Bone sample processing on the RapidHIT ID System with RapidINTEL cartridges

In this application note, we demonstrate:

- Discriminating short tandem repeat (STR) profiles from a variety of bone samples processed on the Applied Biosystems™ RapidHIT™ ID System with the RapidINTEL™ Sample Cartridge Kit
- Streamlined bone preparation protocols for use by forensic laboratories or law enforcement agencies
- An efficient sample-to-answer workflow for time-sensitive investigations such as disaster victim identification (DVI)

Sample-to-answer workflow for bone samples

Extraction of DNA from bone is a laborious process involving physical and chemical techniques that can, depending on the method used, take over a day of hands-on time. In this application note, we explore the viability of protocols that streamline this process and allow laboratories and investigators to obtain interpretable data from medium- to high-quality bone samples in significantly less time.

Rapid DNA technology gives forensic laboratories the latest evolution in DNA analysis tools and law enforcement the ability to investigate crime at the point of action in a fully automated workflow (Figure 1). In this study, we provide guidance on applying this innovative approach to bone testing using the RapidHIT ID System with RapidINTEL cartridges. Three independent laboratories utilized different bone sample preparation methods and successfully demonstrated the ability to produce discriminating DNA results from a range of starting samples. The laboratory protocols required minimal manual effort and no offline extraction steps—both are attractive features for minimizing time-to-results.

Figure 1. Bone sample workflow using the RapidHIT ID System.

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Introduction to the RapidINTEL Sample Cartridge Kit

The RapidINTEL Sample Cartridge Kit expands the capability of the RapidHIT ID System to allow for analysis of samples from crime scenes and skeletal remains. The sample cartridge uses Applied Biosystems™ GlobalFiler™ Express chemistry and enables increased sensitivity compared to other RapidHIT ID protocols. The analysis process consists of two parts, primary and secondary. Primary analysis dictates if the results are suitable for database searches or if they require review by a forensic analyst. Primary analysis results on the RapidHIT ID System are displayed in Table 1.

System threshold values were optimized and validated during development of the RapidINTEL workflow. These threshold values are utilized during primary analysis, which is performed automatically on the RapidHIT ID System by an internal version of the GeneMarker™ HID STR Human Identity Software on the instrument. System threshold values define criteria such as minimum peak height ratios and stutter filter percentages. More information on the validation can be found in the user bulletin for the RapidINTEL Sample Cartridge for Blood and Saliva Samples Validation (Pub. No. MAN0018979).

Secondary analysis is required when primary analysis yields a yellow check mark. In other words, if quality score flags are present, a manual review and editing of the data may be necessary. If a sample is determined to be acceptable after review (for example, a single-source sample that was flagged because of a heterozygote peak height imbalance), the data can be utilized for further comparison or database searching. It is important to consider that a single marker can prevent a sample from passing primary analysis; therefore, a sample could theoretically generate a full genotype and still not pass primary analysis. To obtain a true evaluation of a system's efficiency, it is important to measure not only the primary analysis pass rate but also the number of correctly identified peaks or markers that do not trigger quality flags.

Materials and methods

Three forensic laboratories participated in this study: the Forensic Science Laboratory of the French Gendarmerie, France; DNA Labs International, USA; and the Institute of Legal Medicine: University of Mainz, Germany.* The testing sites each employed a unique bone processing protocol on a variety of sample types and ran the samples directly on the RapidHIT ID System v1.1.3 with RapidINTEL cartridges. Secondary analysis was performed using the guidelines for allele interpretation outlined in the user bulletin for the RapidINTEL Sample Cartridge for Blood and Saliva Samples Validation (Pub. No. MAN0018979) with GeneMarker HID software v2.9.5.

* The bone samples provided by the University of Mainz were processed at the Thermo Fisher Scientific laboratory in Darmstadt, Germany.

Table 1. Primary analysis results on the RapidHIT ID System.

Status	DNA profile is generated	Action
	Yes	• Profile suitable for search by law enforcement. • All peaks and markers in the DNA profile met system threshold criteria.
	Yes	• Profile not suitable for search by law enforcement. • Secondary analysis to be performed by a forensic analyst. • One or more system threshold criteria were not met. An internal quality score flag is triggered.
	No	• DNA profile was not generated.

Bone sample preparation

Three different bone preparation protocols were utilized for testing in the rapid workflow (Figure 2). For each site, bone surfaces were first cleaned by scrubbing or removing any soft tissue or debris adhering to the bone.

Site	Protocol	Sample images
Forensic Science Laboratory of the French Gendarmerie (IRCGN)	1. Using a surgical trepan, remove a cylinder approximately 2 mm in diameter and a few millimeters to 1 cm in length. 2. Place sample directly into the sample cartridge.	
DNA Labs International (DLI)	1. In a vented hood, using a drill with a cutting wheel, cut an approximately 2 inch long section from a long bone or large bone fragment. 2. Using a sanding bit, remove any spongy bone present on the surface of the bone cross-section. 3. Wash the bone cross-section 2–3 times with deionized water. 4. Perform an ethanol wash and allow the bone section to dry at room temperature in the hood for approximately 30 min. 5. In a mortar, using a chisel and hammer, pulverize the bone section until it reaches the consistency of rock salt. 6. If needed, a mixer mill can be utilized for further grinding; however, if the bone is powdered it will clog the cartridge. See the image for the desired consistency. 7. Place the sample directly into the sample cartridge. 8. The bone sample can be removed from the cartridge and stored in a freezer to be reanalyzed on a new cartridge.	
University of Mainz (ILM Mainz)	1. Pressing a 6 mm steel drill at a 180° angle onto the surface of the bone, drill into the bone 2–6 cm. A Bosch™ GSR 12V-15 Professional drill was used. 2. Using a single-use scalpel, collect bone chips sticking to the drill and place them into a tube. At this point, the sample is placed at –20°C until ready for processing. 3. Using a swab or single-use tweezers, take 50–60 mg of bone chips out of the tube and place them directly into the sample cartridge.	

Figure 2. Bone sample preparation steps for three testing sites.

Samples

Table 2 summarizes the number, type, and condition of bone samples at each testing site. Each testing site developed their own criteria for judging the quality of the bone samples, based on the age, amount, and condition of the specimen. Table 3 provides further information on how each lab assessed sample quality.

Table 2. Bone sample details for each test site.

Test site	No. of bones, sample IDs	Bone type	Condition	Sample size
IRCGN	10, G-1 to G-21	Femur, rib	High to low quality	20–99.7 mg
DLI	3, D-1 to D-13	Femur, long bone	Medium to low quality	9.9–501.6 mg
ILM Mainz	18, M-1 to M-18	Femur, tibia, skull, rib	High to low quality	50–60 mg

Table 3. Assessment of bone sample quality from each test site.

Test site	Sample ID	Assessment of quality	Details of condition
IRCGN	G-1 to G-9	High	Slightly burnt DVI bone samples from November 2019 (Figure 3)
	G-10 to G-15	Medium	Fleshy bone samples from corpses discovered from July to December 2019
	G-18 to G-21	Low	Skeletonized bone samples from corpses discovered from November 2018 to December 2019
	G-16, G-17		No information provided
DLI	D-1 to D-9	Medium	Bone sample from a corpse discovered in acidic soil
	D-10, D-11	Low	Bone sample from 1986, buried for several years before exhumed
	D-12, D-13		Bone sample from 2002, from a corpse alongside a creek
ILM Mainz	M-5, M-6, M-10, M-11	High	Bone samples with no extreme exposure, aged 1–2 days
	M-15, M-17		Bone sample from a burned corpse, aged 1–2 days
	M-13	Medium	Bone sample from a corpse discovered in water, aged 1–2 days
	M-18		Bone sample from a corpse discovered at home, aged 11 days
	M-1 to M-4, M-9, M-12, M-14, M-16	Low	Skeletonized bone samples, aged beyond 2 weeks
	M-7		Decomposed bone sample, exposed to 1–2 days in formic acid
	M-8		Decomposed bone sample from a corpse discovered in water, aged 56 days

Figure 3. Three DVI-related femur samples tested by IRCGN.

Results

Of the 46 samples processed in the study that passed primary analysis, full single-source profiles were confirmed for 14 (~30%) of the samples. In all cases where a full profile was confirmed, the sample was of medium or high quality. Two samples (D-6 and M-13) passed primary analysis without the need for secondary review. Partial, unconfirmed, or mixture profiles were obtained for 28% of samples passing primary analysis.

Table 4 summarizes the primary analysis allele recovery rate (the percentage of markers meeting all system thresholds) and secondary analysis allele recovery rate (the percentage of markers that can be confidently called or confirmed with manual review). The “Quality” column offers a generalized description of the bone based on age, condition, and environmental information, and characterized by each laboratory as detailed in Tables 2 and 3. Seven samples are not included in the table. Of these, six samples failed to generate a DNA profile in primary analysis due to an instrument, consumable, or size-quality failure. Inhibition introduced by the sample could not be ruled out for three of the samples that were not rerun (G-21, M-2, and M-7). Three samples from IRCGN (G-14, G-17, and G-20) were run at alternate inputs, which confirmed an issue related to the run rather than a sample issue. A single sample, a femur from a burned body (M-15), generated a profile of at least two contributors and secondary analysis was not performed; all markers had data suitable for interpretation. On average, 22% of markers were recovered after a manual review following interpretation guidelines set forth in the RapidINTEL development validation. For partial profiles, the probability of identity (PI) was calculated using US Caucasian marker frequencies for all confirmed markers except amelogenin. The PI ranged from 4.9×10^{-7} to 2.8×10^{-25} (for G-1 and G-2, respectively).

Table 4. Primary and secondary analysis recovery rates.

Sample ID	Bone	Sample size	Quality	Primary analysis (percentage of unflagged markers)*	Secondary analysis (percentage of markers confirmed after review)	Final result**
G-1	Femur 1	20 mg	High	23%	32%	
G-2		58.7 mg		73%	95%	
G-3		99.7 mg		45%	77%	
G-4	Femur 2	70.2 mg		64%	100%	
G-5		89.6 mg		95%	100%	
G-6		93.9 mg		77%	100%	
G-7	Rib 1	87.6 mg		91%	100%	
G-8		72.5 mg		68%	100%	
G-9		92.3 mg		68%	100%	
M-5	Tibia 1	50–60 mg		91%	100%	
M-6	Femur 5			50%	86%	
M-10	Rib 1			95%	100%	
M-11	Rib 2			95%	100%	
M-17	Femur 10			95%	100%	
G-10 to G-12	Femur 3	20, 88.6, 93.8 mg	Medium	0	0	
G-13	Femur 4	85.4 mg		0	0	
G-15	Femur 5	91.8 mg		0	0	
D-1	Femur 1	9.9 mg		91%	95%	
D-2		9.9 mg second run		4.5%	73%	
D-3		10 mg		95%	100%	
D-4		10 mg second run		82%	95%	
D-5		10 mg third run		0	50%	
D-6		47.3 mg		100%	N/A	
D-7		50.5 mg		95%	100%	
D-8		93.7 mg		86%	91%	
D-9		97 mg		77%	82%	
M-13	Femur 8	50–60 mg		100%	N/A	
M-18	Femur 11			4.5%	73%	
M-1	Femur 1		Low	0	0	
M-3	Femur 3			0	0	
M-4	Femur 4			0	0	
M-8	Femur 8			0	0	
M-9	Skull 1			0	0	
M-12	Skull 2			0	0	
M-14	Skull 3			0	0	
M-16	Skull 4			0	0	
G-18	Femur 7	90.5 mg	Low	4.5%	45%	
G-19	Femur 8	80.5 mg		0	0	
D-10, D-11	Femur 2	121.5, 501.6 mg		0	0	
D-12, D13	Long bone 1	102 mg, 200 µL		0	0	
G-16	Femur 6	86.3 mg	No info	0	0	

* Y indel and DYS391 are not included in the marker recovery evaluation; therefore, the total possible number of markers is 22.

** A full profile with all markers either meeting system thresholds or confirmed during secondary analysis.
Either at least one marker with an allele in the stochastic range or at least one marker with artifact(s) interfering with genotype determination resulted in an unconfirmed marker genotype. However, knowing the correct genotype, it was determined that all alleles were present.
A partial profile that could include markers with complete dropout or a sister allele falling below the analysis threshold.
There were no confirmed markers, with zero to four peaks present in the profile.

Second and third runs were performed with femur 1 from DLI for the 9.9 mg and 10 mg sample inputs (D-2, D-4, and D-5). In these cases, the bone chips were removed from the cartridge and placed into a different cartridge to run. DNA profiles that are acceptable for database searches or reference comparisons were generated for the original and repeat runs.

Long bone 1 from DLI was processed in two ways; the nonchemical protocol described in Figure 2 was used for sample D-12 and yielded no results. The laboratory then predigested a sample from the same bone (D-13) in digest buffer overnight at 56°C in a thermomixer before adding 200 µL of the slurry to the sample cartridge. The addition of the digest step did not yield better results for this long bone.

The DLI femur 1 sample at higher inputs (D-8 and D-9) generated profiles with artifactual data that complicated secondary analysis and resulted in unconfirmed markers. For the IRCGN DVI bones, femur 2 and rib 1, where full profiles were confirmed, the lower sample input amounts resulted in data that required less manual editing of artifacts during secondary analysis. In these cases, high-quality bone did not require increased sample inputs to generate full profiles with minimal manual editing.

Figure 4 displays the electropherograms for different sample results (green, yellow, and orange as indicated in Table 4). Secondary analysis improved marker recovery for all samples where DNA was recovered.

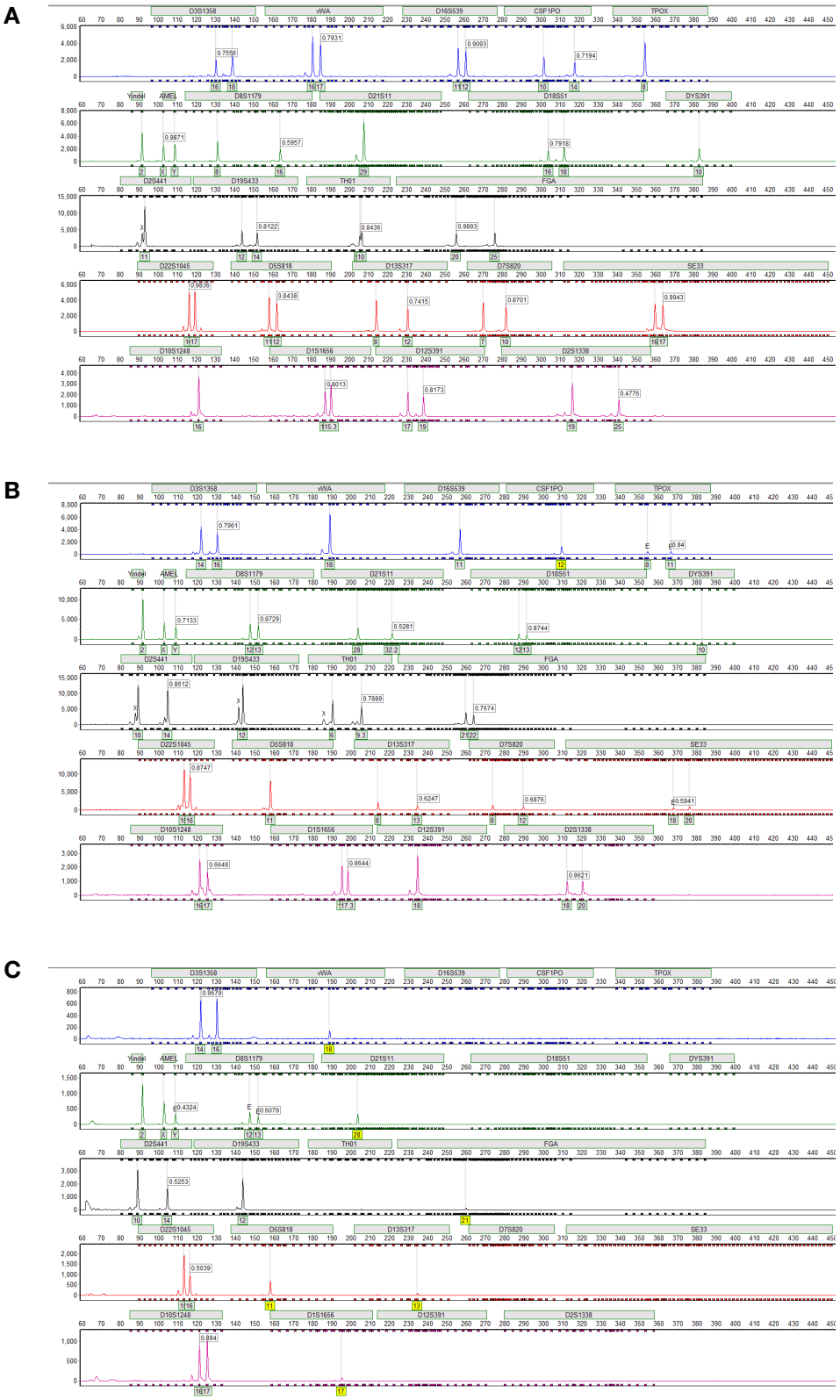

Figure 4. Representative electropherograms for different sample results. (A) G-5 femur 2 (green result, Table 4): 100% of alleles recovered after secondary analysis. Probability of identity calculated from the frequencies of the 21 confirmed markers is 3.7×10^{-26}. **(B)** G-2 femur 1 (yellow result, Table 4): 95% of alleles recovered after secondary analysis. The yellow-flagged allele indicates the marker was not confirmed due to a peak in stochastic range. Probability of identity calculated from the frequencies of the 21 confirmed markers is 2.8×10^{-25}. **(C)** G-1 femur 1 (orange result, Table 4): 32% of alleles recovered after secondary analysis. Yellow-flagged alleles indicate markers that were not confirmed due to peaks in stochastic range. Probability of identity calculated from the frequencies of the 21 confirmed markers is 4.9×10^{-7}.

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Conclusions

Rapid DNA analysis using the RapidHIT ID System with RapidINTEL cartridges provides a fully automated, point-of-action response to identification of human remains.

Each of the three protocols presented here demonstrates the ability to generate suitable DNA profiles from bone. The procedures offer the added benefits of minimal hands-on time and a streamlined workflow for direct introduction of the sample into the RapidHIT ID System for processing with minimal physical and chemical constraints. Additionally, evidence suggests that samples can be reprocessed if needed.

The quality—age, amount, and condition—of DNA from skeletal remains can have a significant impact on the amount of STR data recovered. When bone samples are of medium to high quality, the RapidHIT ID System protocol can produce discriminating, partial to full profiles using GlobalFiler Express chemistry, for database searches or reference comparisons to assist with conclusive identification. With lower-quality samples such as remains exposed to harsh conditions, a traditional capillary electrophoresis workflow may be required.

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