Extraction of Challenging Samples using the HID NIMBUS Presto System

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SHSU was provided instrumentation and kits by Thermo Fisher Scientific, but no remuneration.

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Objective

Examine the utility of the HID Nimbus Presto System for DNA purification from frequently encountered challenging sample types

- Fired Cartridge Casings (FCCs)
- "Touch" Samples
- Challenging Samples from Decomposing Cadavers –
  - Hair
  - Nails
  - Teeth
- Skeletal Samples

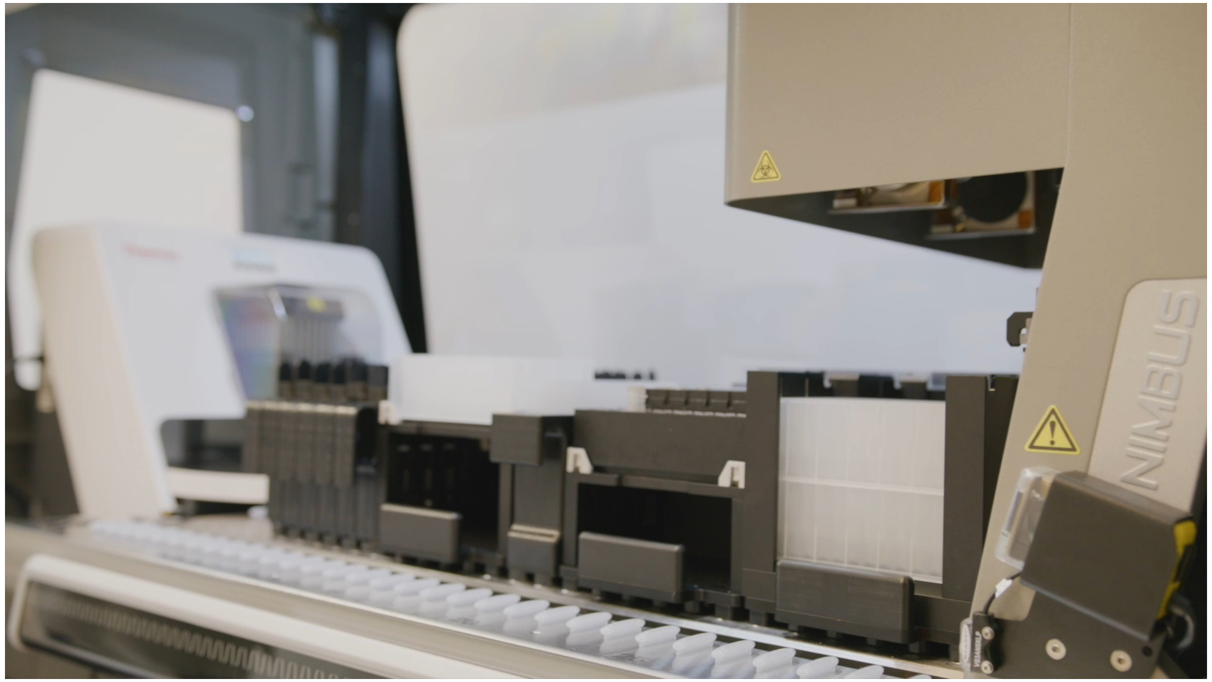



HID NIMBUS Presto System

- KingFisher™ Presto System
  - Magnetic particle-based purification

HID NIMBUS Presto System

- KingFisher™ Presto System
  - Magnetic particle-based purification
- NIMBUS liquid handling system –Can process up to 96 samples in ~90 min
  - Adaptable system
- Scripts for PrepFiler™ and PrepFiler™ BTA Extraction methods
  - Samples lysed manually



Workflow

Brass Casings

Swab moistened with BTMix (n = 12 pooled)

Swab moistened with PrepFiler™ Buffer (n = 6)

Swab moistened with BTA Lysis buffer (n = 6)

N = 24

Swabbed Touched Items

Cell Phone (n = 6)

Keyboard (n = 6)

Water Bottle (n = 6)

N = 18

Challenging samples from decomposed cadavers

Nails (n = 6)

Hair (n = 6)

Teeth (n = 6)

N = 18

Performed in Duplicate

Bones

Burned Remains (n = 6)

Buried Remains (n = 6)

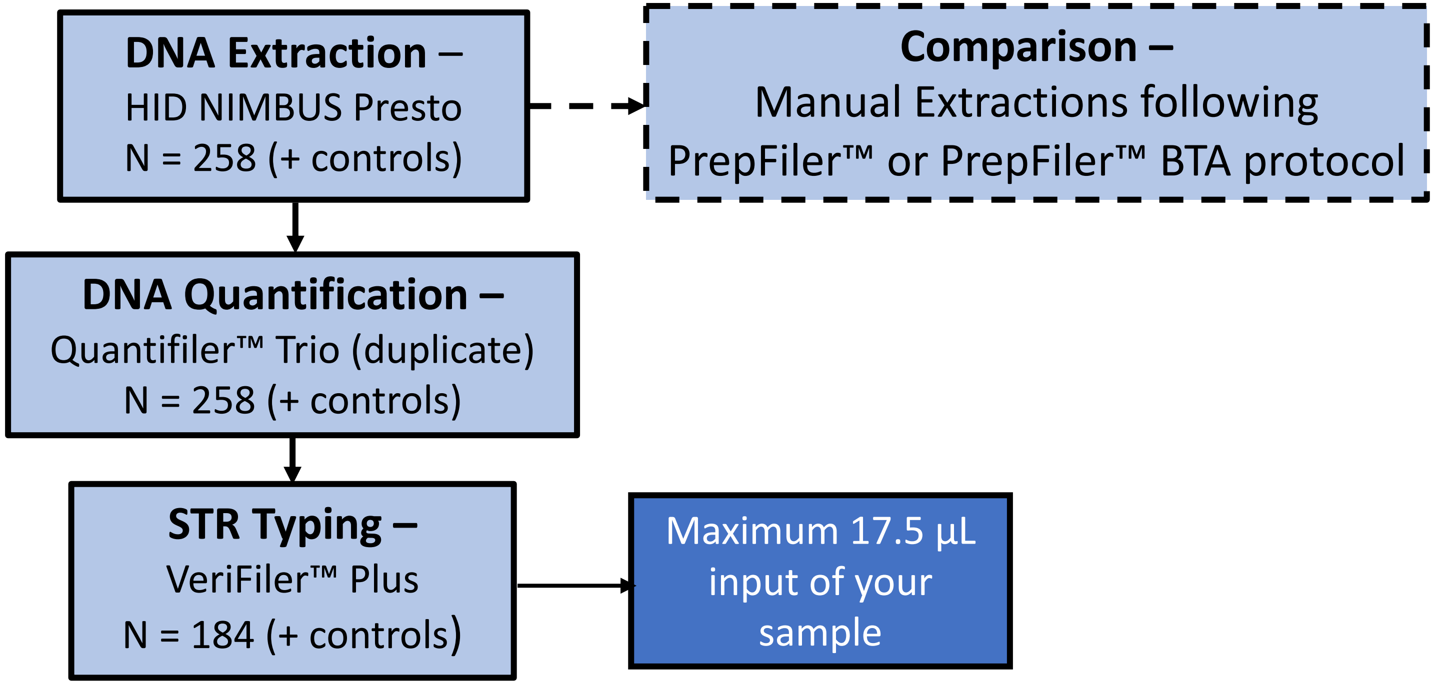
Surface Decomposed Remains (n = 6)

Long Bones (n = 3): Femur, Tibia, Humerus

Alternate Bones (n = 2): Cuneiform, Cuboid

N = 90

Performed in Duplicate



DNA Extraction –
HID NIMBUS Presto
N = 258 (+ controls)

Comparison –
Manual Extractions following
PrepFiler™ or PrepFiler™ BTA protocol

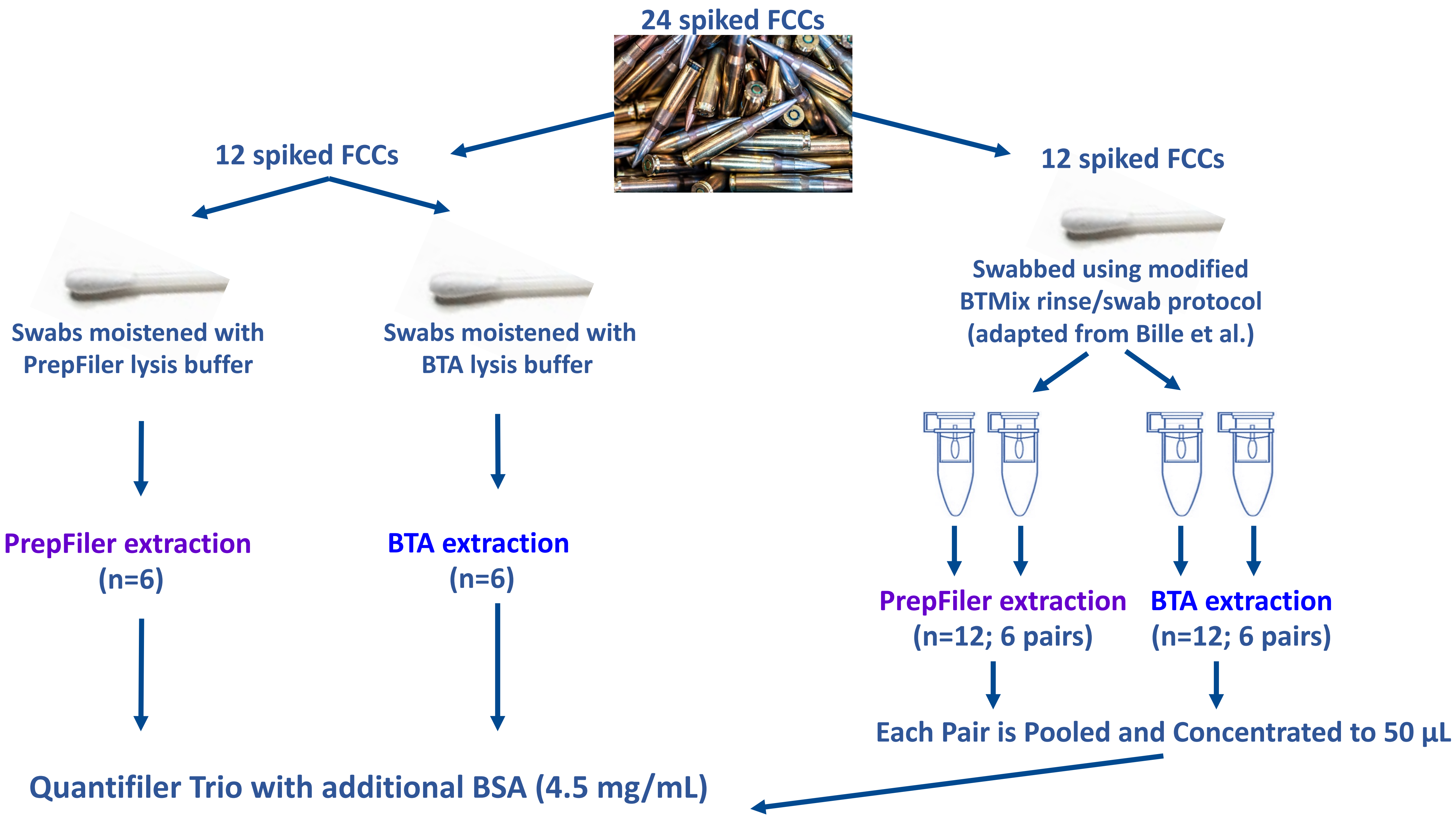
DNA Quantification –
Quantifiler™ Trio (duplicate)
N = 258 (+ controls)

STR Typing –
VeriFiler™ Plus
N = 184 (+ controls)

Maximum 17.5 µL
input of your
sample

Cartridge Casings Methodology

- 48 rounds of UV sterilized 9 mm brass cartridges
  - Sumbro X-Force 9mm Luger 124 grain Full Metal Jacket
- Spotted with 10 ng of buccal cell DNA (~ 217 cells/µL)
- Fired by a male law enforcement officer using a 9mm Glock 19



24 spiked FCCs

12 spiked FCCs

Swabs moistened with PrepFiler lysis buffer

PrepFiler extraction
(n=6)

Swabs moistened with BTA lysis buffer

BTA extraction
(n=6)

12 spiked FCCs

Swabbed using modified BTMix rinse/swab protocol (adapted from Bille et al.)

PrepFiler extraction
(n=12; 6 pairs)

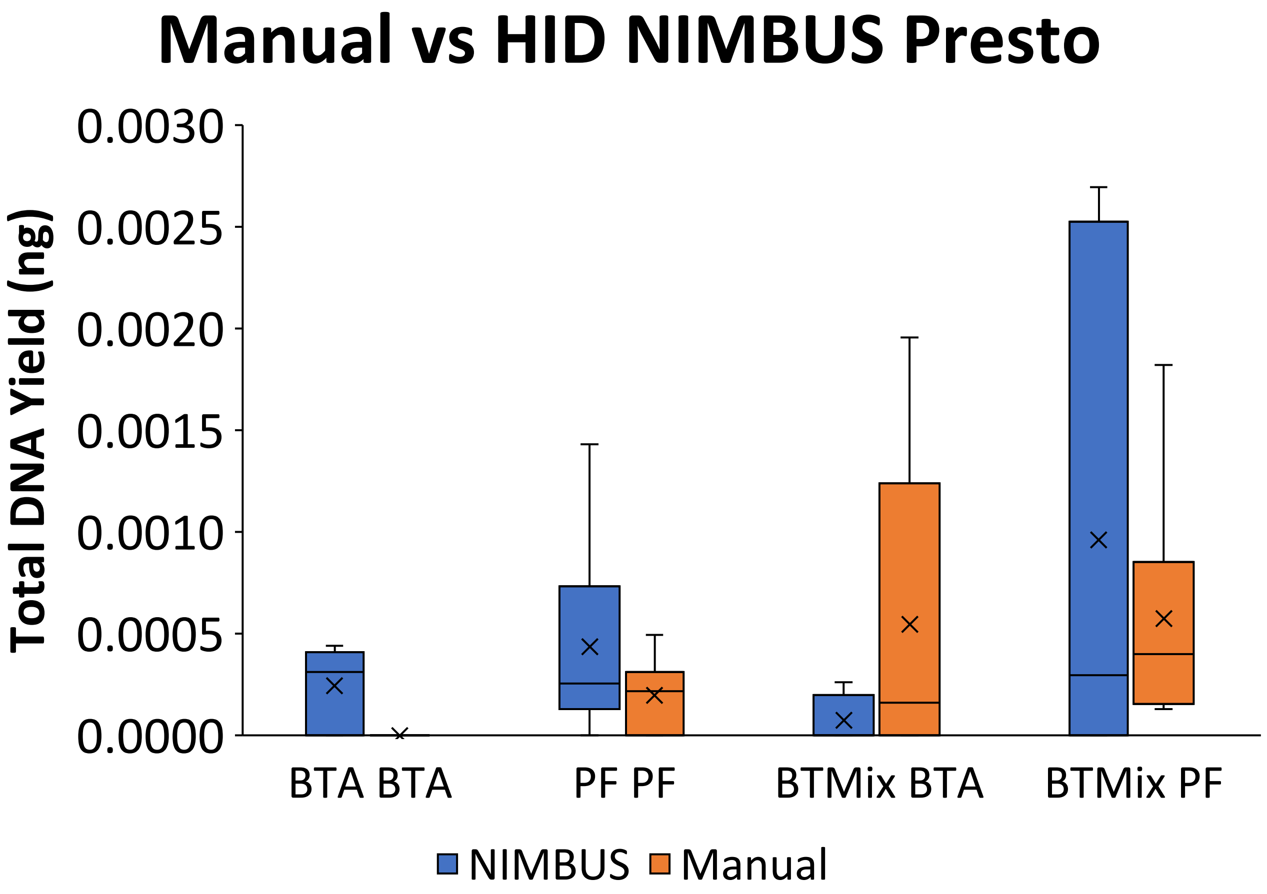
BTA extraction
(n=12; 6 pairs)

Each Pair is Pooled and Concentrated to 50 µL

Quantifiler Trio with additional BSA (4.5 mg/mL)

HID NIMBUS Presto vs Manual

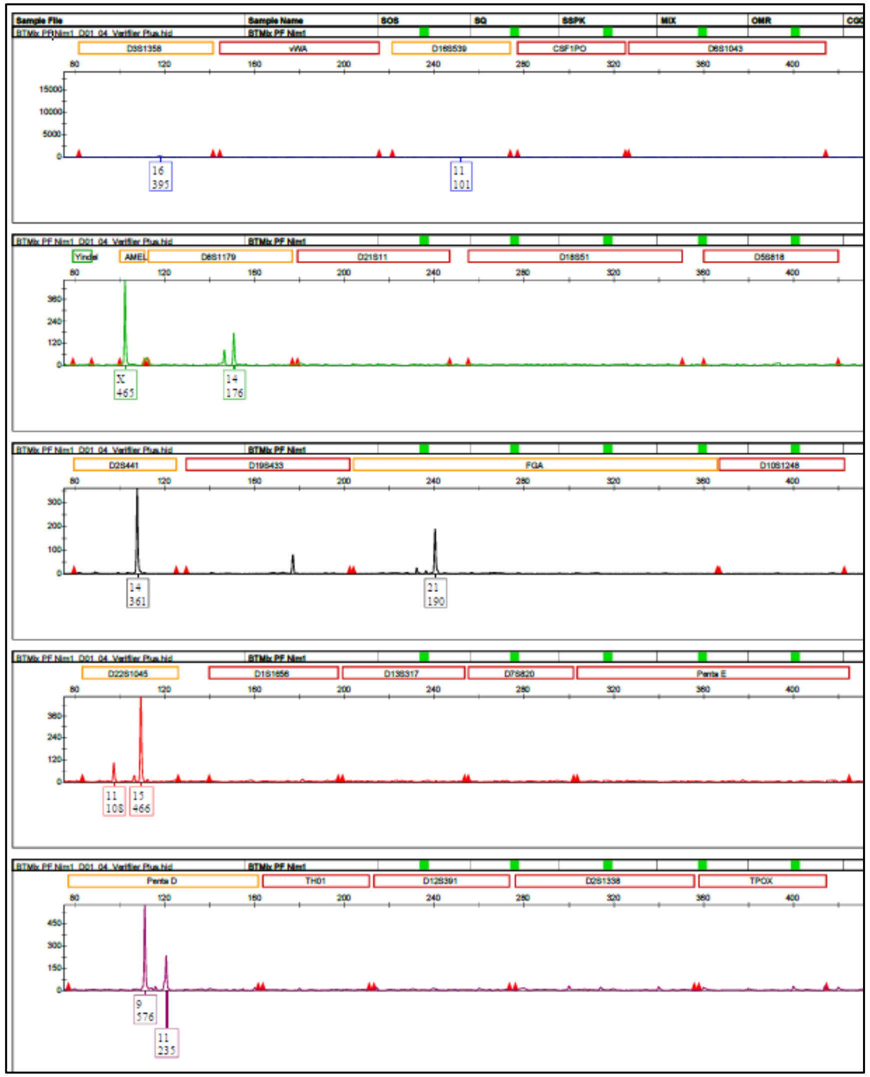
- No inhibition observed, but most samples exhibited some level of degradation
- Most successful combination was collection using BTMix rinse/swab method and extraction using PrepFiler™ using the HID NIMBUS Presto System.



No significant difference in DNA recovery between manual and HID NIMBUS Presto ($p>0.05$)

HID NIMBUS Presto vs Manual

	Swab Solution	Extraction	Concentration (ng/µL)	DNA Input (ng)	Allele Recovery
NIMBUS	PrepFiler	PrepFiler	0.0014	0.0715	17.3%
	BTMix	PrepFiler	0.0026	0.1347	11.4%
	BTMix	PrepFiler	0.0024	0.1233	2.17%



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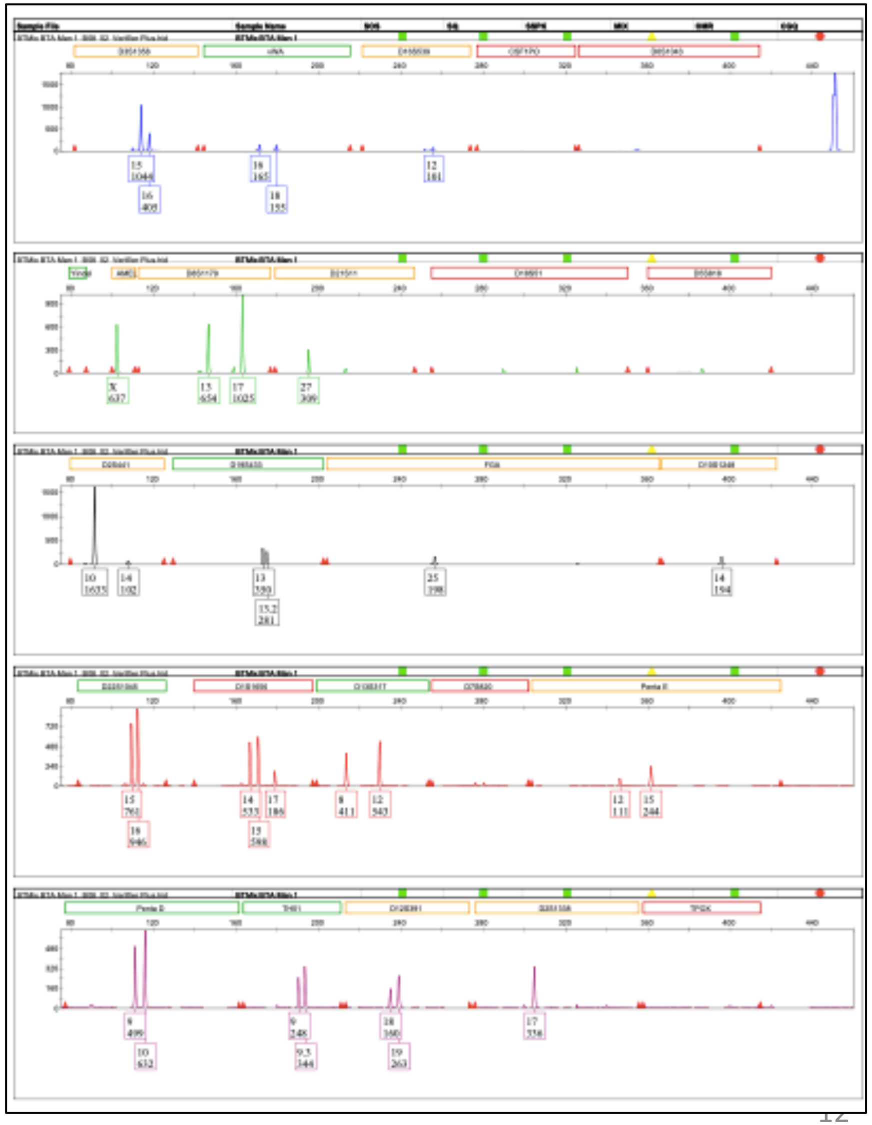
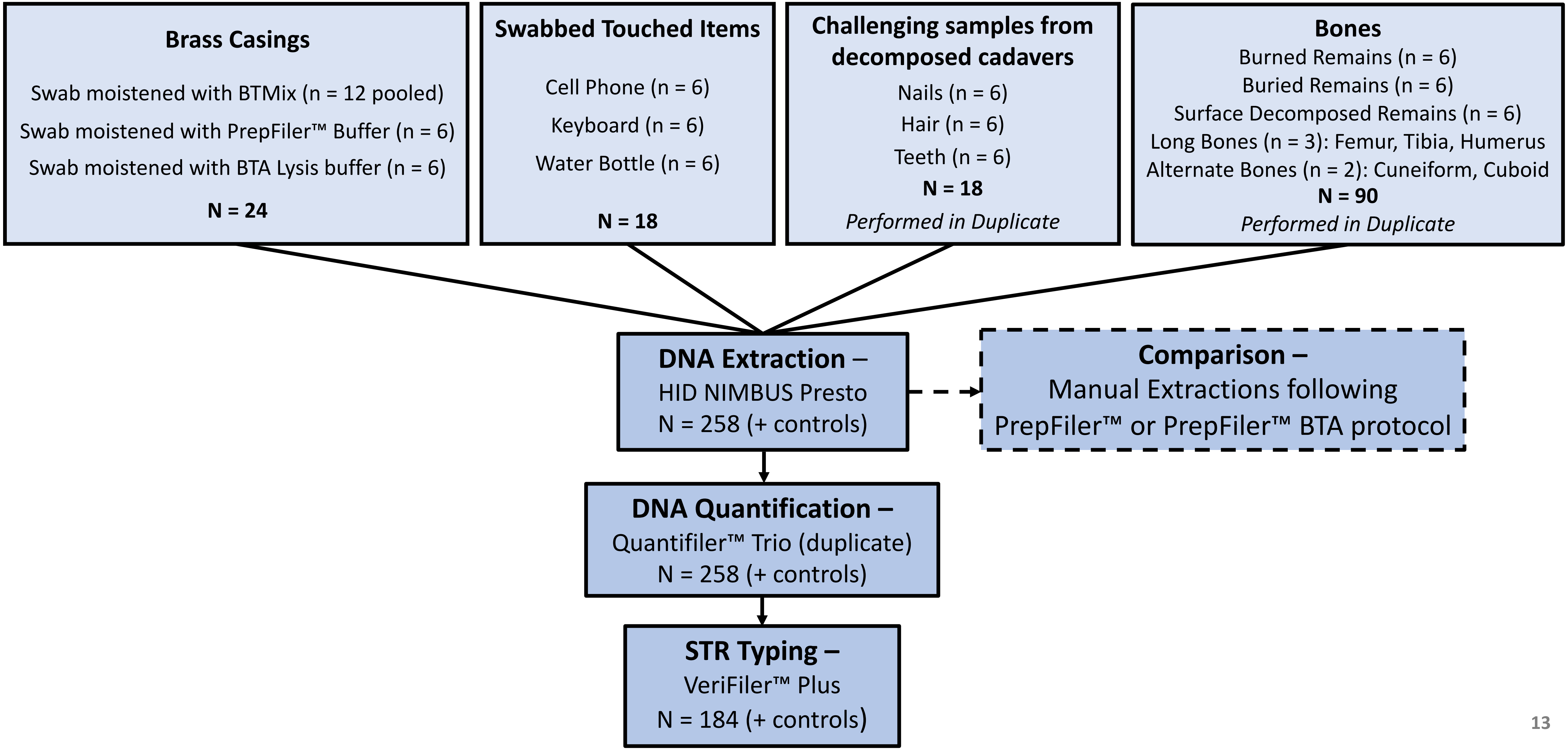
	BTMix	PrepFiler	0.0024	0.1233	2.17%
	Swab Solution	**Extraction**	**Concentration (ng/µL)**	**DNA Input (ng)**	**Allele Recovery**
Manual	BTMix	BTA	0.0018	0.0910	30.4%
	BTMix	PrepFiler	0.0010	0.0501	47.8%
	BTMix	PrepFiler	0.0019	0.0998	23.9%



Workflow



Brass Casings

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N = 24

Swabbed Touched Items

Cell Phone (n = 6)

Keyboard (n = 6)

Water Bottle (n = 6)

N = 18

Challenging samples from decomposed cadavers

Nails (n = 6)
Hair (n = 6)
Teeth (n = 6)
N = 18
Performed in Duplicate

Bones
Burned Remains (n = 6)
Buried Remains (n = 6)
Surface Decomposed Remains (n = 6)
Long Bones (n = 3): Femur, Tibia, Humerus
Alternate Bones (n = 2): Cuneiform, Cuboid
N = 90
Performed in Duplicate

DNA Extraction –
HID NIMBUS Presto
N = 258 (+ controls)

Comparison –
Manual Extractions following
PrepFiler™ or PrepFiler™ BTA protocol

DNA Quantification –
Quantifiler™ Trio (duplicate)
N = 258 (+ controls)

STR Typing –
VeriFiler™ Plus
N = 184 (+ controls)

“Touch” Samples

Cell Phones

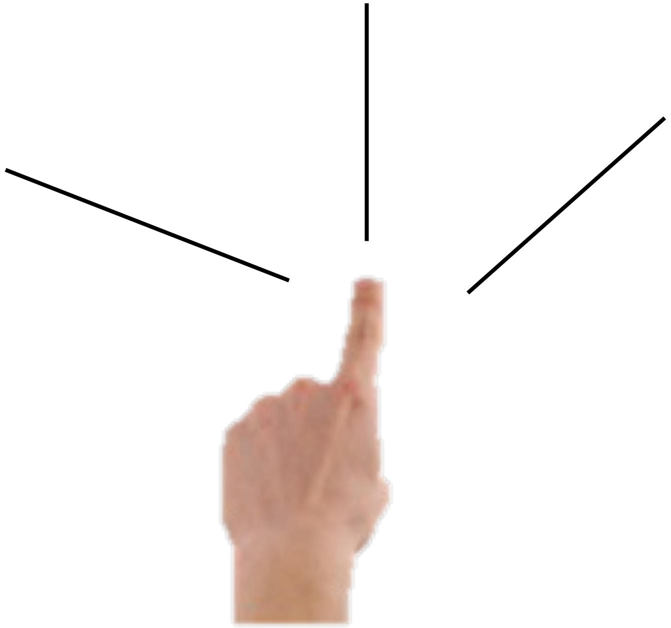
https://www.consumerreports.org/products/cell-phones-200703/smartphones-28726/apple-iphone-11-399694/

Keyboards

https://www.hydroflask.com/32-oz-wide-mouth

Water Bottles

https://www.hydroflask.com/32-oz-wide-mouth#color=Mesa

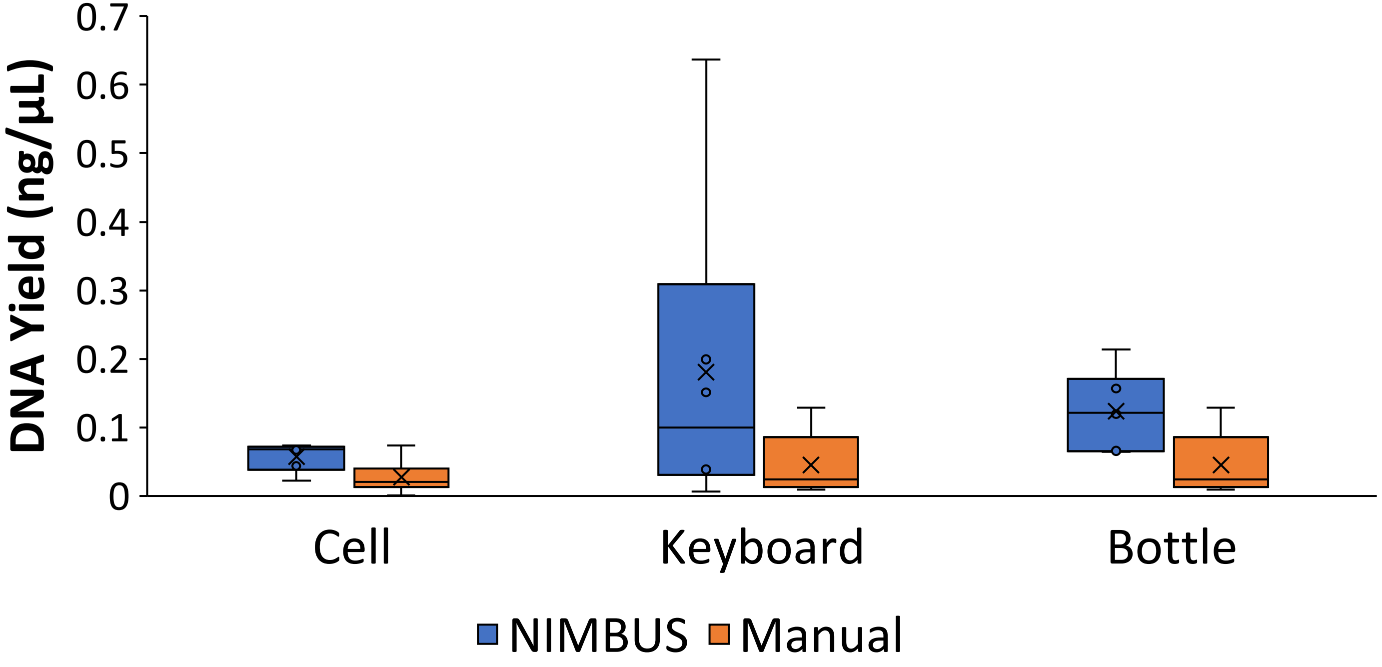


Swabs of the following items were collected from 6 participants (N = 18)

HID NIMBUS Presto vs Manual

- Most DNA recovered from Keyboards using the HID NIMBUS Presto
- Lowest average DNA recovered from cell phones for both methods
- All samples met threshold for STR analysis

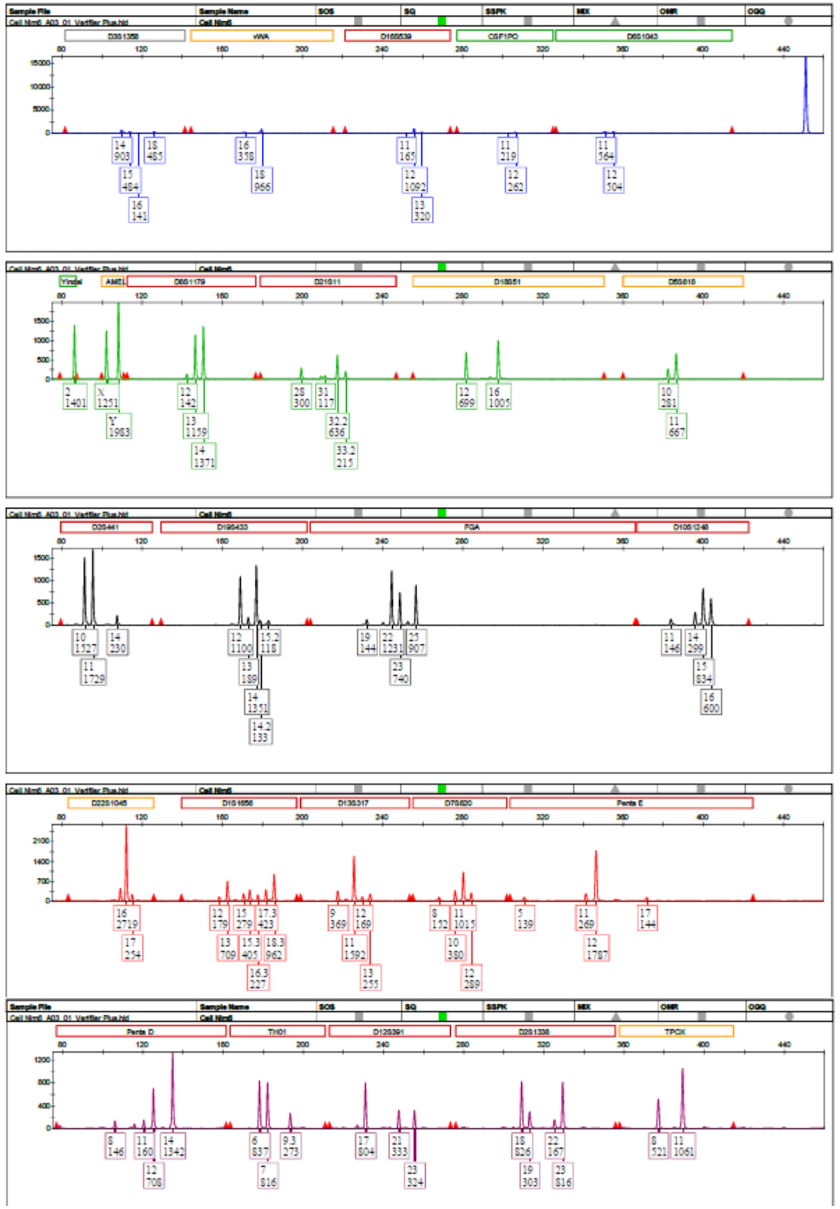
DNA Yield Comparison of Touch Samples



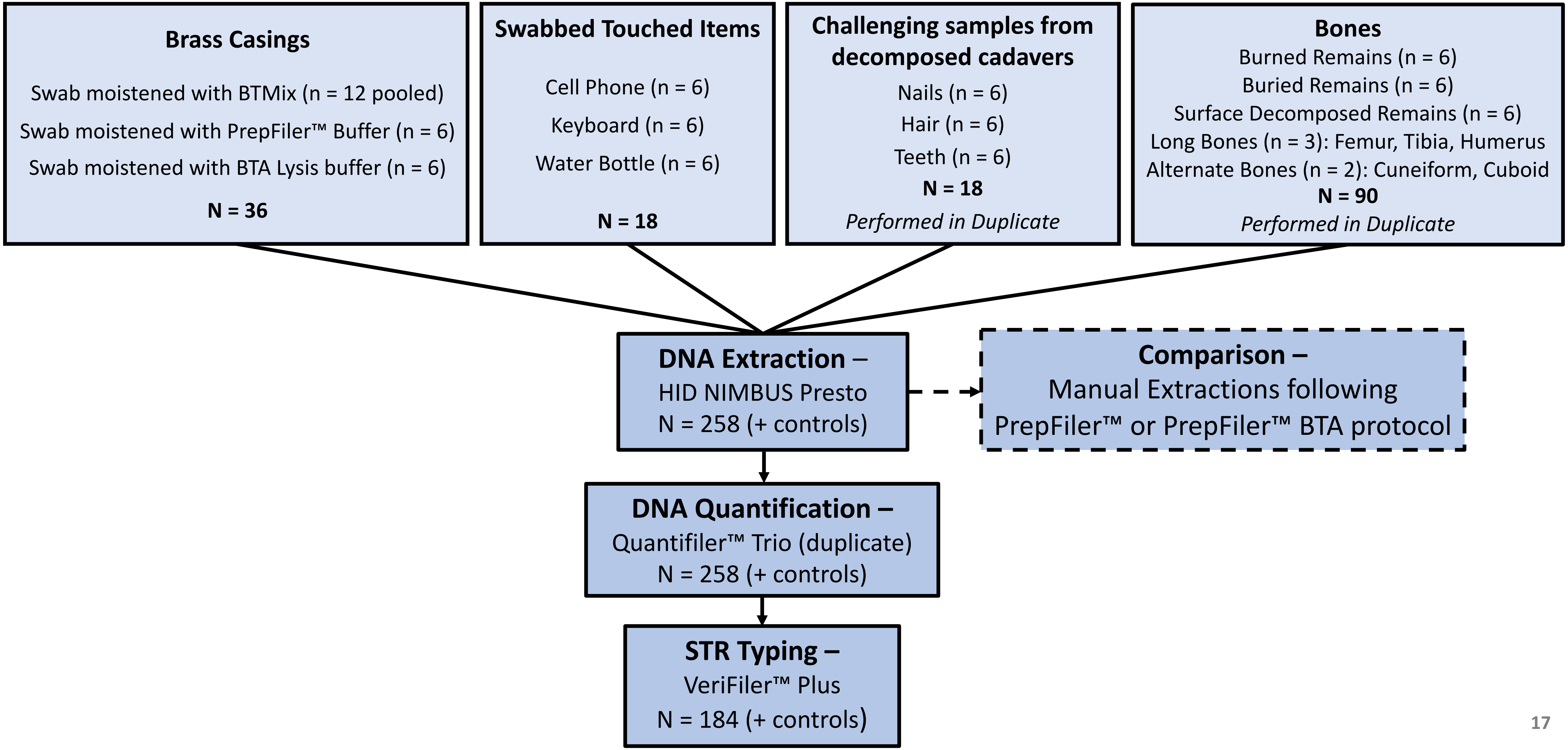
Touch samples extracted with the HID NIMBUS Presto generated extracts with significantly higher DNA Yield ($p < 0.05$)

"Touch" Results

- **13/18** samples using the HID NIMBUS Presto and **15/18** for manual extractions indicated mixtures
- 100% allele recovery from the major contributor (owner of item) for both methods



Workflow



Brass Casings

Swab moistened with BTMix (n = 12 pooled)

Swab moistened with PrepFiler™ Buffer (n = 6)

Swab moistened with BTA Lysis buffer (n = 6)

N = 36

Swabbed Touched Items

Cell Phone (n = 6)

Keyboard (n = 6)

Water Bottle (n = 6)

N = 18

Challenging samples from decomposed cadavers

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Hair (n = 6)

Teeth (n = 6)

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Performed in Duplicate

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Surface Decomposed Remains (n = 6)

Long Bones (n = 3): Femur, Tibia, Humerus

Alternate Bones (n = 2): Cuneiform, Cuboid

N = 90

Performed in Duplicate

DNA Extraction –

HID NIMBUS Presto

N = 258 (+ controls)

Comparison –

Manual Extractions following PrepFiler™ or PrepFiler™ BTA protocol

DNA Quantification –

Quantifiler™ Trio (duplicate)

N = 258 (+ controls)

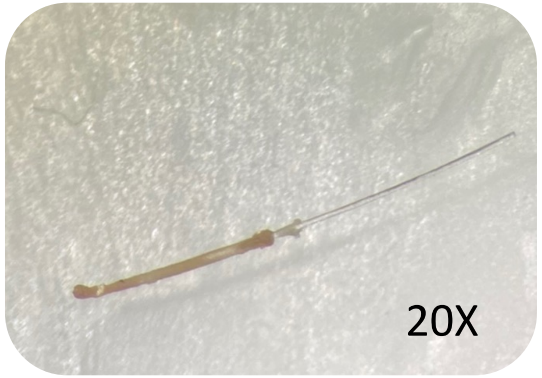
STR Typing –

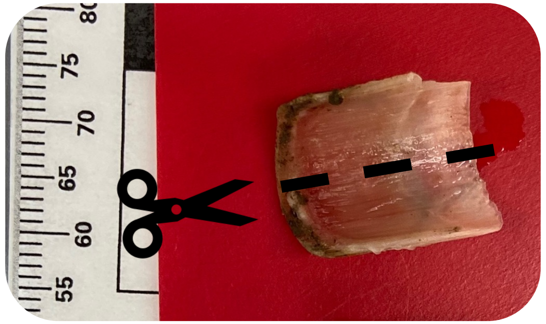
VeriFiler™ Plus

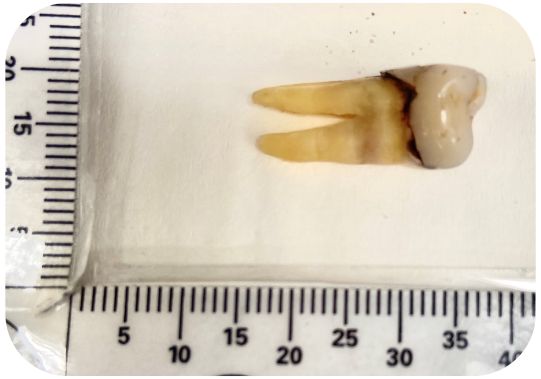
N = 184 (+ controls)

Challenging Samples Prep and Extraction

- **Hair**
    - Cut to 3-5mm
    - Washed with Tergazyme™, water, then ethanol
    - PrepFiler™ protocol
- **Nails**
    - Cut to ~5mm
    - Washed for 15 min in sterile water at 50°C by shaking followed by 3 min in ethanol
    - PrepFiler™ protocol
- **Teeth**
    - Premolars and Molars
    - Washed with 10% Bleach (3 sec), water, and ethanol
    - Wrapped in Kimwipe and pulverized with a mallet before being powdered with the Freezer Mill SPEX
    - PrepFiler™ BTA protocol

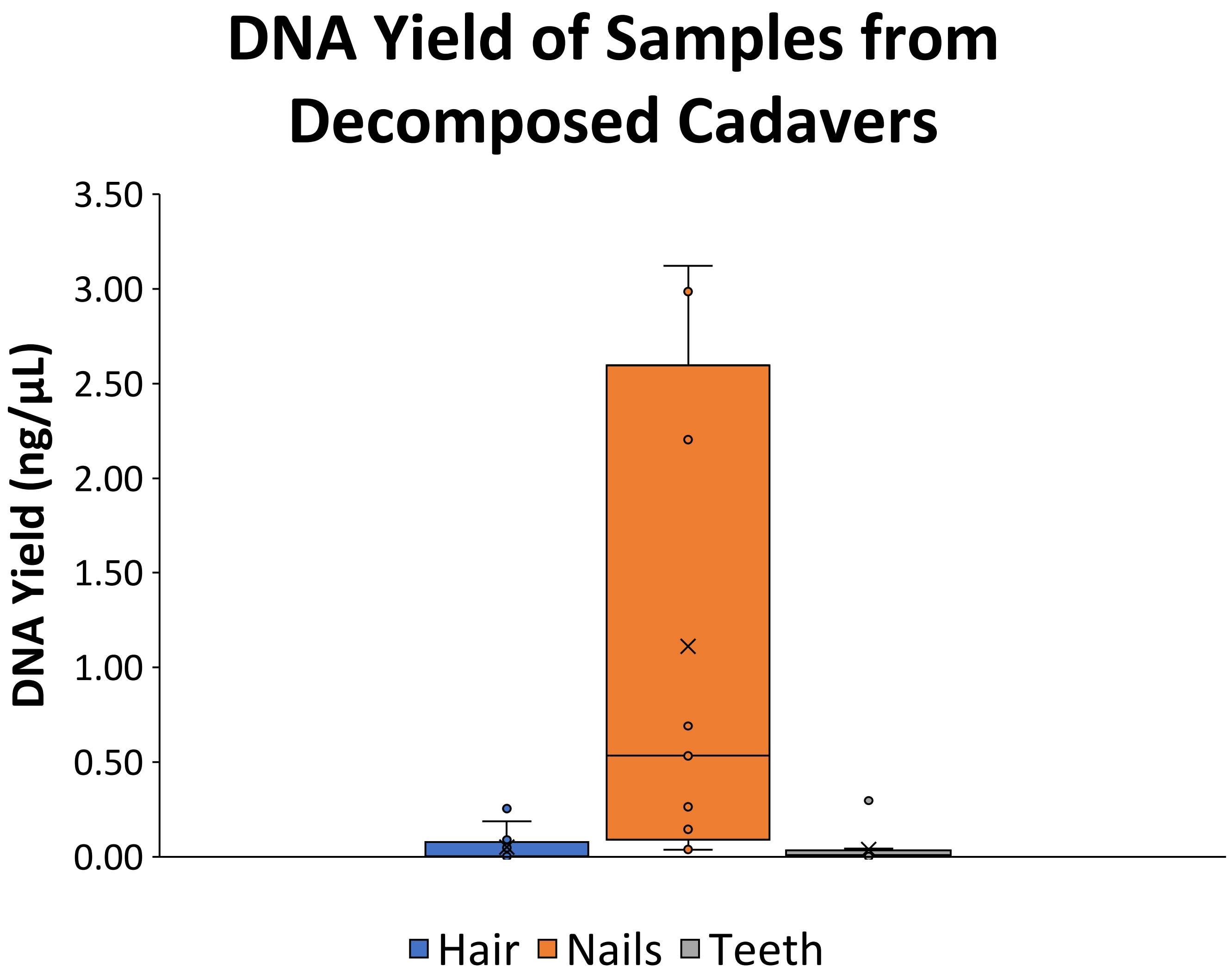






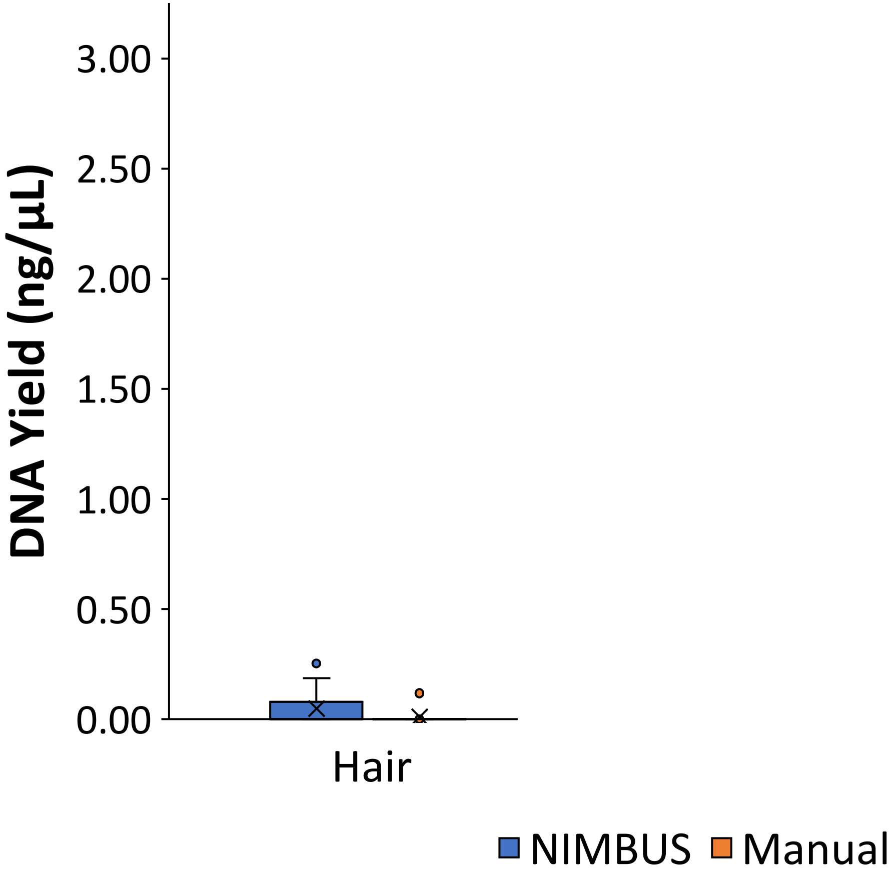
Challenging Samples Results

- Highest amount of DNA was recovered from nails
    - Nail beds
- No inhibition detection, but DNA degradation was observed in nail and teeth samples



HID NIMBUS Presto vs Manual

DNA Yield of Challenging Samples from Decomposed Cadavers



	HID NIMBUS Presto	Manual
Average hair DNA yield (ng/µL)	**0.0488***	0.0099
Hair samples amplified	6/12	2/12

* $p < 0.05$

HID NIMBUS Presto vs Manual

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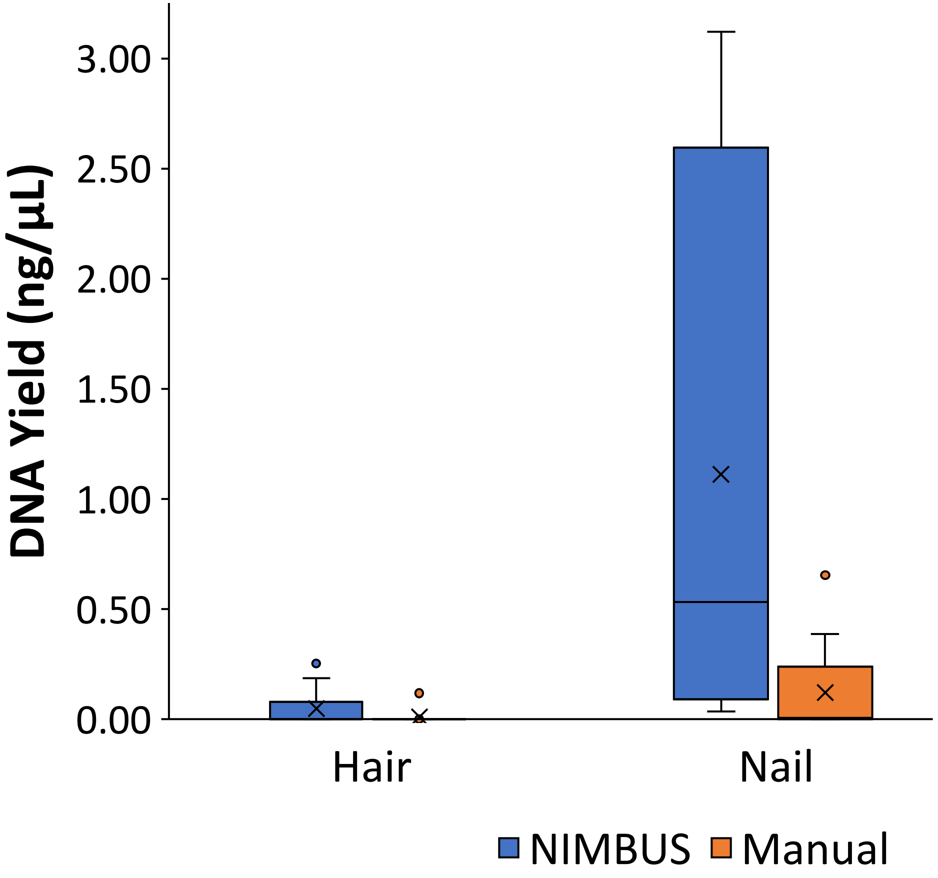

	HID NIMBUS Presto	Manual
Average hair DNA yield (ng/µL)	**0.0488***	0.0099
Hair samples amplified	6/12	2/12
Average nail DNA yield (ng/µL)	**1.11***	0.1221
Nail samples amplified	12/12	11/12

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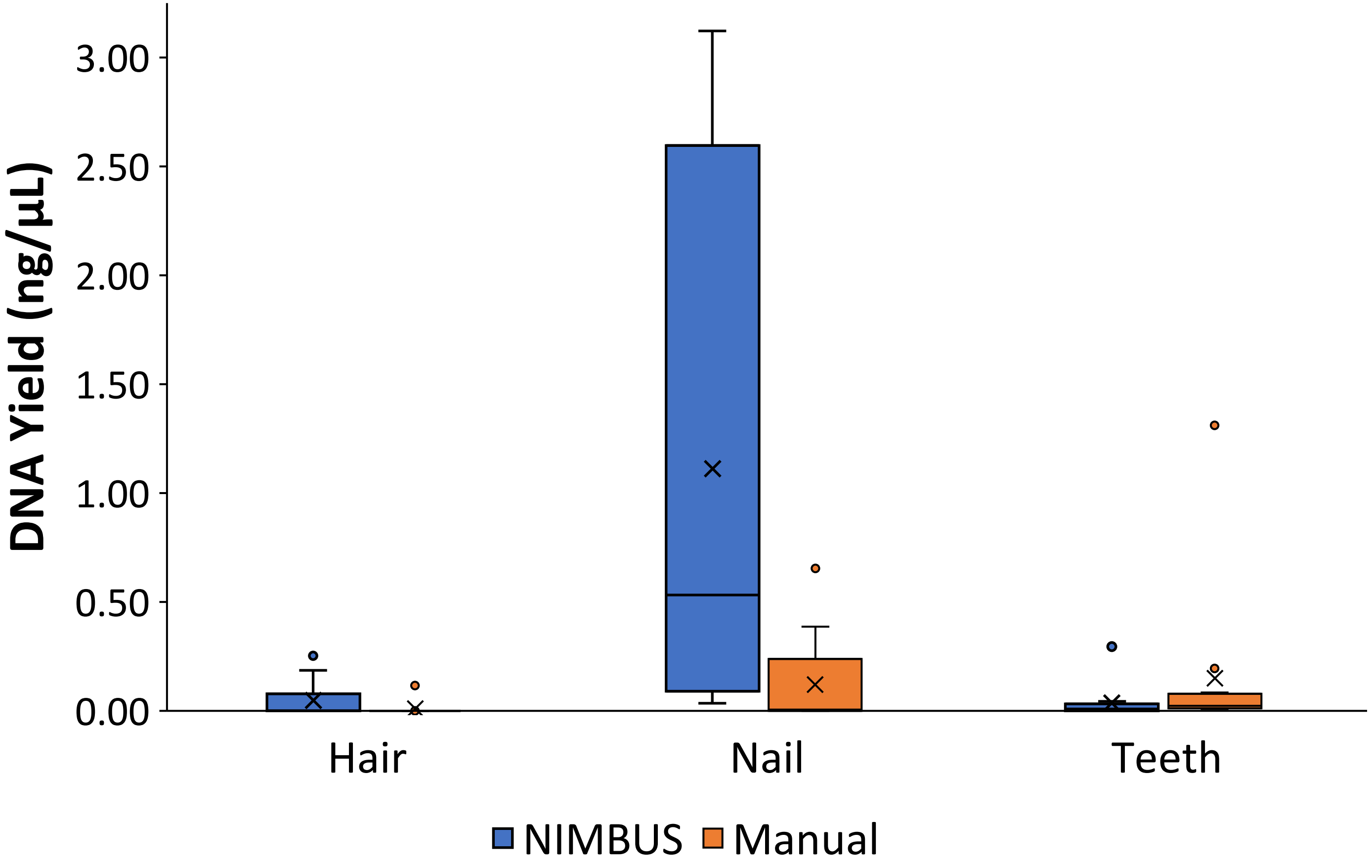
HID NIMBUS Presto vs Manual

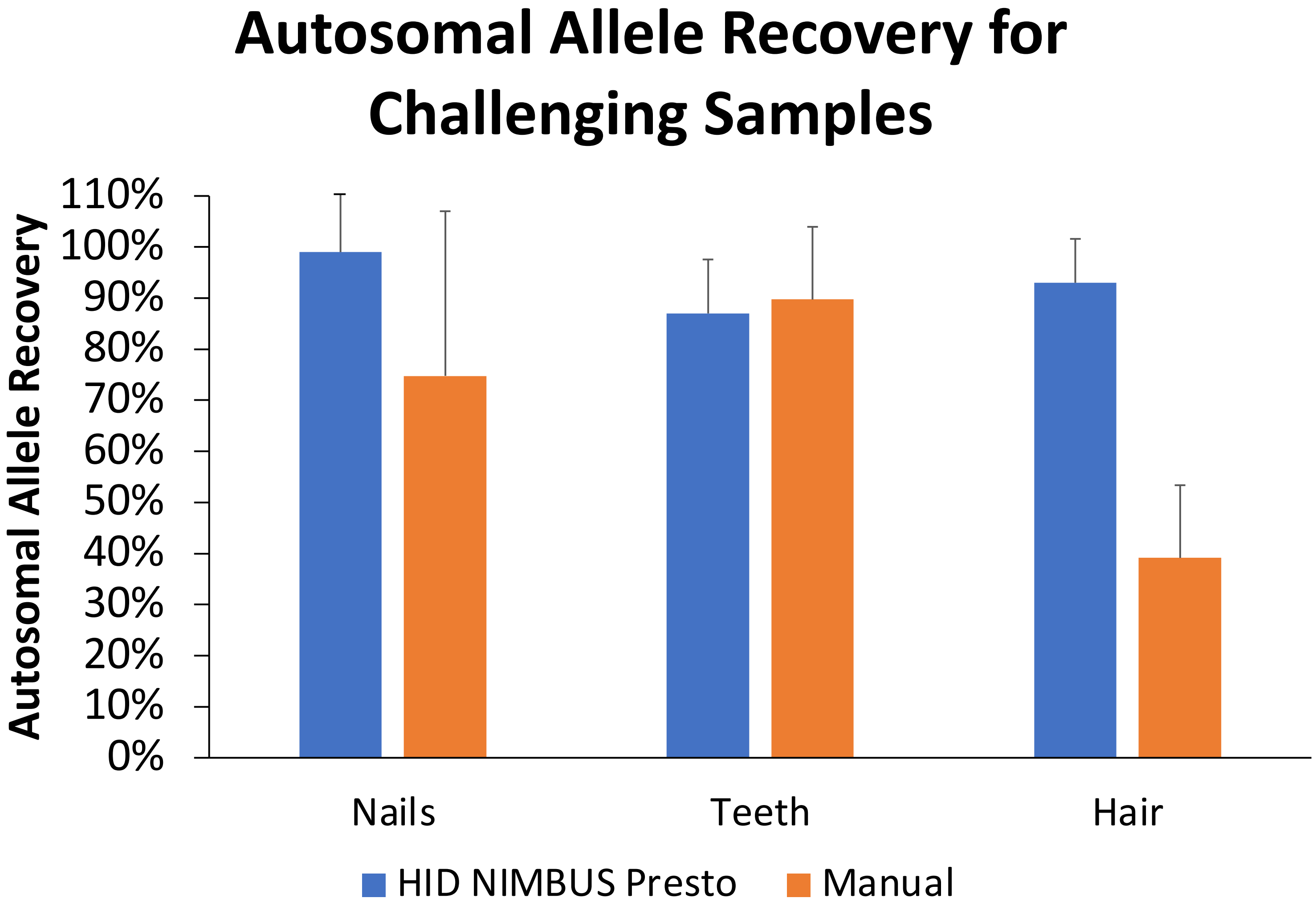
DNA Yield of Challenging Samples from Decomposed Cadavers

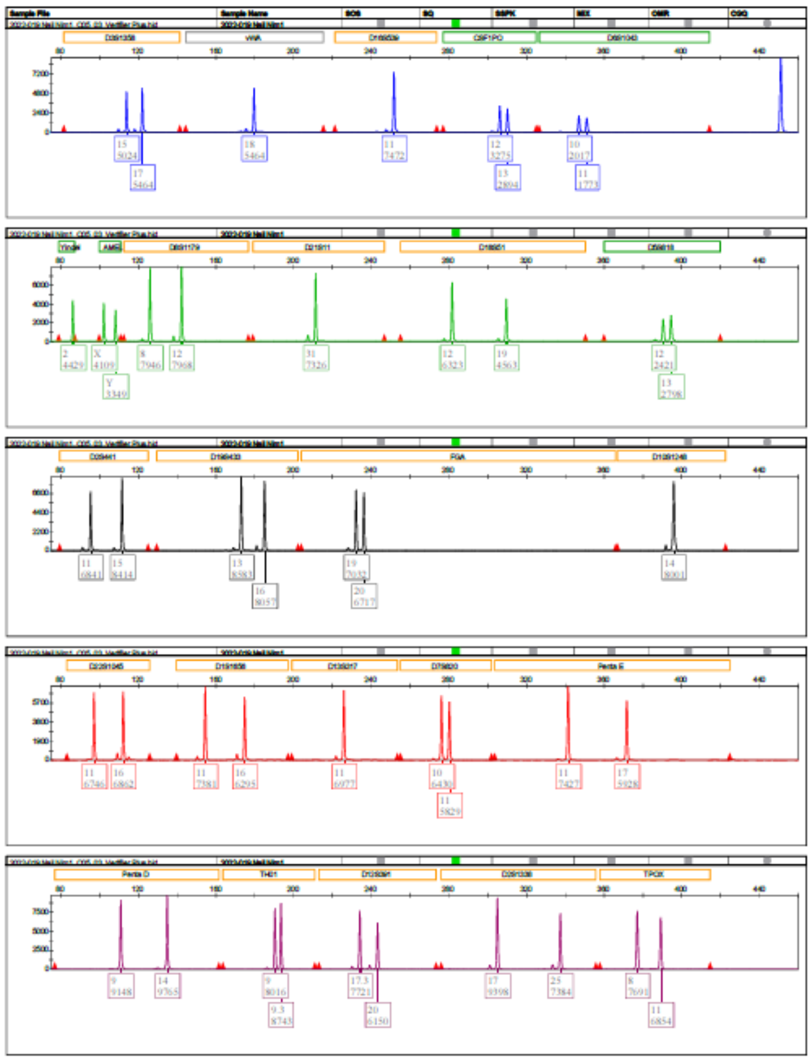


	HID NIMBUS Presto	Manual
Average hair DNA yield (ng/µL)	**0.0488***	0.0099
Hair samples amplified	6/12	2/12
Average nail DNA yield (ng/µL)	**1.11***	0.1221
Nail samples amplified	12/12	11/12
Average Teeth DNA Yield (ng/µL)	0.0371	0.1512
Teeth samples amplified	10/12	12/12

* $p < 0.05$

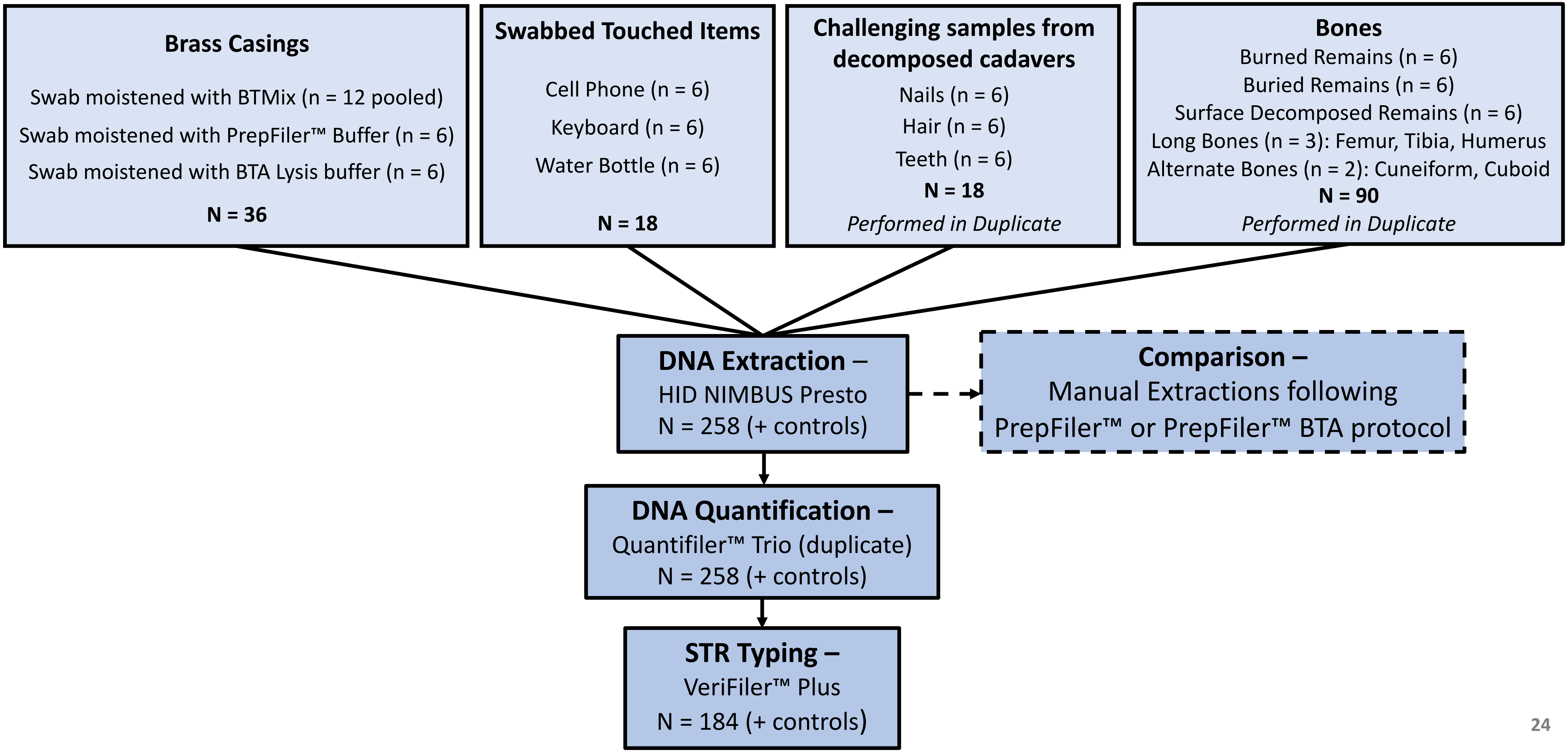
HID NIMBUS Presto vs Manual


Autosomal Allele Recovery for Challenging Samples


Nail Sample

Samples processed with the HID NIMBUS Presto produced statistically significantly higher allele recovery for nail and hair samples ($p < 0.05$)

Workflow



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Performed in Duplicate

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Alternate Bones (n = 2): Cuneiform, Cuboid

N = 90

Performed in Duplicate

DNA Extraction –

HID NIMBUS Presto

N = 258 (+ controls)

Comparison –

Manual Extractions following PrepFiler™ or PrepFiler™ BTA protocol

DNA Quantification –

Quantifiler™ Trio (duplicate)

N = 258 (+ controls)

STR Typing –

VeriFiler™ Plus

N = 184 (+ controls)

Skeletal Samples

- Samples collected from STAFS
  - 3 insults – surface, burned, buried
  - 18 total cadavers
    - 5 skeletal elements per cadaver

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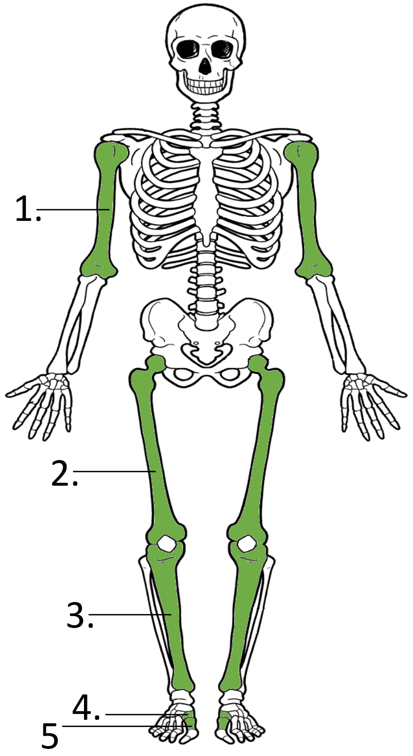
Skeletal Samples

Surface Remains

(n=6 cadavers)

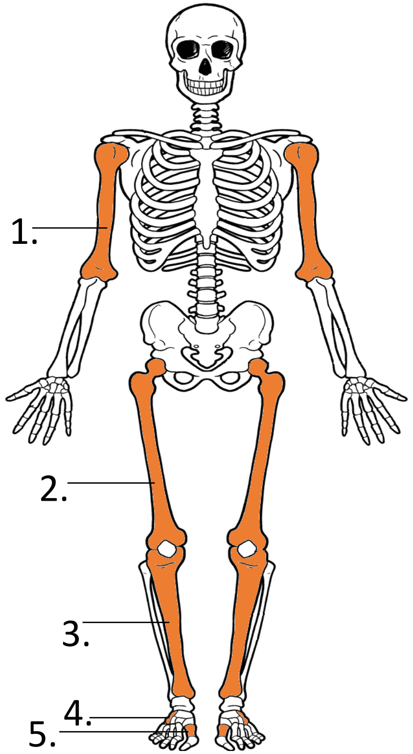
1. Humerus
2. Femur
3. Tibia
4. Medial Cuneiform
5. First Metatarsal



Burned Remains

(n=6 cadavers)

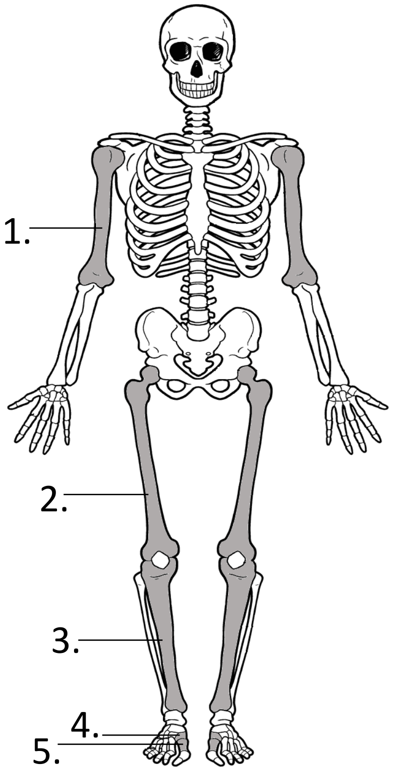
1. Humerus
2. Femur
3. Tibia
4. Cuboid
5. First Metatarsal



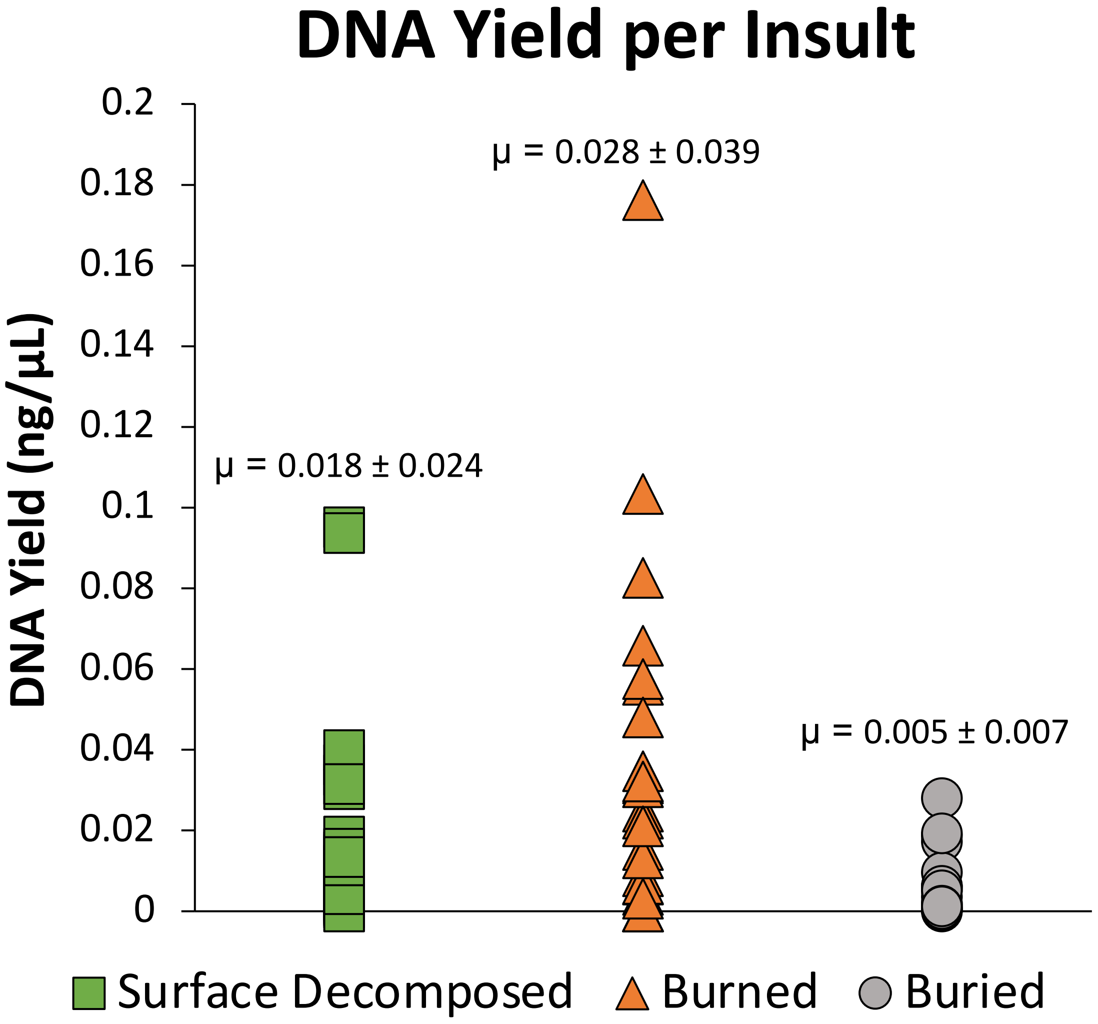
Buried Remains

(n=6 cadavers)

1. Humerus
2. Femur
3. Tibia
4. Medial Cuneiform
5. First Metatarsal



Skeletal Samples



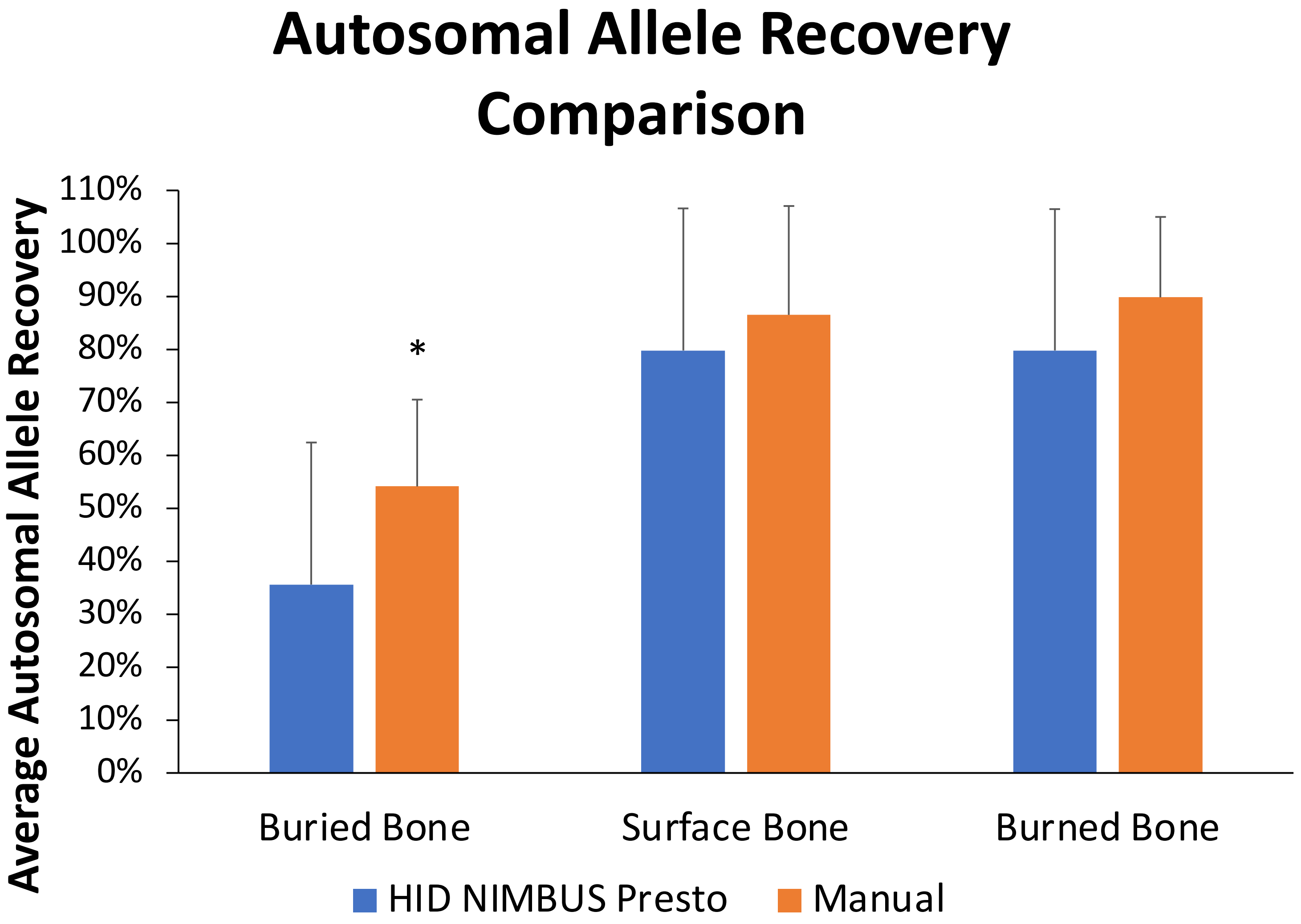
Of the **180** extracts, **133** proceeded to downstream processing based on DNA yield

- **57** surface decomposed samples
- **44** burned samples
- **32** buried samples

HID NIMBUS Presto vs Manual Extraction

Samples Meeting 1 pg/µL Threshold		
Insult Categories	**HID NIMBUS Presto System**	**Manual Extraction**
Surface Decomposed (N = 60)	**57**	48
Burned (N = 60)	44	44
Buried (N = 60)	**32**	18
TOTAL (N = 180)	**133**	110

HID NIMBUS Presto vs Manual Extraction



- **No significant difference** between the two methods for **Burned** and **Surface Decomposed** samples
- Manual extraction performed **significantly better** than the HID NIMBUS Presto for STR allele recovery $p < 0.05$ for **Buried** Samples, but only 18 buried samples met the input threshold

Summary

- The HID Nimbus Presto System successfully purified DNA from a variety of challenging sample types
- Performed similarly to manual extraction methods
- Saves analyst time

Acknowledgments

Thermo Fisher
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Thank You!

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- **Bones:** Jennifer Snedeker- jls191@shsu.edu

- Dr. Sheree Hughes- sxh039@shsu.edu

- **HID NIMBUS Presto:** Hanh Le- hanh.le@thermofisher.com