Application of Rapid DNA Analysis for Victim Identification in Mass Fatality Incidents

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1. Introduction

- DNA is one of the primary identifiers during Disaster Victim Identification.
- Standard DNA workflow requires 6 to 24 hours to generate a DNA profile depending on the sample type.
- The RapidHIT™ ID System can generate a DNA profile within 90 minutes from sample insertion. The instrument may be particularly useful when investigators need to swiftly identify bodies and body parts for urgent and sensitive cases.

2. Objective

To assess the performance of the RapidHIT™ ID System for the analysis of muscle and bone marrow samples.

3. Materials & Methods

a) Sample Preparation

- Buccal cells were collected from volunteers with 5 buccal swipes using cotton swabs (n=6).
- De-identified human tissue samples (**Fig. 1**) stored at -20°C for > 10 years were retrieved, and 80 mg of tissue was taken from each sample (n=8 for muscle tissues; n=13 for bone marrow).
- Waste pads in used cartridges were recovered as described in **Fig. 2**.

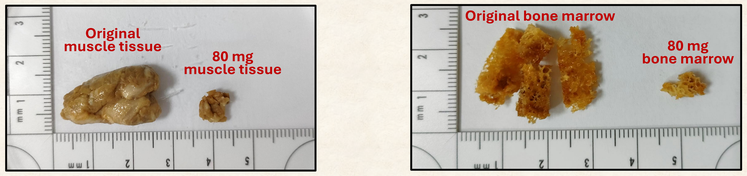


Figure 1: Examples of muscle (**left**) and bone marrow (**right**) samples used. 80 mg of tissue was sampled from the original piece of tissue and was analysed with either RapidHIT or the standard laboratory workflow.

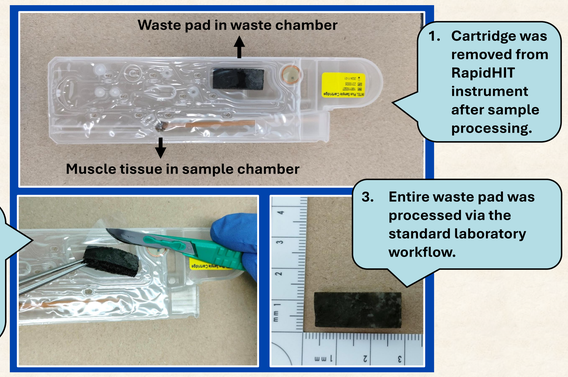


Figure 2: Removal of waste pad from a used sample cartridge.

b) RapidHIT Analysis

- Buccal swabs were processed with either ACE GlobalFiler™ Express cartridge (ACE) or RapidINTEL™ Plus (INTEL+) cartridge with the General Protocol. Tissue samples were processed with INTEL+ only.
- Samples were processed in RapidHIT™ ID System with the RapidLINK™ v2.0 software.

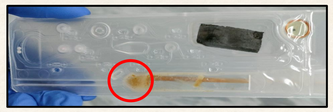


Figure 3: INTEL+ cartridge from a failed sample run. The lysate failed to enter the PCR chamber and remained in the sample chamber (circled in red), due to bone marrow residue clogging the microfluidic channels.
Note: RapidHIT may be unsuitable for samples with fine particulate matter.

c) Standard Laboratory Workflow (Std)

- DNA extraction: Promega Maxwell® FSC instrument with the DNA IQ™ kit.
- Quantitation: ABI Quantifiler™ Trio Kit on the QuantStudio™ 7 Flex.
- Amplification: 1 ng of DNA template, GlobalFiler™ Amplification Kit.
- Detection: ABI 3500xL with injection parameters of 3 µL DNA, 1.2 kV, 24 s.

d) Data Analysis

- Analytical thresholds were 35 RFU and 110 RFU for RapidHIT and Std respectively.
- Height of a homozygous peak was divided by 2 for mean peak height calculations.
- Intra-colour balance for each dye channel was calculated by dividing the sum of peak heights in largest-sized marker, over the sum of peak heights in smallest-sized marker.

4. Results

(A) INTEL+ yielded ~ 2x the peak height from buccal swabs compared to ACE

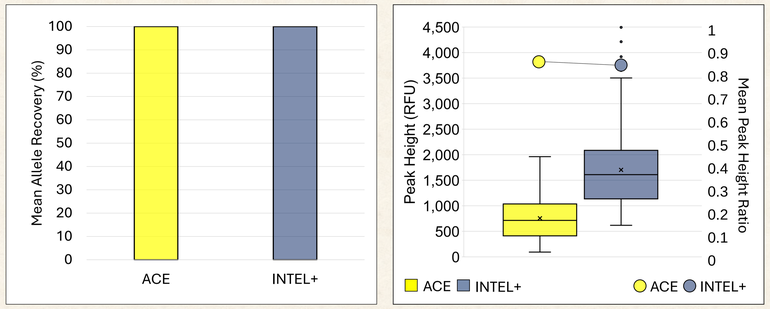
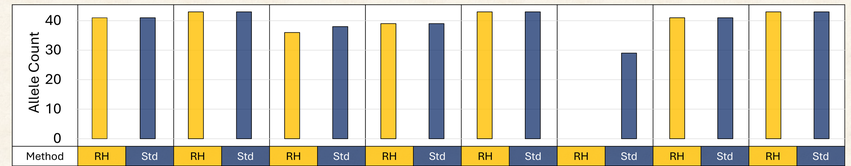


Figure 4: Mean allele recovery, mean peak height (box and whisker) and mean peak height ratio (PHR, dot) from 6 buccal swabs analysed by RapidHIT.

- **The INTEL+ cartridge displayed higher sensitivity than ACE as shown by the higher peak heights. This can be expected with 2 additional PCR cycles and more PCR template as compared to the ACE cartridge.**
- **Given its higher sensitivity, INTEL+ was used for subsequent analysis of tissue samples.**

(B) RapidHIT yielded satisfactory profiles for muscle tissue



Method	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std
Muscle	M1		M2		M3		M4		M5		M6		M7		M8	
Mean PH ±SD (RFU)	3,568 ± 4,800	4,874 ± 2,242	2,724 ± 3,416	5,233 ± 2,112	1,926 ± 2,648	2,776 ± 3,959	2,294 ± 4,512	4,209 ± 1,260	3,119 ± 3,661	5,883 ± 1,626	None	295 ± 226	1,687 ± 1,834	2,871 ±1,636	2,361 ± 3,605	4,366 ± 1,342
Mean ICB	0.25	0.43	0.39	0.65	0.02	0.09	0.14	0.64	0.65	0.79	None	0.66	0.10	0.40	0.27	0.96
Mean PHR±SD	0.78 ± 0.14	0.90 ± 0.07	0.69 ± 0.18	0.67 ± 0.17	0.47 ± 0.21	0.41 ± 0.23	0.77 ± 0.16	0.82 ± 0.13	0.78 ± 0.11	0.89 ± 0.08	None	0.63 ± 0.27	0.74 ± 0.16	0.85 ±0.12	0.81 ± 0.12	0.85 ± 0.10

Figure 5: Allele count, mean peak height (PH), mean intra-colour balance (ICB), and mean peak height ratio (PHR) from 8 muscle samples analysed by RapidHIT (RH) or the standard laboratory workflow (Std). No allele was detected from M6 for RH.

- **Both RapidHIT and Std processing generated profiles with comparable number of alleles.**
- **Profiles from RapidHIT show higher variability (through mean PH SD) in peak heights between loci, as compared to that from Std.**
- **Profiles of sufficient quality can be expected from muscle tissues. RapidHIT may be employed for the analysis of muscle tissues for human identification.**

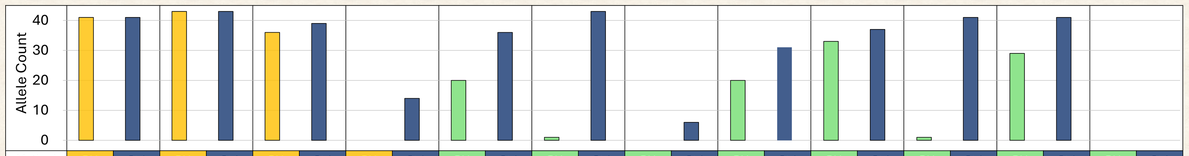
(C) Variable performance for bone marrow samples using RapidHIT analysis

Method	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std
Bone Marrow	BM1		BM2		BM3		BM4		BM5		BM6		BM7		BM8		BM9		BM10		BM11		BM12		BM13	
Mean PH ±SD (RFU)	2,618 ± 4,887	2,625 ± 3,607	1,390 ± 2,907	3,261 ± 2,603	324 ± 372	2,959 ± 2,071	38	3,626 ± 2,438	None	390 ± 304	2,881 ± 4,261	6,033 ± 2,173	502 ± 690	562 ± 513	2,692 ± 5131	2,642 ± 2,642	93	1,794 ± 1,988	353 ± 429	2,754 ± 2,163	None	197 ± 63	2,384 ± 4,643	4,796 ± 4,165	41	169 ± 75
Mean ICB	0.14	0.29	0.06	0.28	0.12	0.29	None	0.31	None	0.42	0.17	0.49	0.25	0.38	0.06	0.16	None	0.24	0.10	0.18	None	0.94	0.07	0.24	None	None
Mean PHR ±SD	0.79 ± 0.13	0.74 ± 0.16	0.78 ± 0.13	0.88 ± 0.12	0.81 ± 0.12	0.81 ± 0.12	None	0.84 ± 0.10	None	0.63 ± 0.16	0.77 ± 0.12	0.85 ± 0.09	0.83 ± 0.16	0.76 ± 0.15	0.64 ± 0.15	0.76 ± 0.10	None	0.77 ± 0.14	0.69 ± 0.22	0.81 ± 0.14	None	None	0.71 ± 0.17	0.83 ± 0.14	None	None

Figure 6: Allele count, mean peak height (PH), mean intra-colour balance (ICB), and mean peak height ratio (PHR) from 13 bone marrow samples analysed by RapidHIT (RH) or the standard laboratory workflow (Std). No allele was detected from samples BM5 and BM11 for RH.

- **Bone marrow samples via RapidHIT generally yielded much lower PH and lower allele recovery (~ 40% lower) than that via Std.**
- **High variability (through mean PH SD) in peak heights between loci, and lower ICB for RapidHIT, as compared to that from Std.**
- **One bone marrow sample (refer to Fig.3) caused analysis failure due to clogging of microfluidic channels by fine particulate matter.**

(D) Waste pad processing from used cartridges produced good quality profiles



Method	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std	RH	Std
Sample	M1	M1 Waste	M2	M2 Waste	M3	M3 Waste	M6	M6 Waste	BM1	BM1 Waste	BM4	BM4 Waste	BM5	BM5 Waste	BM7	BM7 Waste	BM8	BM8 Waste	BM9	BM9 Waste	BM10	BM10 Waste	BM11	BM11 Waste		
Mean PH ±SD (RFU)	3,568 ± 4,800	9,860 ± 4,495	2,724 ± 3,416	7,620 ± 3,585	1,926 ± 2,648	1,844 ± 2,163	None	184 ± 101	2,618 ± 4,887	1,916 ± 3,141	38	5,514 ± 8,225	None	419 ± 513	502 ± 690	846 ± 817	2,692 ± 5131	5,650 ± 7,666	93	2,332 ± 2,310	353 ± 429	1,196 ± 764	None	None		
Mean ICB	0.25	0.56	0.39	0.82	0.02	0.26	None	0.80	0.14	1.37	None	0.50	None	0.71	0.25	1.08	0.06	0.20	None	1.03	0.10	0.55	None	None		
Mean PHR±SD	0.78 ± 0.14	0.86 ± 0.09	0.69 ± 0.18	0.73 ± 0.15	0.47 ± 0.21	0.45 ± 0.26	None	0.62	0.79 ± 0.13	0.84 ± 0.13	None	0.55 ± 0.28	None	0.12	0.83 ± 0.16	0.66 ± 0.19	None	0.65 ± 0.15	None	0.80 ± 0.15	0.69 ± 0.22	0.72 ± 0.15	None	None		

Figure 7: Allele count, mean peak height (PH), mean intra-colour balance (ICB), and mean peak height ratio (PHR) from 12 tissues samples (**orange for muscle, green for bone marrow**) processed with RapidHIT (RH). Corresponding waste pads from these 12 samples were retrieved after RH processing and processed using standard laboratory workflow (Std). Results from waste pad Std processing (**blue**) were compared. No allele was detected from samples M6, BM5, BM11 by RH, and BM11 waste by waste pad processing via Std.

- **DNA recovery from waste pad processing via Std higher (~ 67% higher) than that from corresponding tissue samples via RapidHIT.**
- **Considerably higher peak heights and ICB obtained from waste pad processing via Std, in contrast to tissue samples via RapidHIT. Waste pad processing via Std also offers the advantage of having control over the DNA input amount for analysis.**
- **Waste pad processing via Std managed to rescue alleles for tissues with poor allele recovery via RapidHit (M6, BM4, BM9).**
- **Subsequent waste pad processing via Std can be considered if RapidHit analysis fails/repeat analysis required for result verification.**

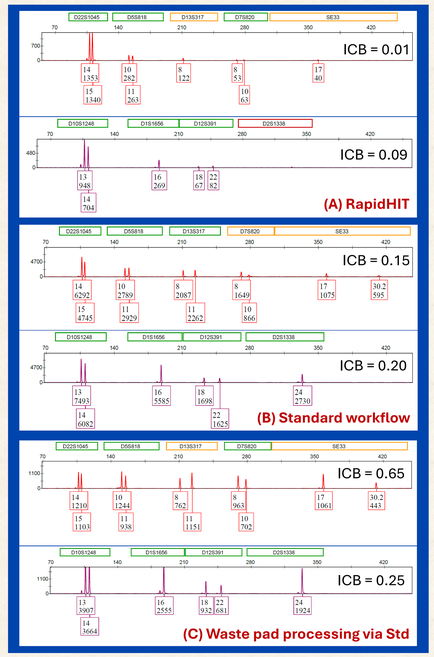


Figure 8: Example of electropherograms obtained from the analysis of bone marrow samples (BM10) for each respective analysis methods stated. Intra-colour balance (ICB) is shown for red (**top**) and purple (**bottom**) channels for each EPG.

Ski-slope effect was least pronounced from waste pad processing via standard workflow. Ski-slope effect more pronounced for RapidHIT.

5. Conclusion

- RapidHIT generally works well with muscle tissue samples; lesser extent for bone marrow samples.
- RapidHIT may not work well with highly degraded samples due to low ICB/high ski-slope effect.
- Waste pad processing a good supplement to RapidHIT.

6. Acknowledgements

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