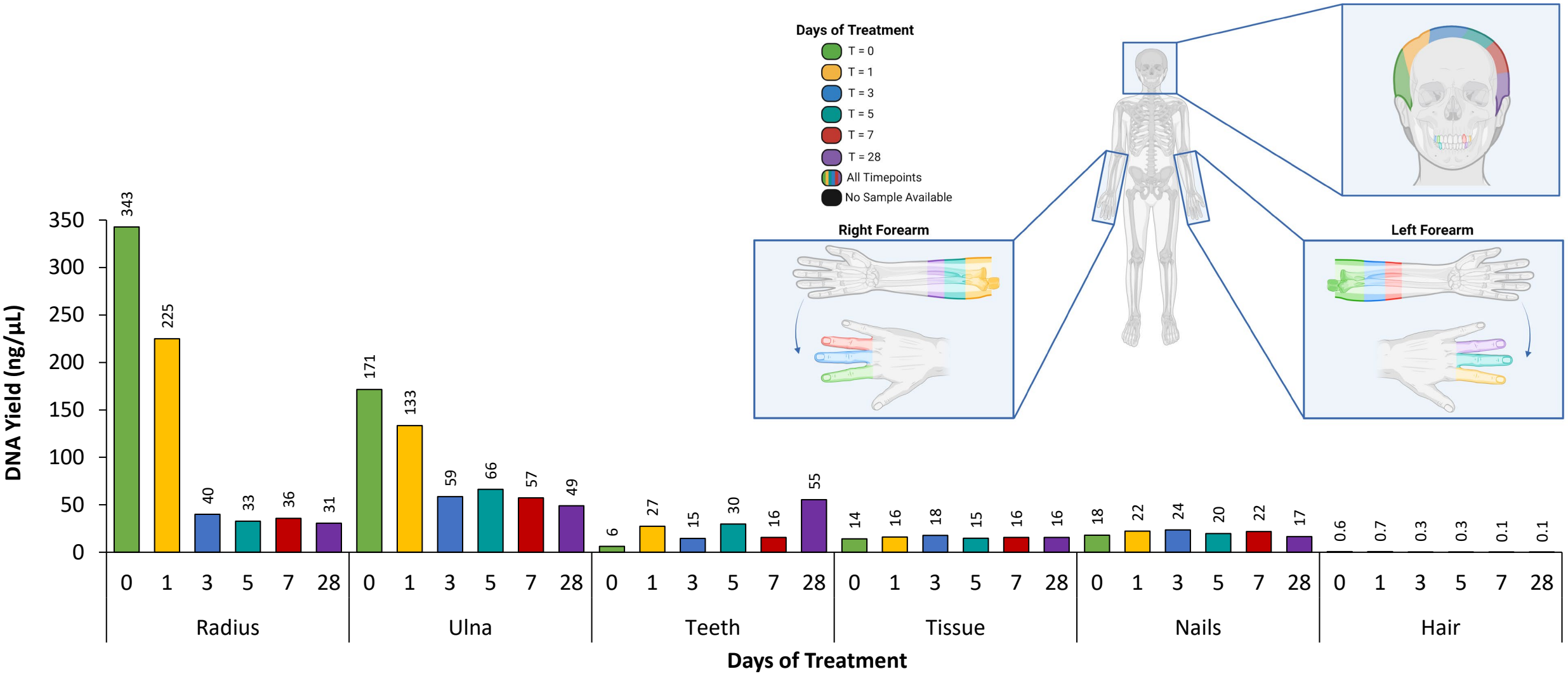
Tissue turned yellow/brown



Solution became viscous

Minimal change after day 1

Bleach Results

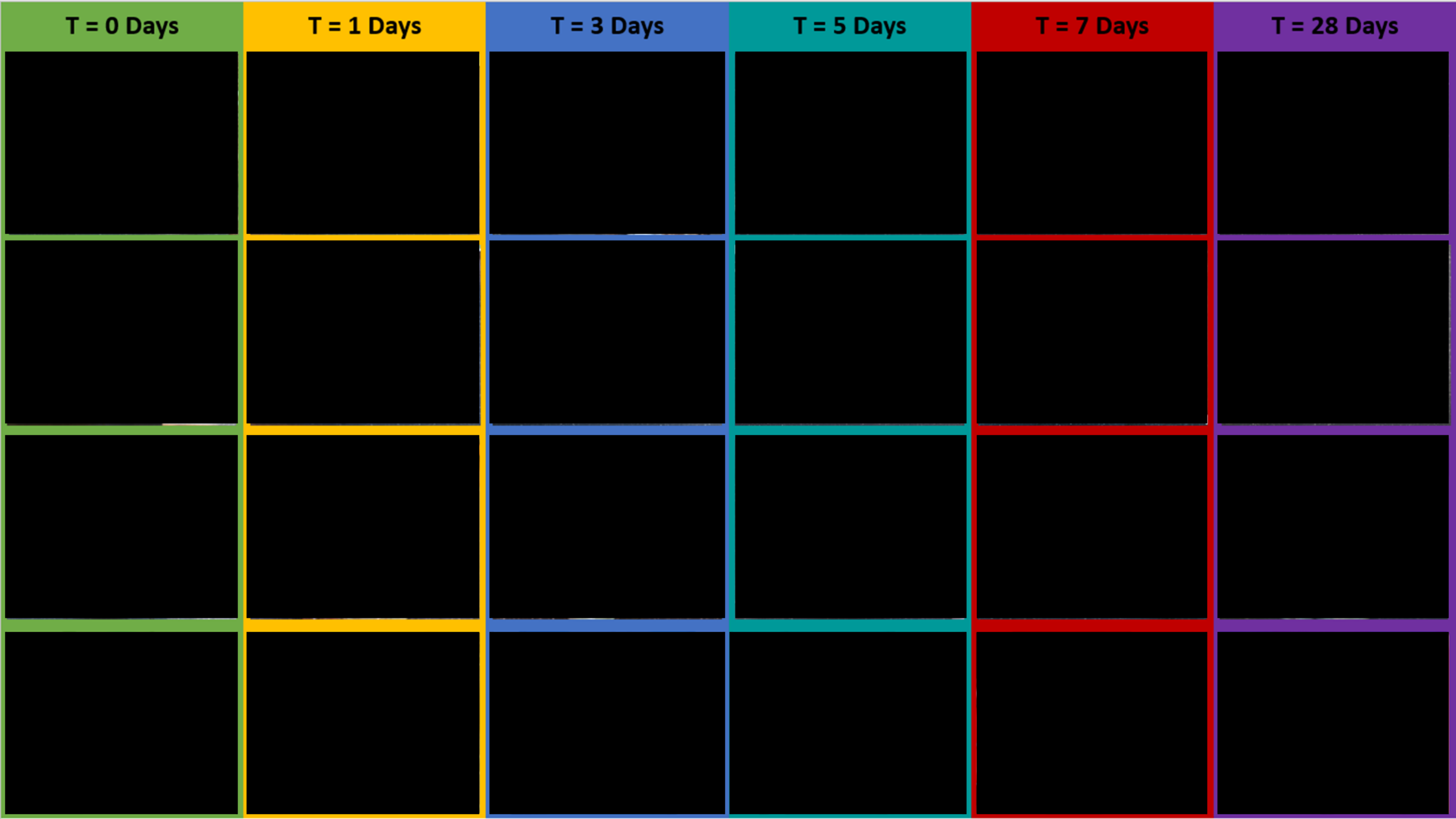


Recovered sufficient DNA to produce **full, concordant** profiles for **ALL** samples

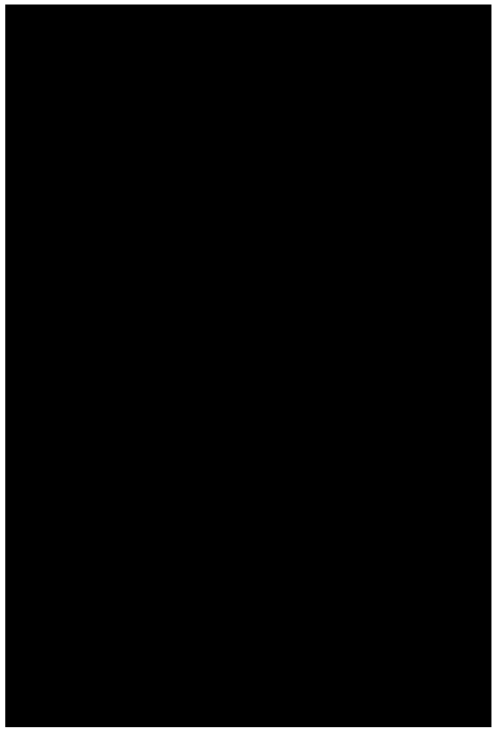
Rid-X®

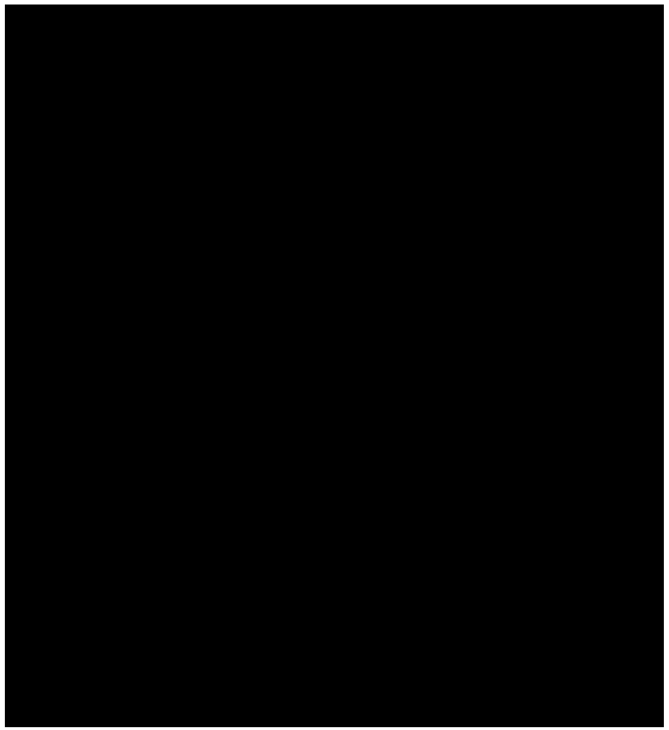
- **Active Ingredients:** bacteria and enzymes
- **Concentration:** proprietary
- **pH:** 6.3 – 8.8

https://images.thdstatic.com/productImages/88ba42f7-8960-4d0f-b662-09f06c24a50d/svn/rid-x-drain-cleaners-19200-89447-64_1000.jpg

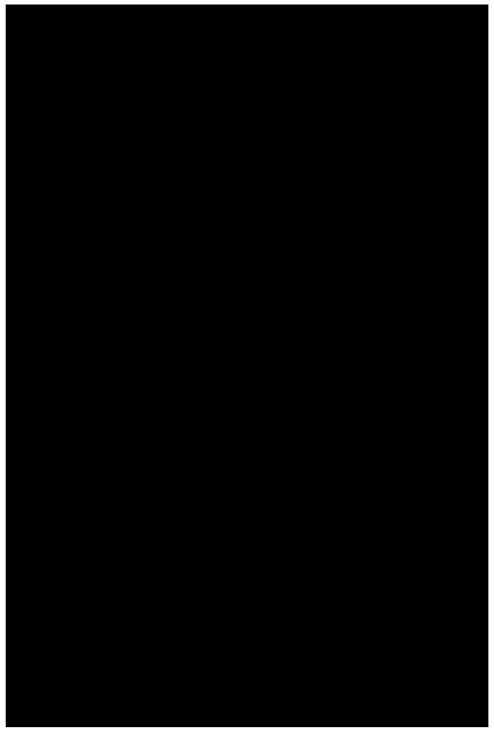

T = 0 Days
T = 1 Days
T = 3 Days
T = 5 Days
T = 7 Days
T = 28 Days

Rid-X® Observations



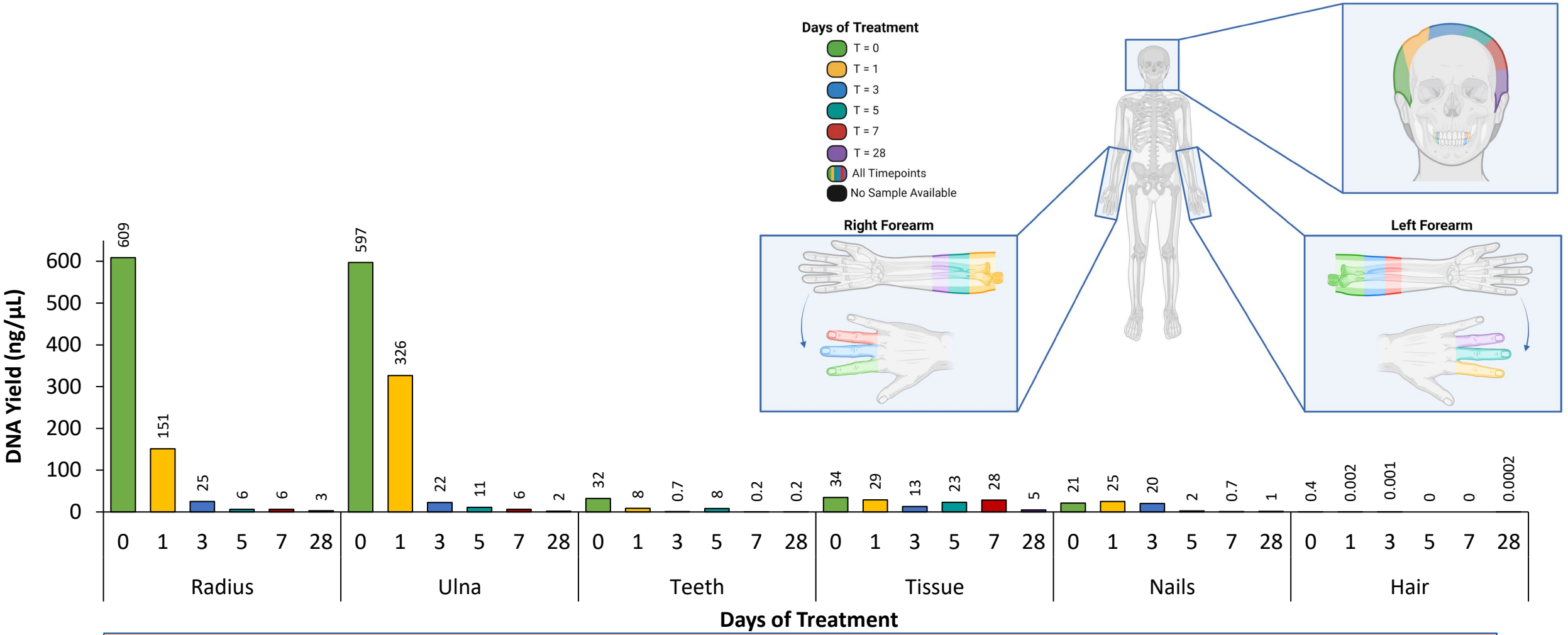


Blue/green discoloration –
Started in areas of skin slippage but progressed to all samples



Mold growth –
Between week 1 and 2 of treatment

Rid-X® Results



Recovered sufficient DNA to produce **full, concordant** profiles for **ALL SAMPLES, EXCEPT HAIR.** MtDNA analysis of hair was successful.

Lye

- **Active Ingredients:** sodium hydroxide
- **Concentration:** 1:1 mixture with water; 25 M
- **pH:** 13 – 14

https://images.thdstatic.com/productImages/a6c03c8d-6cf3-4368-ab37-fa7886b0d1ae/svn/instant-power-drain-cleaners-1650-64_1000.jpg

T = 0 Days	T = 1 Days	T = 3 Days	T = 5 Days	T = 7 Days	T = 28 Days
					
					

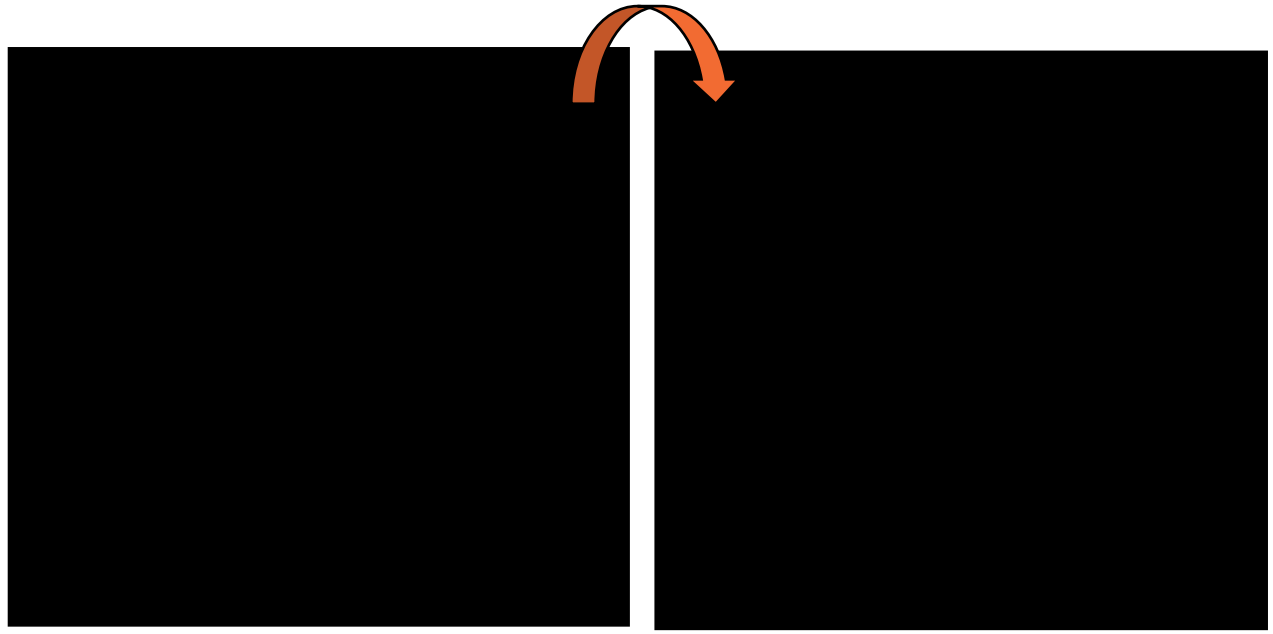
Lye Observations

Drastic deterioration after 1 day of exposure

Exothermic reaction – at least 70°C

Bone embedded within undissolved lye and detergent from continuous saponification of adipose tissue

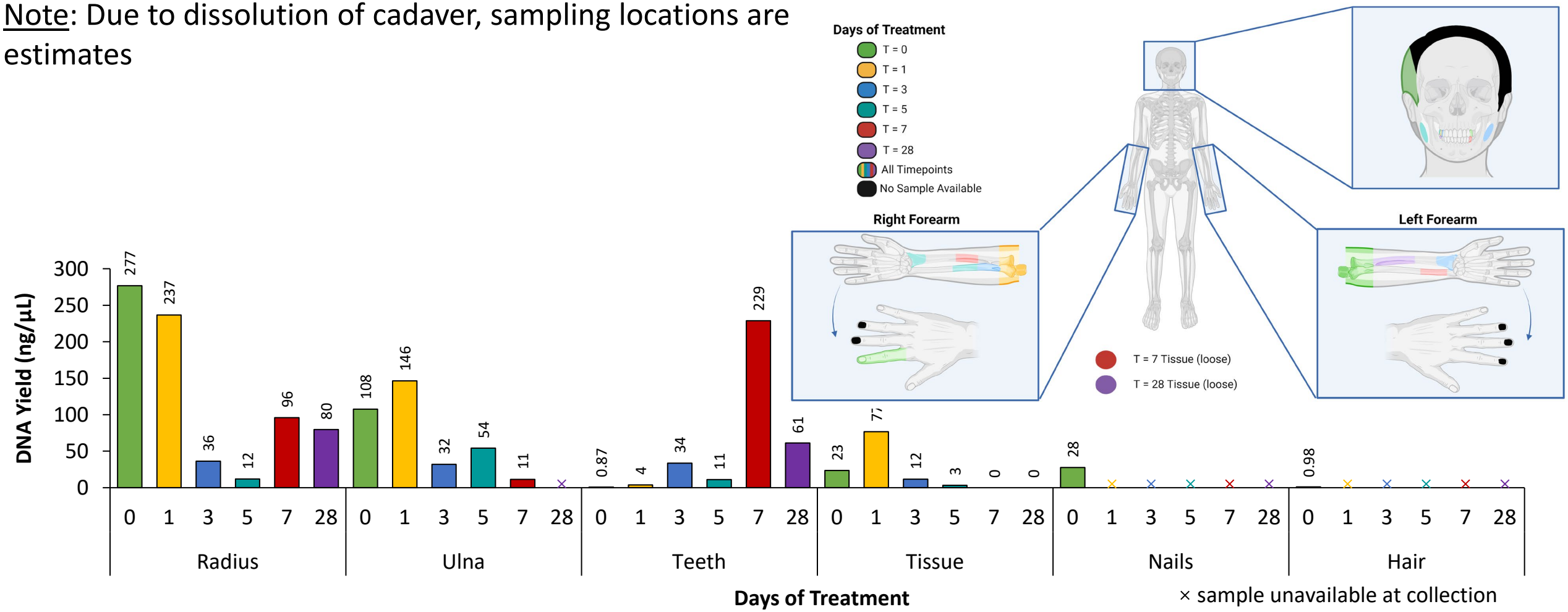
Periosteum began flaking – bone appeared translucent



Hardened top layer but mixture of undigested lye, detergent, bone, and tissue underneath

Lye Results

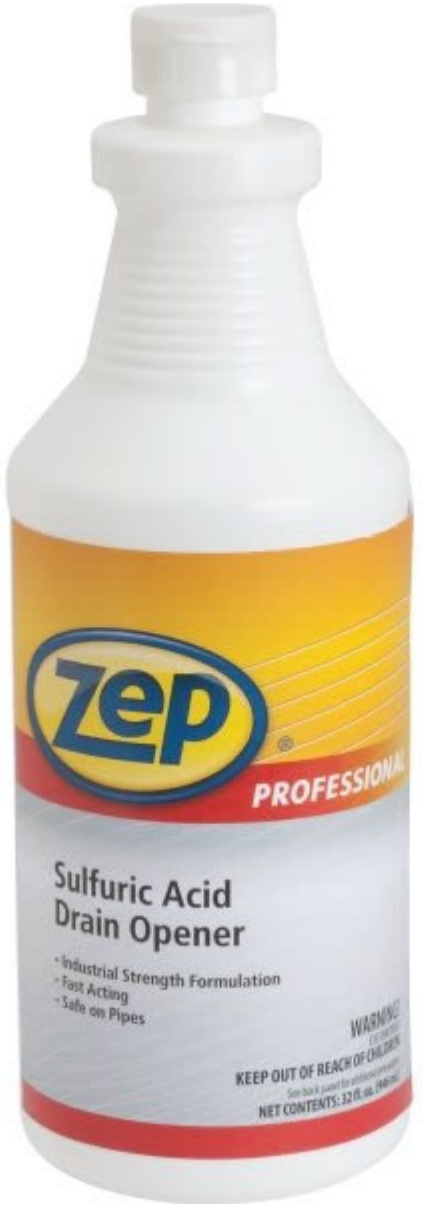
Note: Due to dissolution of cadaver, sampling locations are estimates



Recovered sufficient DNA to produce **full, concordant** profiles for **ALL TIMEPOINTS** through skeletal samples (bone/teeth). MtDNA analysis unsuccessful on tissue 7 and 28 samples.

Sulfuric Acid

- **Active Ingredients:** sulfuric acid
- **Concentration:** 90-100%
- **pH:** <1



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T = 0 Days	T = 1 Days	T = 3 Days	T = 5 Days	T = 7 Days	T = 28 Days
			No Forearm Samples Remained		
					All Remaining Fingernails Taken at T = 7
				No Head Samples Remained	
					

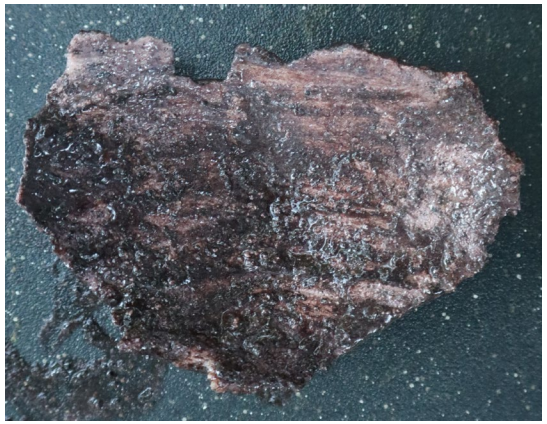
Sulfuric Acid Observations

Only skull fragments, brain tissue, and teeth remained after exposure to sulfuric acid

First chemical with signs of corrosion to teeth

Chemical became dark, and viscous – like motor oil

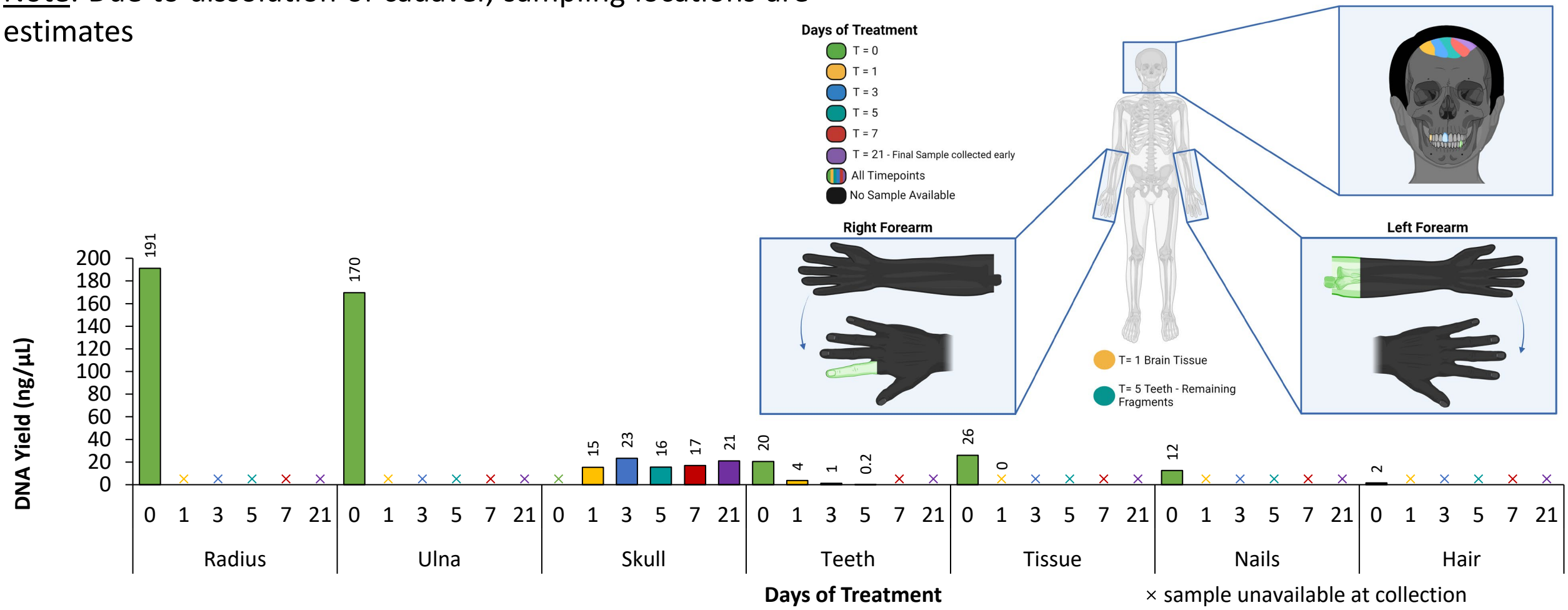
Exothermic reaction – at least 60°C



Skull fragment floated; remained for at least 21 days

Sulfuric Acid Results

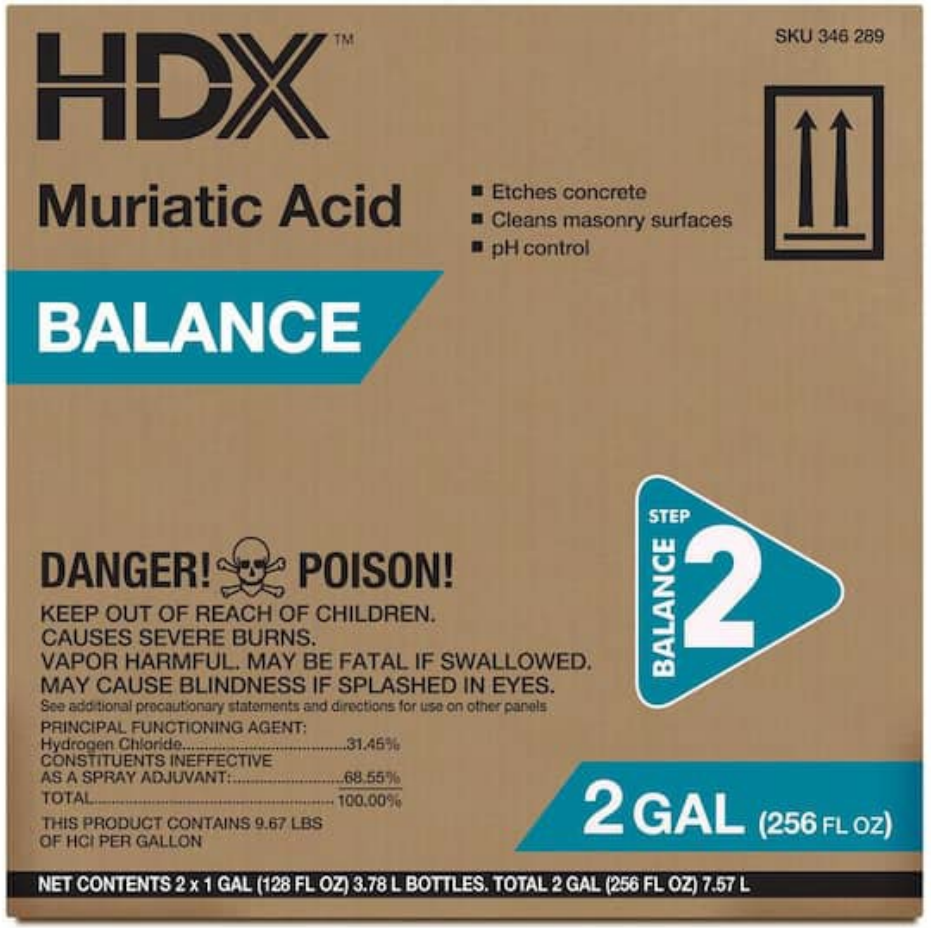
Note: Due to dissolution of cadaver, sampling locations are estimates



Recovered sufficient DNA to produce **full, concordant** profiles for **ALL SKELETAL SAMPLES**. **HID POSSIBLE UP TO 3 WEEKS**. MtDNA analysis unsuccessful for tissue samples.

Hydrochloric Acid

- **Active Ingredients:** hydrogen chloride
- **Concentration:** 25-35%
- **pH:** <1



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T = 0 Days	T = 1 Days	T = 3 Days	T = 5 Days	T = 7 Days	T = 28 Days
			No Forearm Samples Remained		
					All Remaining Fingernails Taken at T = 7
				No Head Samples Remained	
					

Hydrochloric Acid Observations

Most tissue dissolved after one day, but bone remained

Tissue turned a light green

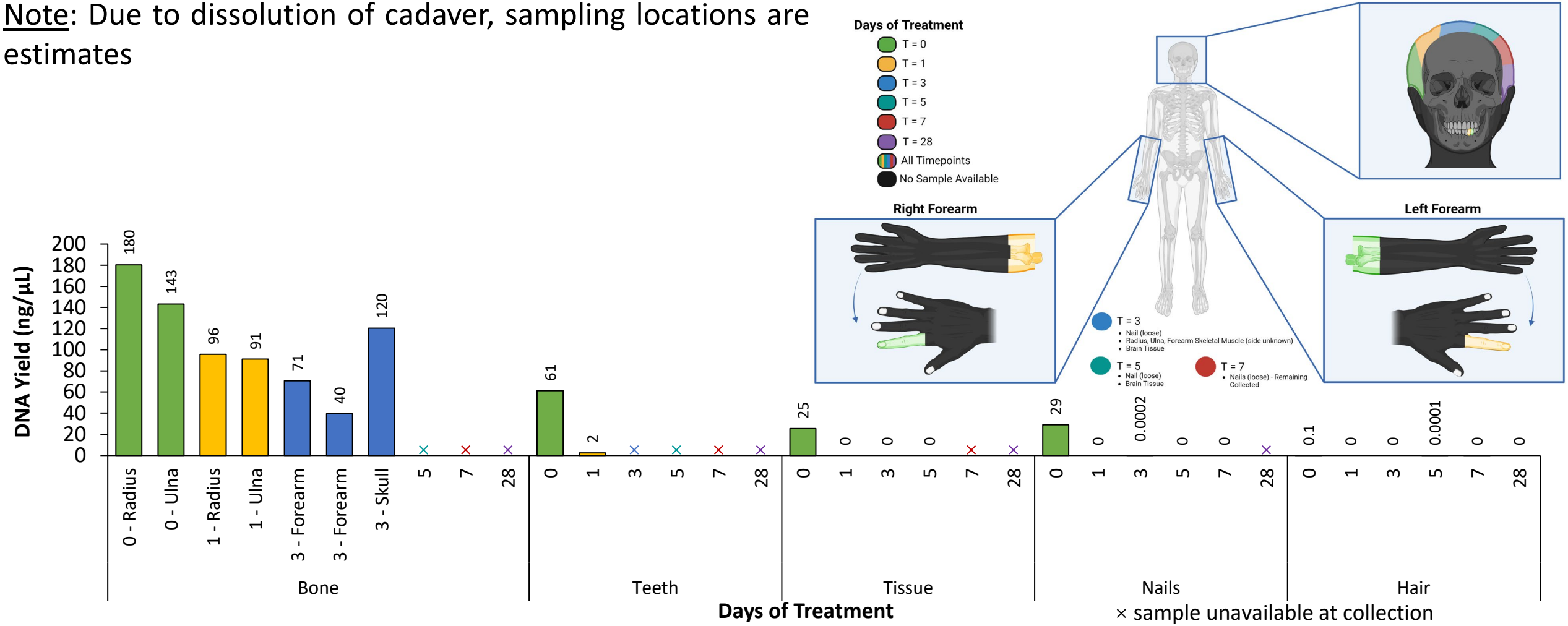
Bone appeared ribbed then frayed

Only restorative dental material remained at 3 days of exposure

Fingernail fragments remained until at least 7 days – removed at this point

Hydrochloric Acid Results

Note: Due to dissolution of cadaver, sampling locations are estimates



Recovered sufficient DNA to produce **full, concordant** profiles for **ALL SKELETAL SAMPLES**. **HID POSSIBLE UP TO 3 DAYS**. MtDNA analysis unsuccessful for hair, nails, and tissue.

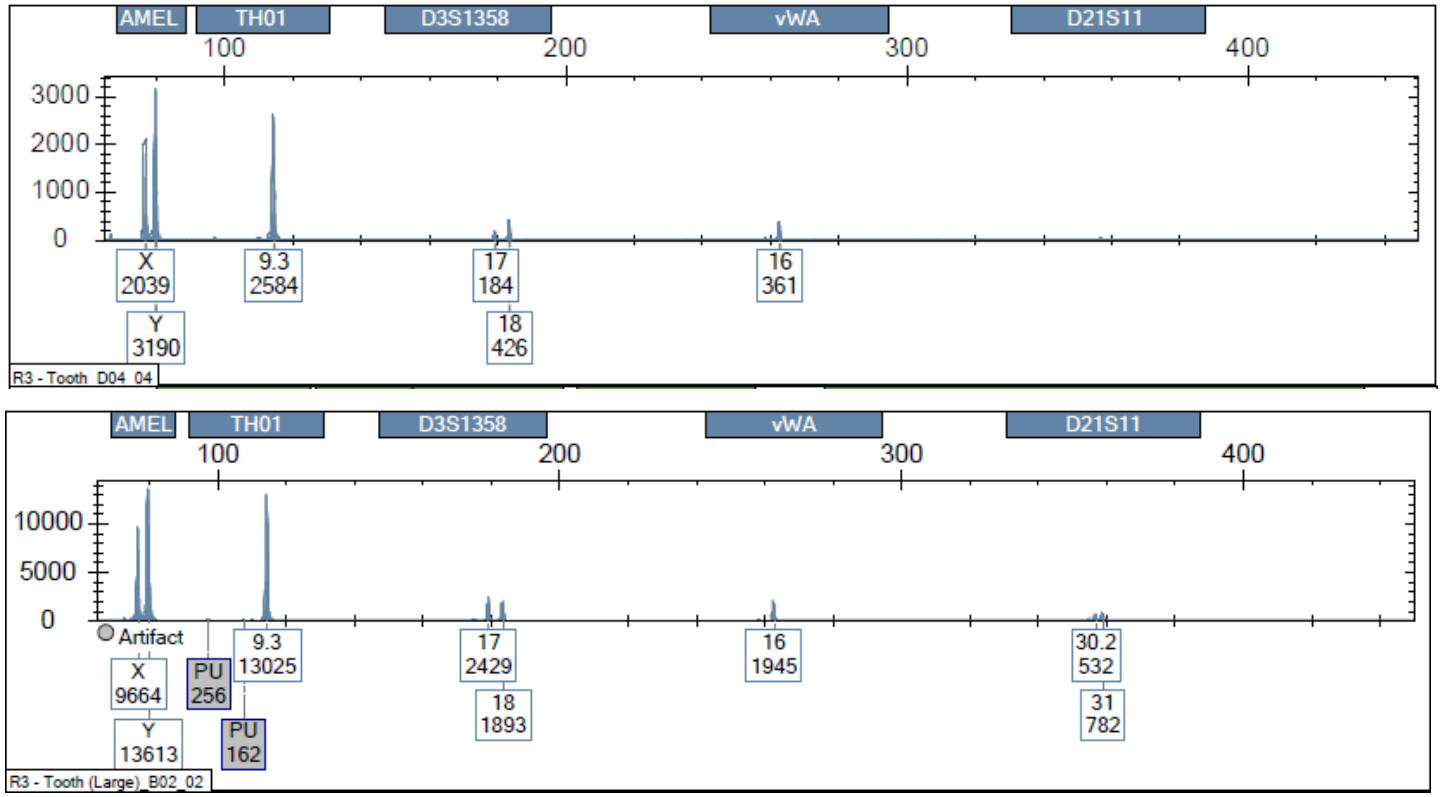
PCR Amplification Modifications

- "Composite" profiles required for several samples
- High amounts of small fragment DNA – required dilutions for PCR amplification
- Extreme degradation, so larger amplicons were being lost – despite sufficient yield
- Solution – Two PCR amplifications

Normalized using [Small Target]

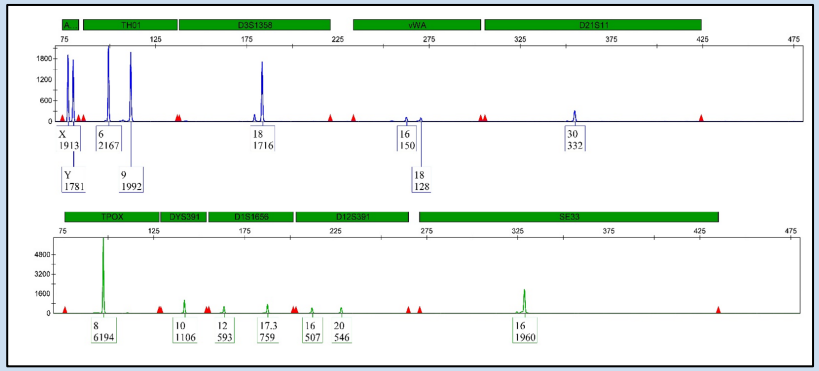
– Then –

Normalized using [Large Target]



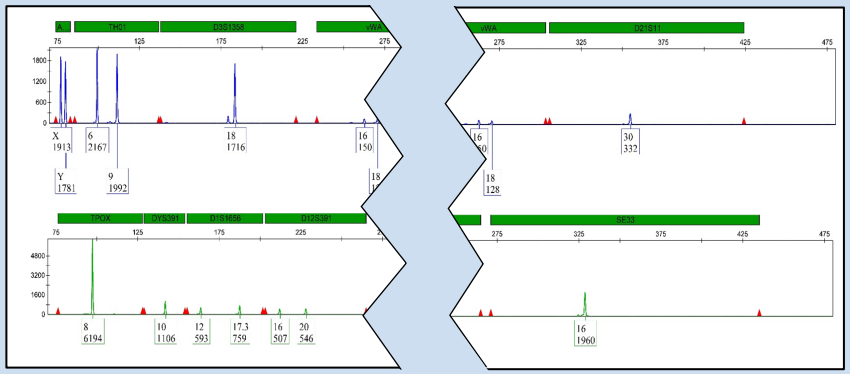
HID Success

Successful HID



Traditional STR Typing

36%



Composite STR Profiles

42%

MtDNA Analysis

5%

Unsuccessful HID

17%

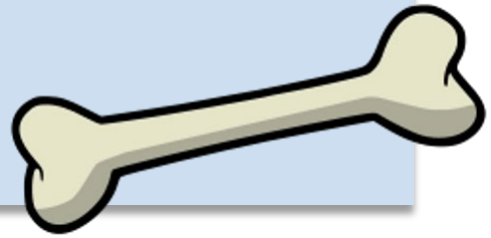
Duration of Success

	SUN	MON	TUE	WED	THU	FRI	SAT
Week 1	X	X	Hydrochloric Acid ✓	X	X	X	X
Week 2	X	X	X	X	X	X	X
Week 3	X	X	X	X	X	X	Sulfuric Acid ✓
Week 4	X	X	X	X	X	X	Bleach, Rid-X, & Lye ✓

Major Findings

Each chemical had a distinct appearance and effect on tissue

100% success in HID with skeletal elements (bone or teeth)



Thank you!

- Bode Technology, Manzar Ahmed
- STAFS Facility, the donors, and their family
- Forensic Science Foundation - Lucas Grant
- SHSU Forensic Science Department and

Team DNA-Yay!

References

1. Holden DHS, Simpson DK. The Acid-Bath Murder(s): Rex v. John George Haigh. The Police Journal 1950;23(3):190–202. https://doi.org/10.1177/0032258X5002300306

2. Replogle J. Hope Dwindles For DNA Evidence At Tijuana Body Dump. KPBS. 2014. https://www.kpbs.org/news/2014/sep/03/hope-dwindles-dna-evidence-tijuana-body-dump/

3. Booth W. “Stewmaker” stirs horror in Mexico. NBC News. 2009. https://www.nbcnews.com/id/wbna28868152

4. Ryan P. Real-life crimes echo “Breaking Bad.” USA Today. 2013. https://www.usatoday.com/story/life/tv/2013/09/19/breaking-bad-real-life-crimes/2782635/

5. Loreille OM, Diegoli TM, Irwin JA, Coble MD, Parsons TJ. High efficiency DNA extraction from bone by total demineralization. Forensic Sci Int Genet 2007;1(2):191–5. https://doi.org/10.1016/j.fsigen.2007.02.006.

Questions?

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