Not All Samples Are Equal: Testing Bone Methods and Prioritizing Challenging Human Tissues for Rapid DNA Success

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INTRODUCTION

Rapid DNA can be deployed for in-field human identification in cases such as mass disasters. The RapidHIT ID (RHID) system by Thermo Fisher Scientific has been used to identify victims and process familial reference samples during events such as the 2021 Surfside, Florida, condominium collapse (1) and the Kaua'I flooding (2). The RHID system has also been tested in mock DVI exercises (3,4)

When blood or soft tissues are unavailable from skeletal remains, alternative biological materials such as bone or teeth must be processed. However, standardized "rapid" protocols for these challenging samples have not been established.

The RHID system processes one sample in ~90 minutes. The recently developed RapidINTEL Plus cartridge is optimized for challenging samples, incorporating a larger filter to capture more DNA, increased number of PCR cycles, and a reduced lysis volume to minimize dilution.

This study aimed to assess whether pretreatment (25% Prep-n-Go, PrepFiler™ BTA lysis buffer, or complete demineralization buffers) and different bone consistencies (powder, granule, or chip) could improve DNA yield and STR recovery compared to putting larger bone chips directly into the RHID cartridge. Additionally, we evaluated the success of different cadaveric tissues at different stages of decomposition to test which samples may be the most suitable for rapid DNA processing.

An off-instrument "mock rapid" protocol was developed to manually replicate the RHID parameters, allowing evaluation of pre-treatment chemistries on bone and cadaveric samples. This approach enabled assessment of STR success rates expected on the RHID while substantially reducing costs by avoiding instrument runs of low-quality samples.

MATERIALS & METHODS

Skeletal Samples

Two femora from the willed body program at the Southeast Texas Applied Forensic (STAFS) facility at Sam Houston State University were used to test parameters and were prepared in three ways to reflect varied coarseness and subjected to three pretreatments in varying amounts (Fig. 1) Each pretreatment was tested in duplicate for each bone fragment type and amount, using sufficient buffer volume to adequately submerge and mix the entire bone sample (Table 1).

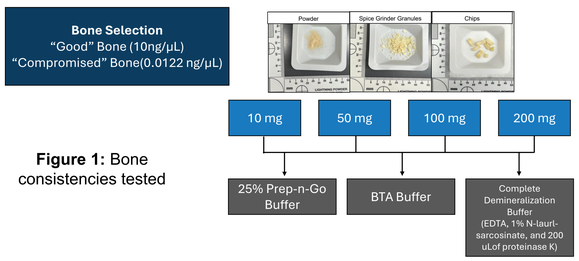

Figure 1: Bone consistencies tested

Table 1: Bone consistencies tested

Bone Amount	Powder (µL)	Spice Grinder (µL)	Chips (µL)
10mg	100	100	N/A
50mg	200	200	200
100mg	400	300	300
200mg	600	400	400

*** 10 mg chip samples were not possible, as individual chips weighed more than the target amount**

RESULTS & DISCUSSION

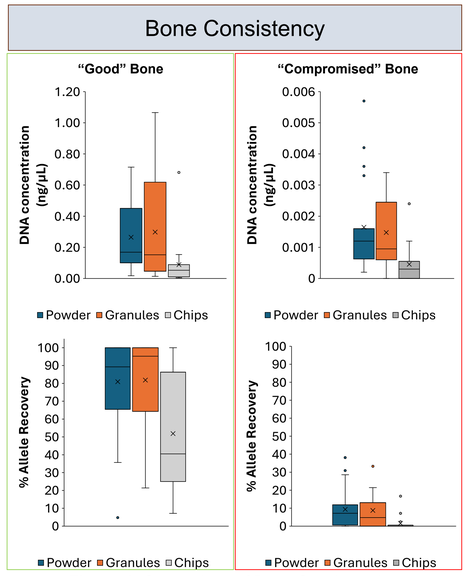

Figure 4: Comparison of Bone Consistencies

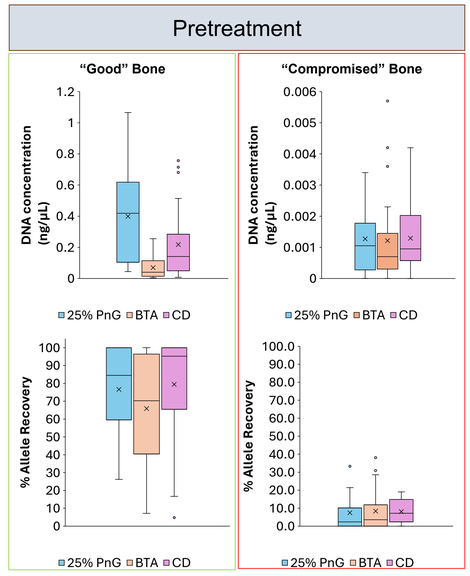

Figure 5: Comparison of Bone Pretreatments

- For "good" bone samples, bone consistency had a significant effect on DNA concentration ($p < 0.05$) (Fig. 4).
- Granules provided the most consistent results for DNA yield and STR recovery when using 50 mg or more of bone input (Fig. 6B), with chips performing the least consistently overall (Fig. 4 & 6C).
- 25% Prep-n-Go buffer produced significantly higher DNA yields for "good" bone ($p<0.05$) compared to other pretreatments, but no significant differences among pretreatments for "compromised" bones ($p > 0.05$). (Fig. 5).
- Although no statistical difference in DNA yield or STR success was observed based on the amount of bone powder used, 10 mg powder seemed to perform the most consistently among all pretreatments with "good" bone samples (data not shown).

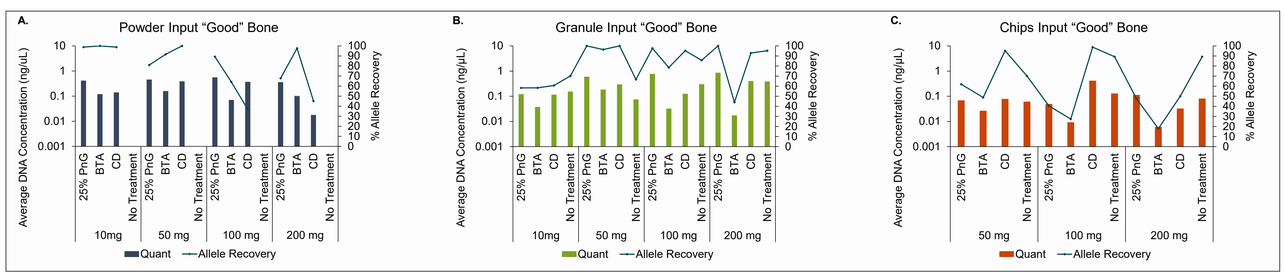

Figure 6: Comparison of Bone Consistencies and Pretreatments for "Good" Bone A) Powder, B) Granules C) Chips

Table 2: Comparative results from samples using the RHID and Mock Rapid Methods

Consistency	Pretreatment	Allele Recovery (%)		Small Target amount (ng)		Large Target amount (ng)		DNA Degradation		PCR Inhibition	
		Mock Rapid	RHID	qPCR	RHID	qPCR	RHID	qPCR DI	RHID Flag	qPCR ΔIPC	RHID Flag
Granule	No pretreatment	97.6	95.2	1.9365	4.99	0.04	0.068	126.6	Yes	-0.09	No
	25% Prep-n-Go	97.6	100	4.3	4.613	0.2137	0.022	20.1	Yes	-0.29	No
	BTA	17.9	44.3	0.03	0.209	0.0005	*ND	3.1	Yes	-0.06	No
Chip	Complete demineralization	50	92.5	0.1635	0.44	0.0063	0.048	38.8	Yes	-0.27	No

- Overall, the mock rapid method resulted in comparable results to when the samples were processed directly on the RHID system (Table 2) indicating this mock rapid method does adequately "predict" the success of the RHID.

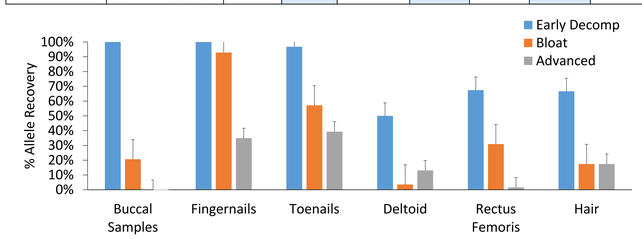

Figure 7: STR Results for Cadaveric Samples

- Of the cadaveric samples tested, buccal swabs were the most suitable for generating the most informative profiles during early decomposition, while nails were a viable option during the bloat stage (Fig. 7).
- Samples collected during advanced decomposition would most likely require traditional DNA extraction and STR typing methods for more complete profile (Fig. 7).

MATERIALS & METHODS

A subset of "good" bone samples 200 mg granules pretreated with 25% Prep-n-Go Buffer, 200 mg no pretreatment, and 200 mg chips pretreated with BTA and complete demineralization buffer were tested on the RHID system to evaluate if results were consistent with the mock rapid method.

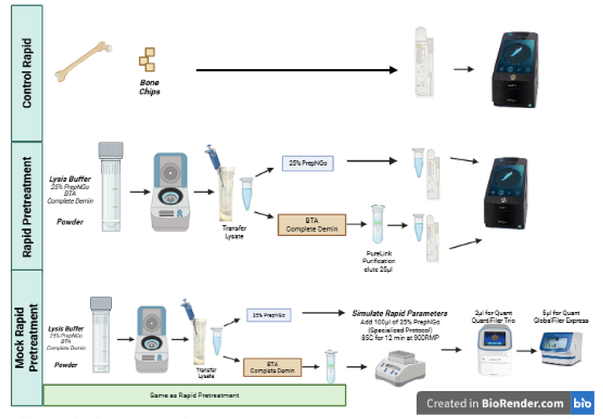

Figure 2: Schematic of rapid, rapid with pretreatment, and mock rapid workflows

Cadaveric Samples

Various cadaveric samples were collected from 3 donors at three stages of decomposition (early decomposition, bloat, and advanced decomposition). These samples were processed using the mock rapid method (Fig. 3).

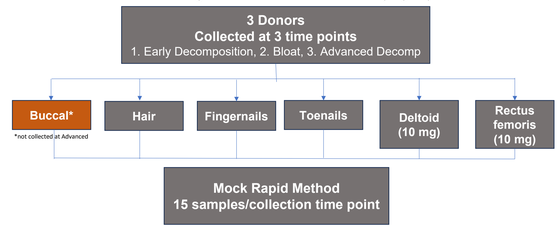

Figure 3: Schematic of cadaveric samples tested

CONCLUSIONS

- Mock rapid method serves as a suitable option and triage tool.
- Processing bones with a spice grinder to produce granules smaller than chips by larger than powder seemed to be the best option.
- Pretreatment did not improve DNA yield or STR results for "compromised" bones.
- Samples collected at the advanced decomposition stage are more likely to need traditional DNA extraction and STR typing methods.

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