FSF FORENSIC SCIENCES FOUNDATION
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Evaluation of DNA Recovery from Chemically Treated Human Remains

Jenn Snedeker, PhD; Rachel Houston, PhD; Sheree Hughes, PhD

Department of Forensic Science

Sam Houston State University

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Funding/Disclosures

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Content Warning

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

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

What do these three men have in common?

John George Haigh[1]

"Acid Bath Murderer"

Santiago Lopez[2,3]

"El Pozolero" or "Stew Maker"

Jason Hart[4]

"Breaking Bad Killer"

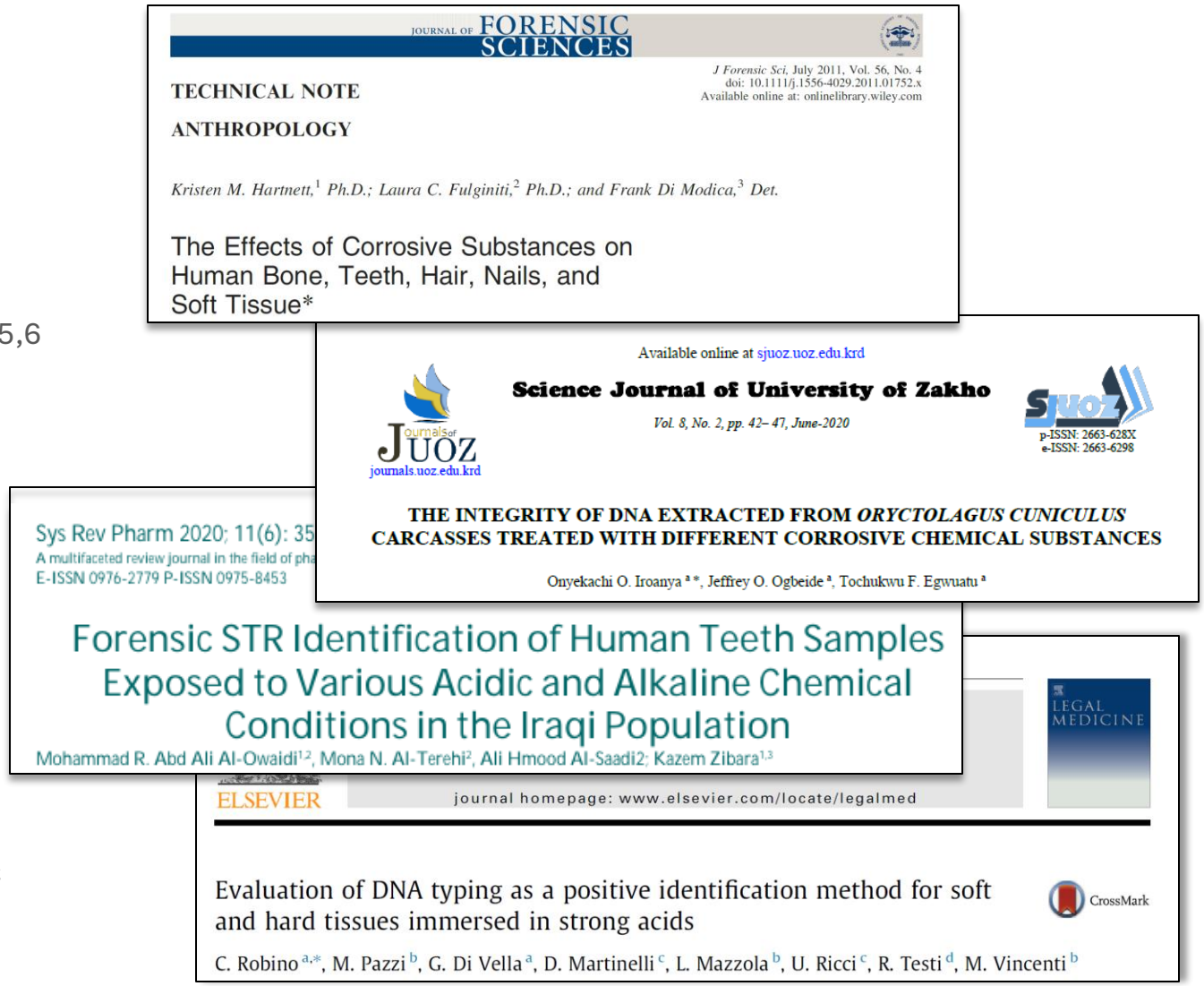
All used chemicals to dissolve human remains

Prevalence

- This practice spans decades
- Includes everyone from drug cartel members to TV show fanatics
- Little to no research discussing DNA recovery from these challenging samples
  - Lack of knowledge in field

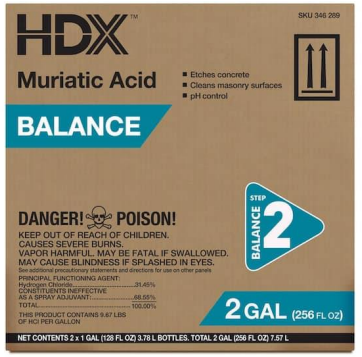
Limitations of Current Studies

1. Most work focuses on morphological changes of isolated fragments of tissue[5,6]
   - Do not address DNA
2. Use animal proxies[7,8]
3. Only examine isolated human teeth[9,10]
4. Many use laboratory grade chemicals[8]
   - Not readily accessible to the public



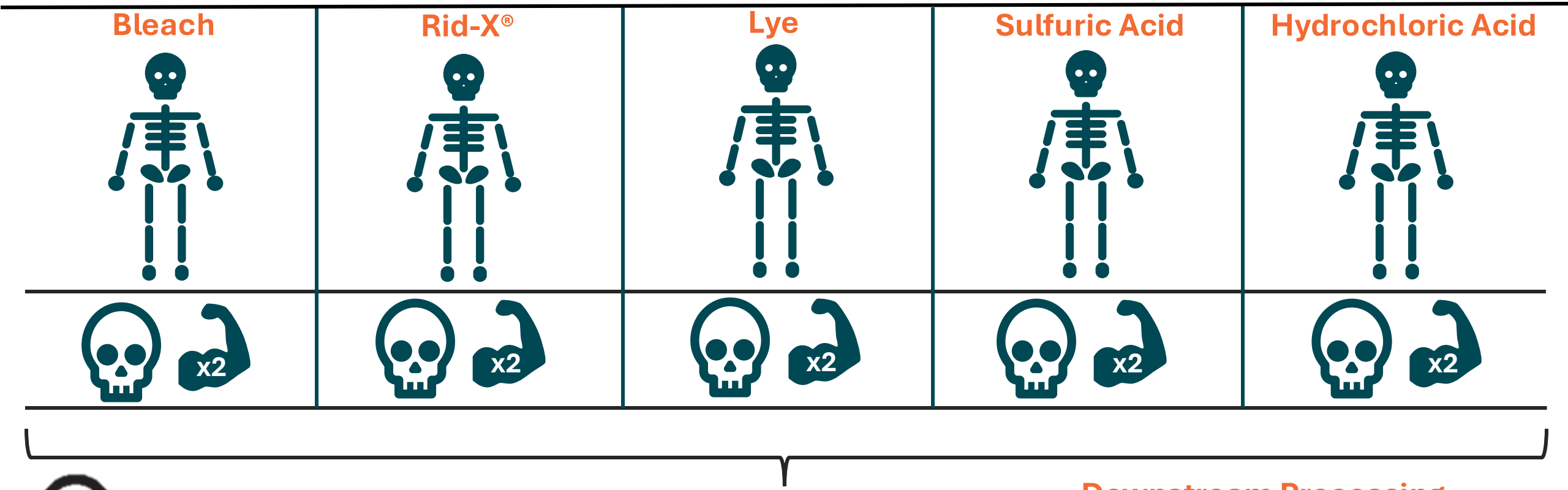
Chemicals

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

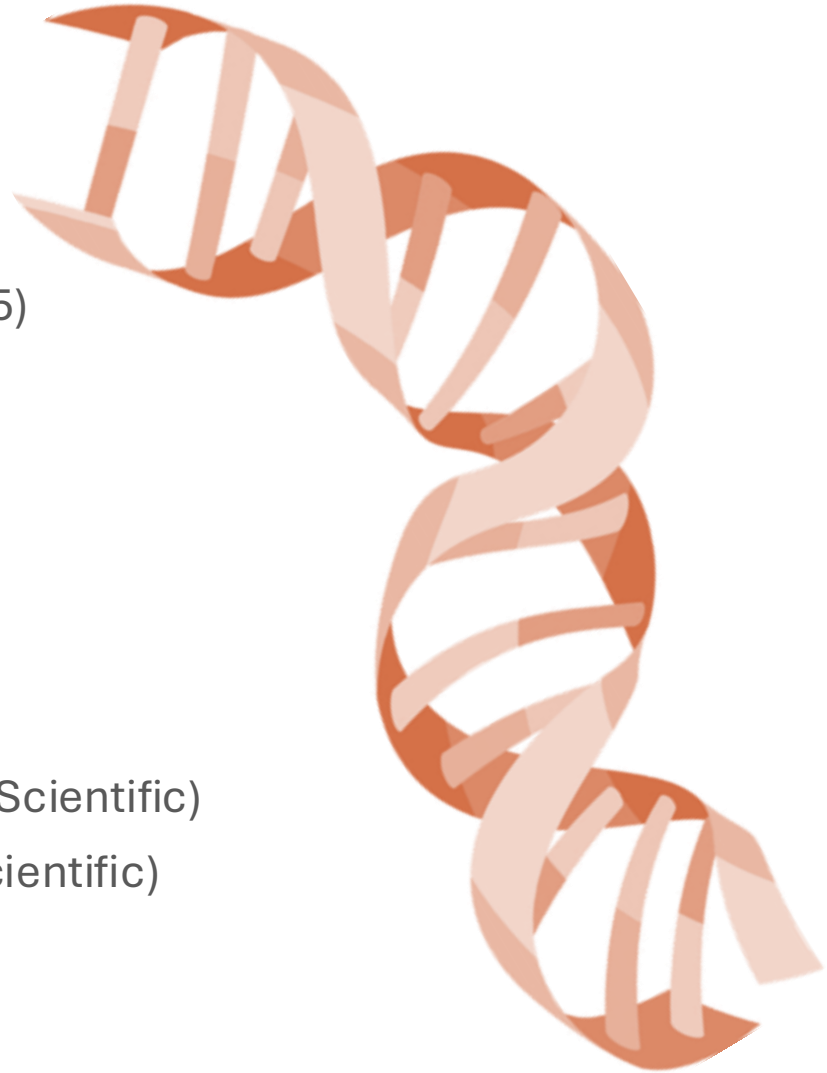


Sampling Design

- 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 and 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[11]
    - 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), ABI 7500 (Thermo Fisher Scientific)
- **STR Typing:** Investigator 24plex QS (QIAGEN), ABI 3500(Thermo Fisher Scientific)
- **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)

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

SOUTHEAST TEXAS APPLIED FORENSIC SCIENCE FACILITY

Chemical Damage Sampling

Measure & Sample

Sampling Setup

PPE

Bleach

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

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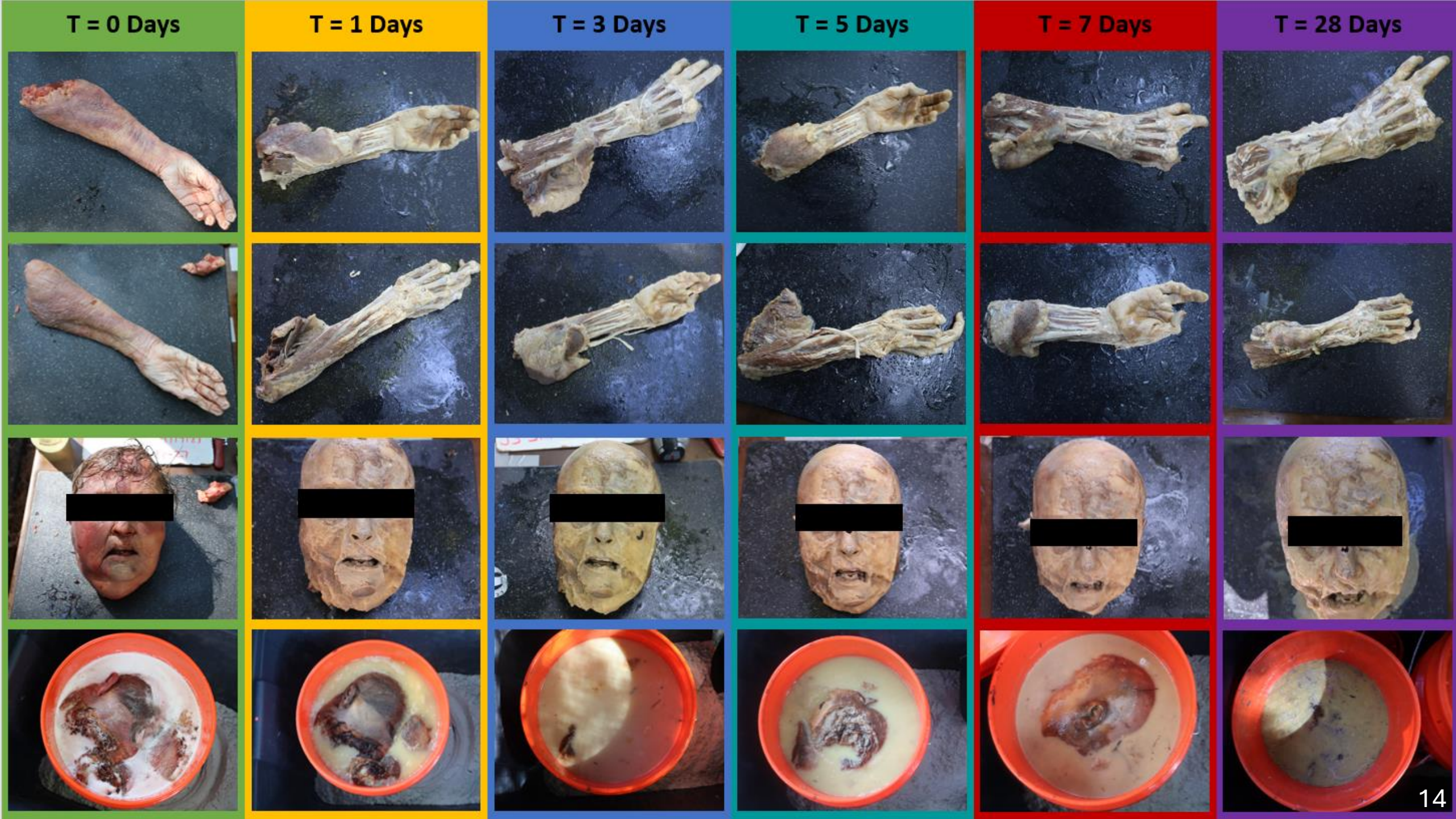
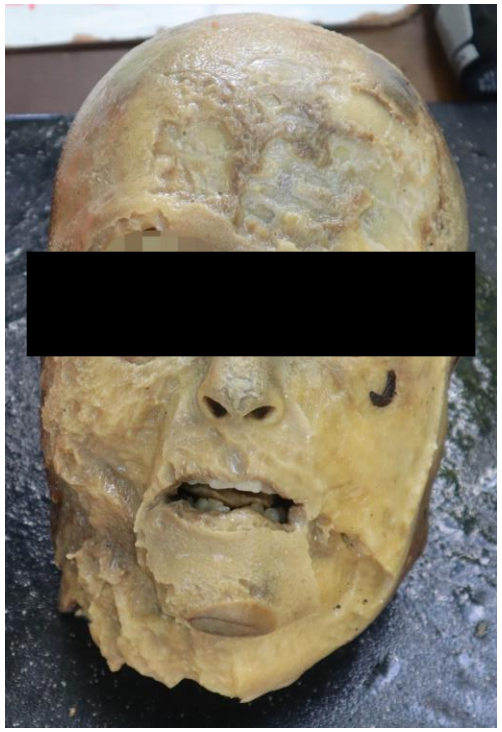

T = 0 Days
T = 1 Days
T = 3 Days
T = 5 Days
T = 7 Days
T = 28 Days

Bleach Observations

Bone exposure after one day

Fingernails became soft/gummy, but edges protected by tissue

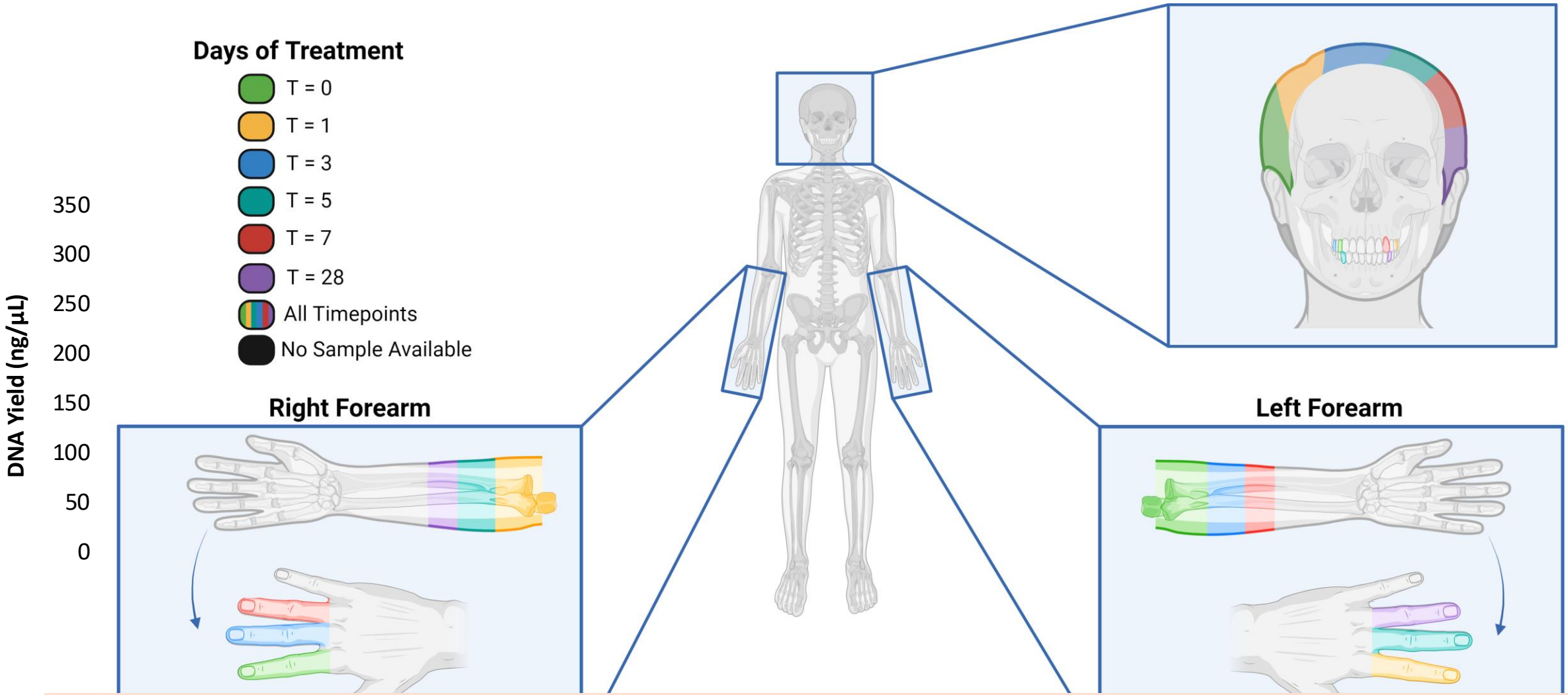


Tissue turned yellow/brown

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

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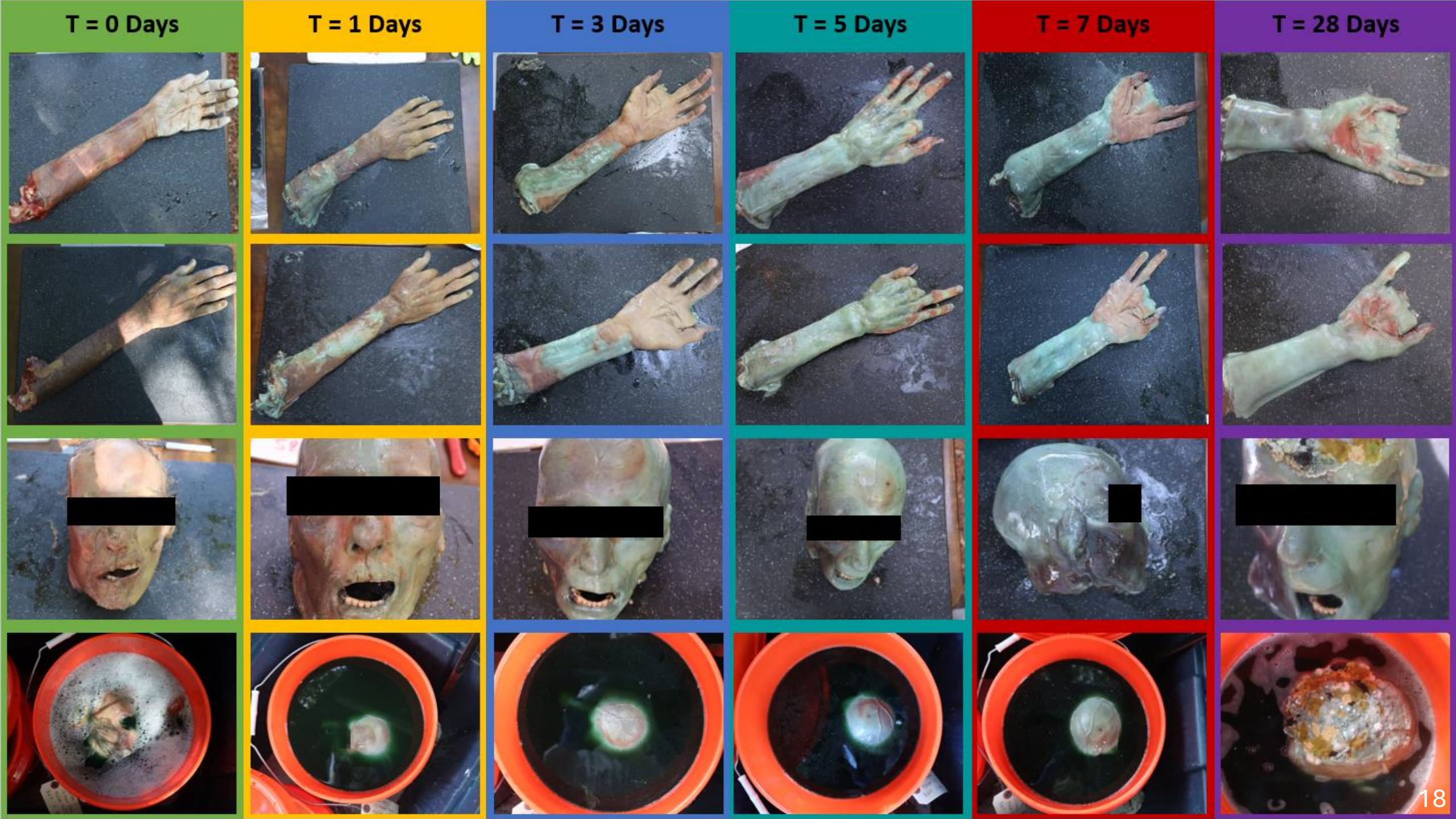
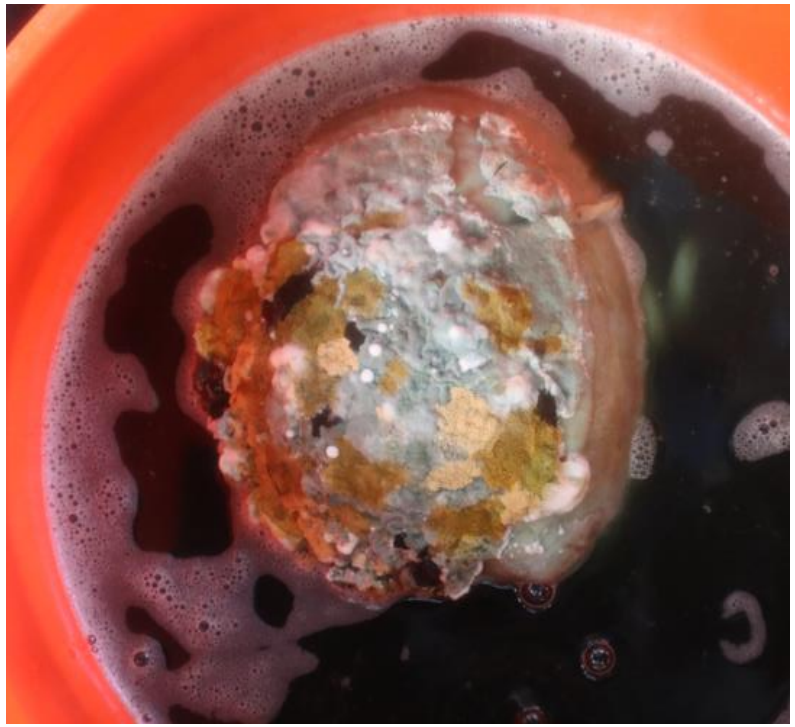
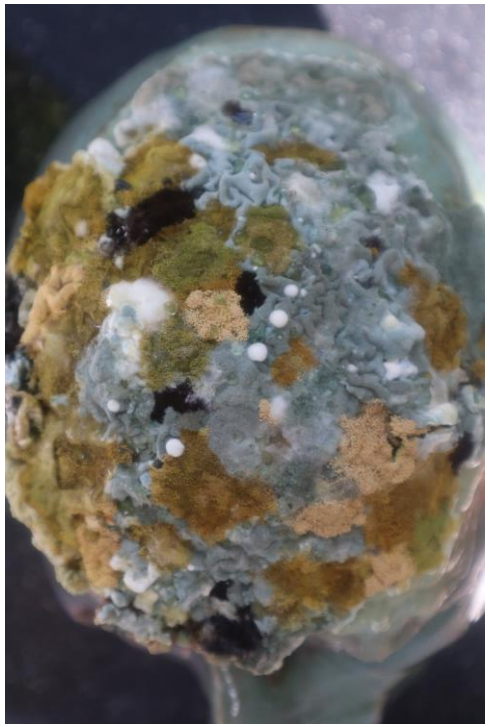

T = 0 Days
T = 1 Days
T = 3 Days
T = 5 Days
T = 7 Days
T = 28 Days

Rid-X® Observations

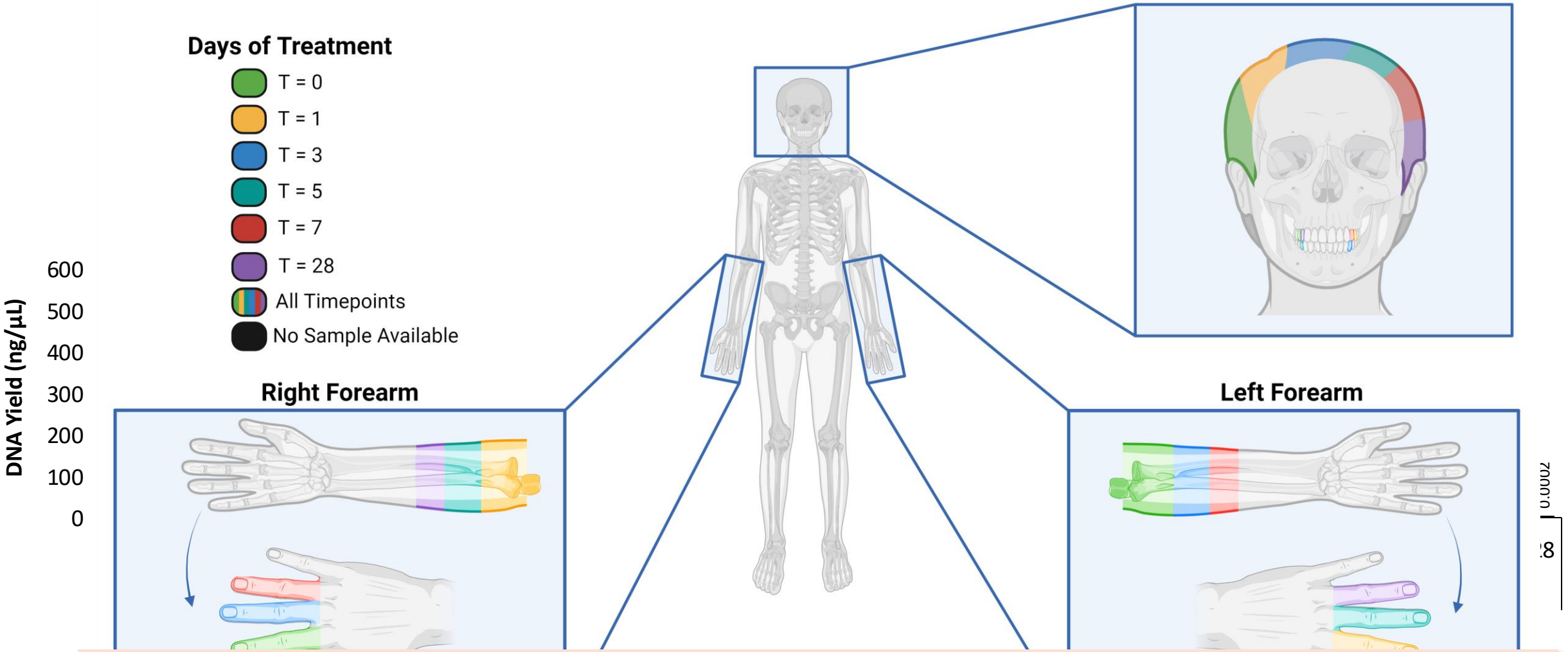
Blue/green discoloration –
Started in areas of skin slippage but progressed to all samples





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

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

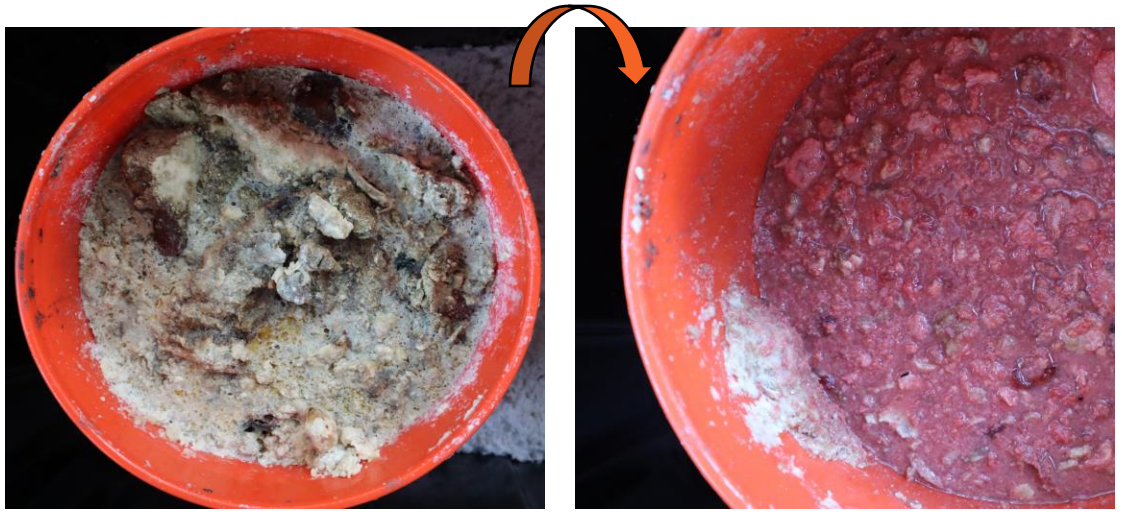
Lye Observations

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

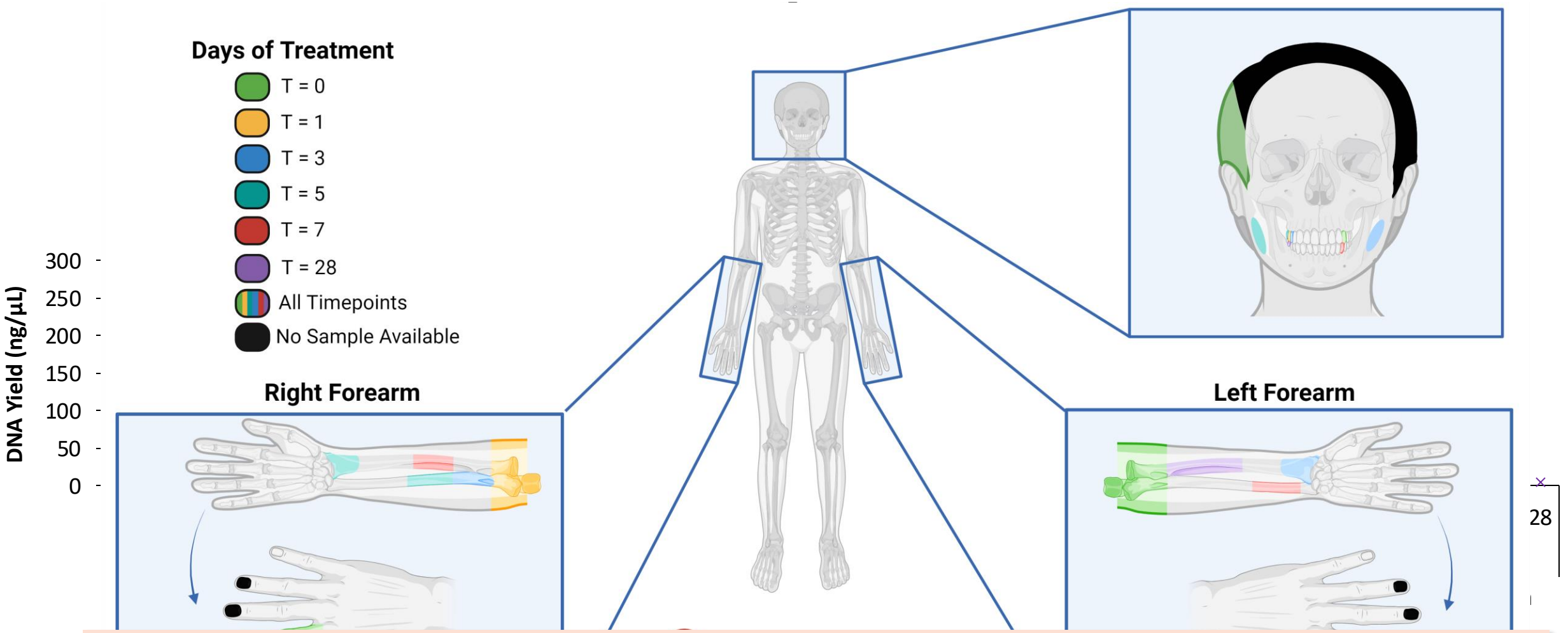
Periosteum began flaking – bone appeared translucent



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

Lye Results

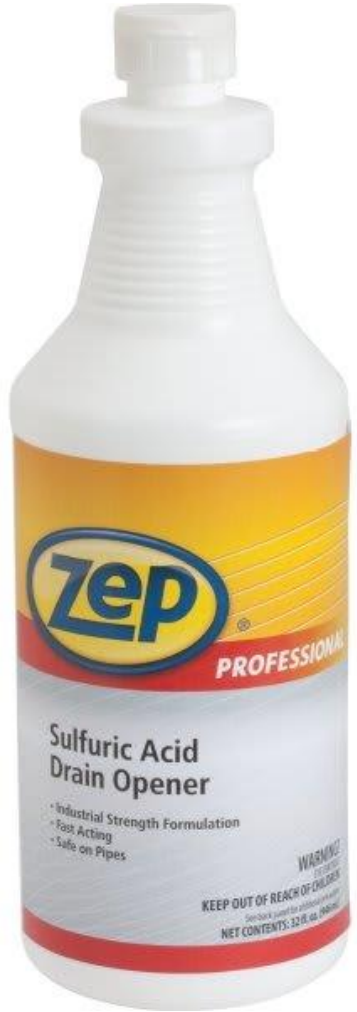
Note: Due to dissolution of cadaver, sampling locations are estimates



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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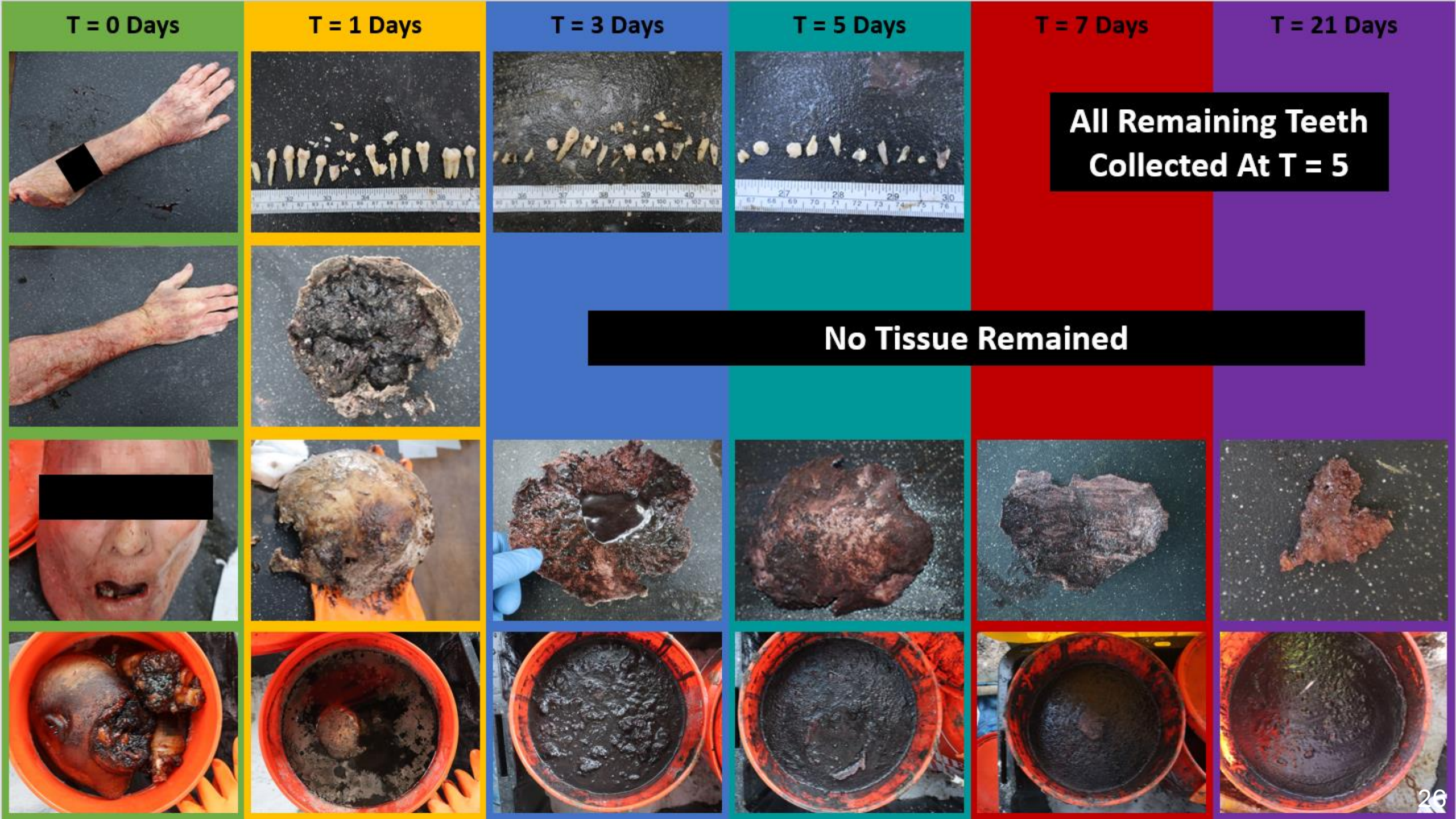
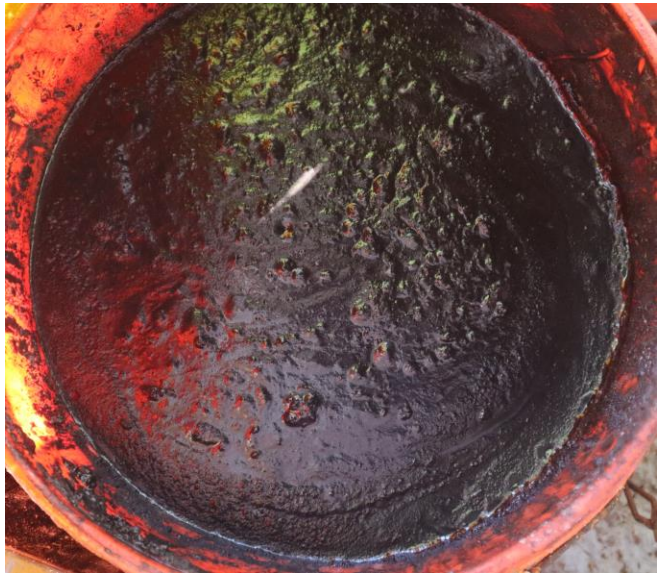

T = 0 Days
T = 1 Days
T = 3 Days
T = 5 Days
T = 7 Days
T = 21 Days
All Remaining Teeth Collected At T = 5
No Tissue Remained

Sulfuric Acid Observations

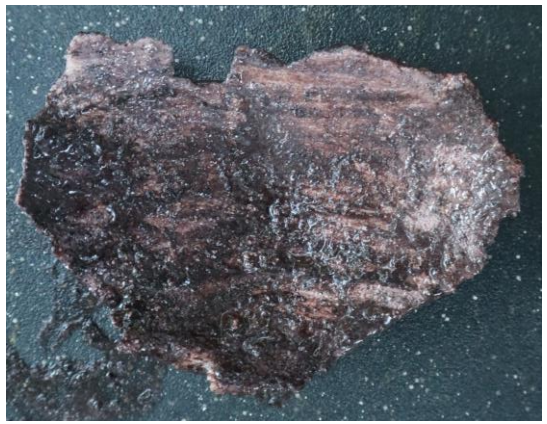
Only skull fragments, brain tissue, and teeth remained after exposure to sulfuric acid

First chemical with signs of corrosion to teeth



Chemical became dark, and viscous – like motor oil

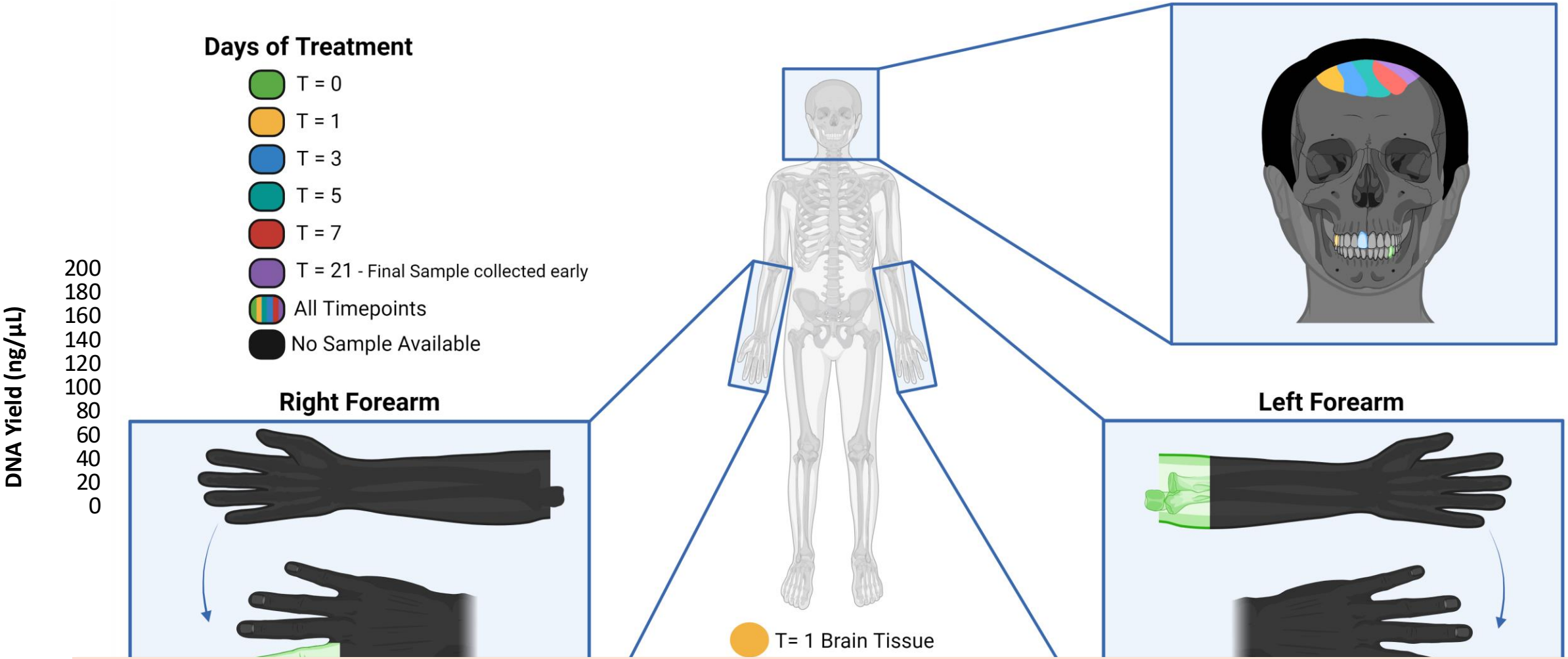
Exothermic reaction – at least 60°C



Skull fragment floated; remained for at least 21 days

Sulfuric Acid Results

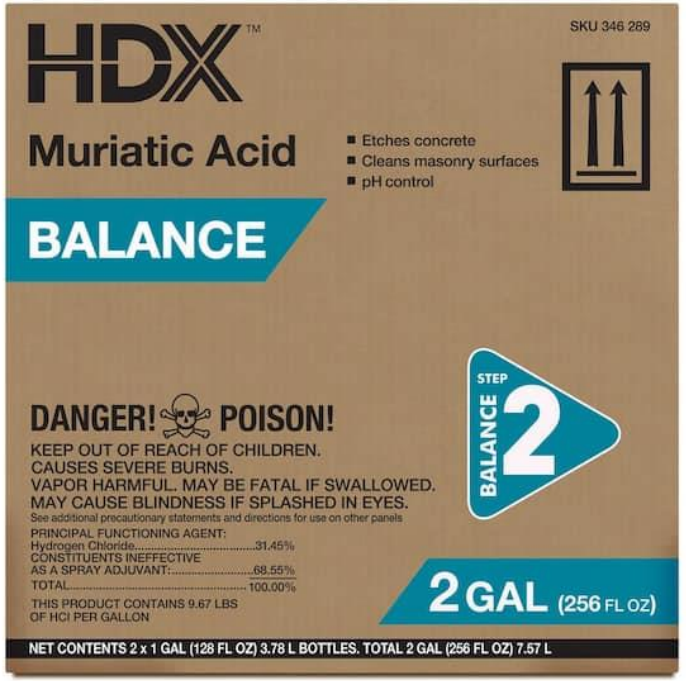
Note: Due to dissolution of cadaver, sampling locations are estimates



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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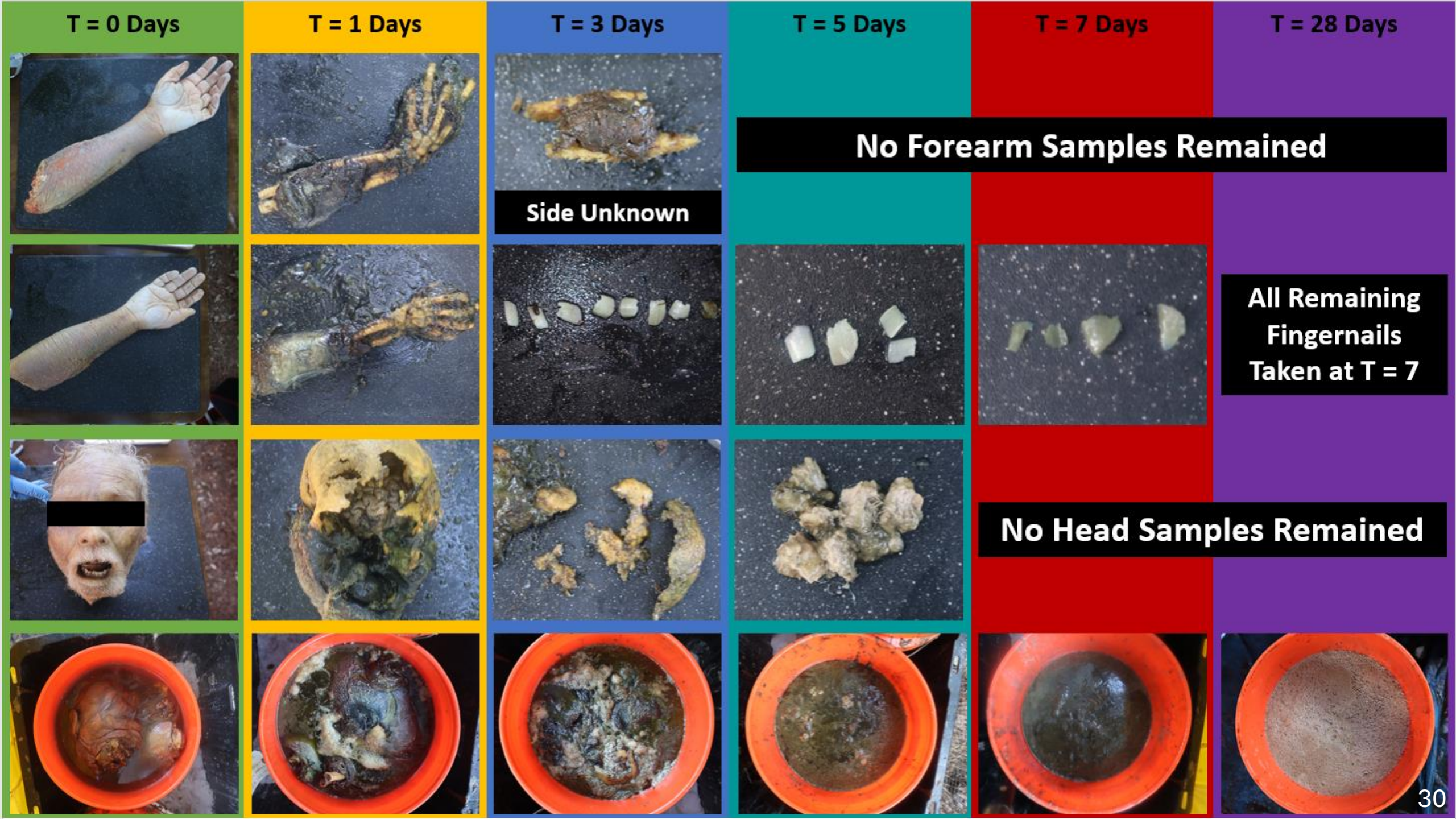

T = 0 Days
T = 1 Days
T = 3 Days
T = 5 Days
T = 7 Days
T = 28 Days
Side Unknown
No Forearm Samples Remained
All Remaining Fingernails Taken at T = 7
No Head Samples Remained

Hydrochloric Acid Observations

Most tissue dissolved after one day, but bone remained

Tissue turned a light green

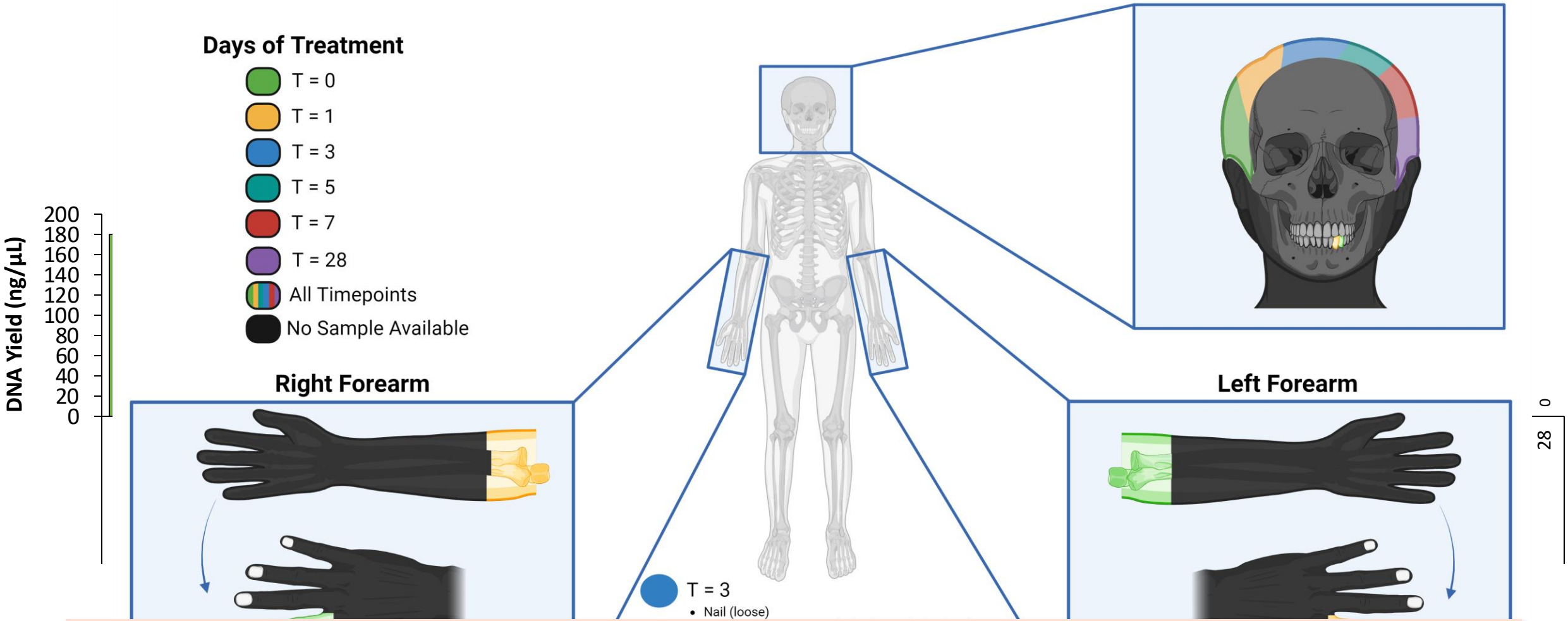
Bone appeared ribbed then frayed

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

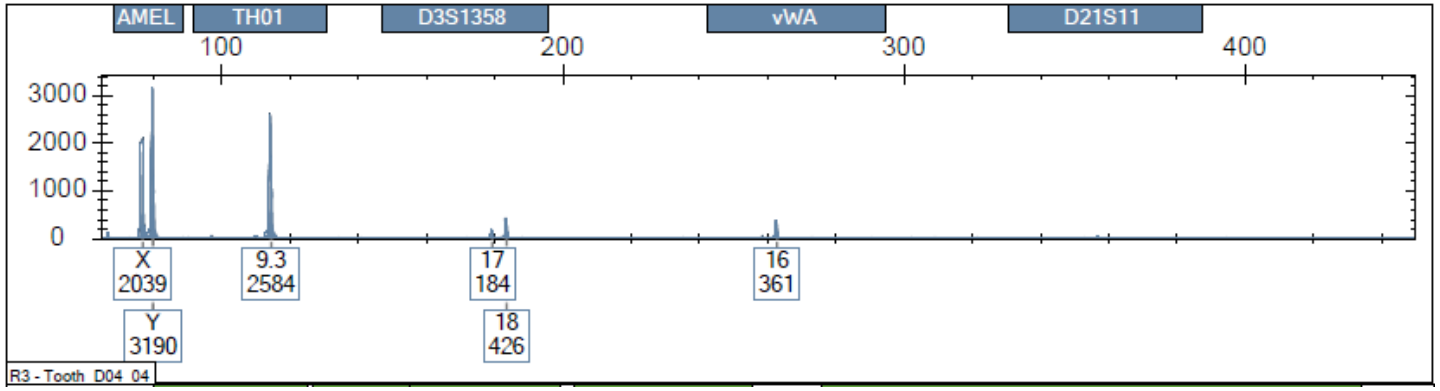


Recovered sufficient DNA to produce **full, concordant** profiles for **ALL SKELETAL SAMPLES. HID POSSIBLE UP TO 3 DAYS.** mtDNA analysis unsuccessful for hair, nails, and tissue.

PCR Amplification Modifications

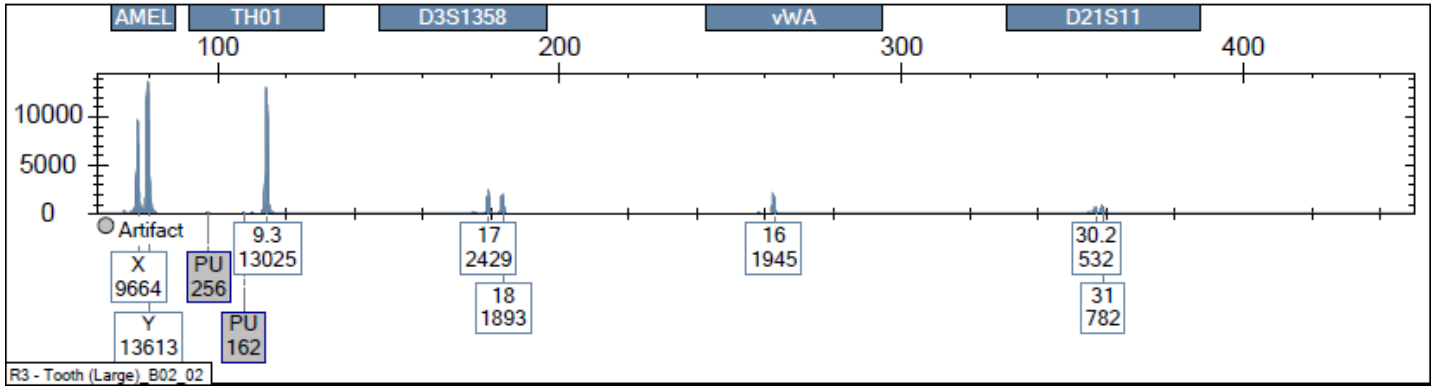
- "Composite" profiles required for several samples
- High amounts of small fragment DNA – required dilutions for PCR amplification
- Extreme degradation, so larger amplicons were being lost – despite sufficient yield
- Solution – Two PCR amplifications

Normalized using [Small Target]



– Then –

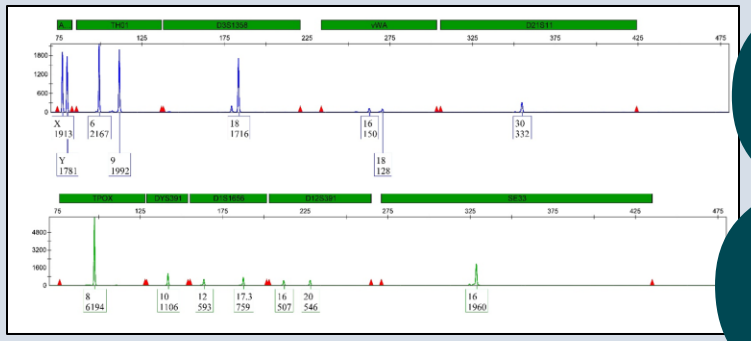
Normalized using [Large Target]



HID Success

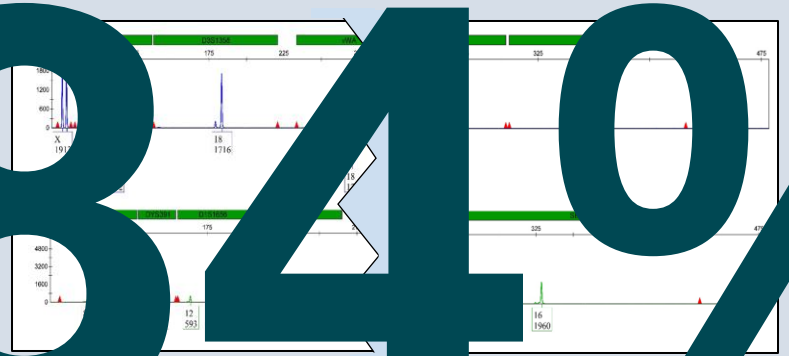
Successful HID

84%



Traditional STR Typing

37%



Composite STR Profiles

42.5%

MtDNA Analysis

5.5%

Unsuccessful HID

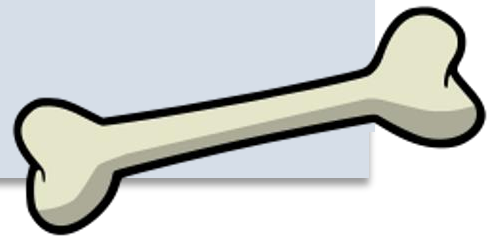
16%

Major Findings

Variation in duration for successful DNA recovery

- **Bleach, Rid-X®, Lye:** 28 days
- **Sulfuric Acid:** 21 days
- **Hydrochloric Acid:** 3 days

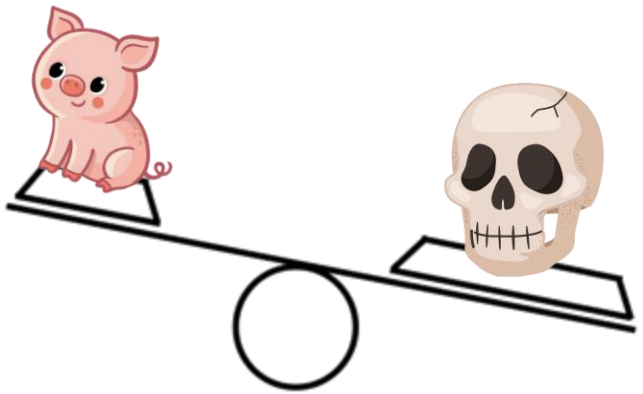
100% success in HID with skeletal elements (bone or teeth)



JFS Manuscript

Twenty-eight days later: The recovery of DNA from human remains submerged in aggressive household chemicals

- Comparison to previous studies
- Preliminary study using porcine ribs
- Variation between small animal studies and our large fragment human study



Thank you!

- STAFS Facility, the donors, and their family
- Forensic Science Foundation – Lucas Grant
- SHSU Forensic Science Department and ***Team DNA-Yay!***

SOUTHEAST TEXAS APPLIED FORENSIC SCIENCE FACILITY

FSF FORENSIC SCIENCES FOUNDATION

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Questions?

Jenn Snedeker: *jls191@shsu.edu*

STAFS: *stafs@shsu.edu*