Human identification

Enhancing forensic efficiency: automated casework workflows with the HID NIMBUS Presto QNA System

In this application note, we demonstrate the following:

- Flexible and reliable automated quantification and amplification workflows for casework samples using the Applied Biosystems™ HID NIMBUS™ Presto QNA System
- Faster turnaround times and easy, walk-away workflows for purification, quantification, and amplification setup
- Results from automated workflows are comparable to or better than those obtained through traditional methods

The HID NIMBUS Presto QNA System can efficiently manage your case workload

Samples processed by forensic laboratories are inherently unique, with their quality and quantity often unpredictable, particularly in challenging cases. Consequently, when considering workflow solutions, one must take into account several factors that can impact results, such as sample degradation, inhibitors, and low amounts of biological material. In this respect, it is essential to prioritize the optimization of DNA yield, minimize contamination, prevent human error, and enhance the accuracy and consistency of results.

The HID NIMBUS Presto system is a proven automated platform that enables high-quality extraction from various sample types. Laboratories often prioritize operational efficiency through advanced technology as the demand for forensic analysis continues to increase. In response to this need, the capabilities of the HID NIMBUS Presto system have been expanded to include quantification setup, normalization, and amplification setup (QNA). The HID NIMBUS Presto QNA System provides an automated workflow for casework to assist with the effective management of larger caseloads. Additionally, the instrument features user-friendly software that assists during instrument loading with intuitive visual and written instructions.

This application note demonstrates a fast turnaround time and results comparable to those of traditional sample processing methods when using the new quantification and amplification workflows of the HID NIMBUS Presto QNA System in conjunction with the Applied Biosystems™ Quantifiler™ Trio DNA Quantification Kit and the GlobalFiler™ IQC PCR Amplification Kit. The work

was performed by Dr. Sheree Hughes, Associate Professor of Forensic Science at Sam Houston State University, and Kayli Carillo from her graduate student team.

Materials and methods

The Applied Biosystems™ PrepFiler™ BTA Automated Forensic DNA Extraction Kit was used to process bone, hair, nail, chewing gum, and blood mock casework samples (Table 1) on the HID NIMBUS Presto QNA System using the latest validated script v3.2.2 and Applied Biosystems™ HID NIMBUS™ System software.

Table 1. Casework sample types and quantities included in the study.

Sample type	Quantity
Bone*	12
Hair	4
Nail	3
Gum	5
2 µL blood on swab	1
Total	25

* Bone samples were subjected to the following conditions: burial (BD, n = 4), burning (BRN, n = 4), or surface decomposition (SD, n = 4).

The Quantifiler Trio DNA Quantification Kit was used to assess the quantity and quality of the DNA isolated from the 25 mock casework samples and a sensitivity series across 3 runs using the HID NIMBUS Presto QNA System with the latest validated script v1.2.2 and HID NIMBUS System software for the quantification workflow. This included male DNA control 007 dilutions at 4 different input amounts (0.625 ng, 2 ng, 10 ng, and 20 ng). Two standard curves were included in each run, resulting in a total of six standard curves. Serial dilutions were set up by the HID NIMBUS Presto QNA System. All samples were analyzed as duplicates.

Subsequently, a PCR reaction plate containing duplicates for each sample was set up with GlobalFiler IQC chemistry on the HID NIMBUS Presto QNA System using the latest validated script v1.2.2 for the amplification workflow. A 29-cycle amplification protocol was used with a normalized 1 ng target DNA input. A maximum volume of 15 µL was added to the PCR reaction for samples with lower concentrations.

The purified DNA samples were reanalyzed using a manual quantification and amplification process with the same consumables and conditions to compare turnaround time (data not shown). All amplified products were run on an Applied Biosystems™ 3500 Genetic Analyzer using Thermo Scientific™ Data Collection Software v4.0 and Applied Biosystems™ GeneMapper™ ID-X v1.6.

Results

The quantification standard curves prepared using the HID NIMBUS Presto QNA System returned the expected performance metrics. All the averaged R^2 values between standard curve replicates were ≥0.99, indicating a close fit between the standard curve regression line and the individual C_t data points. Averaged slope values were close to the expected –3.3 value for 100% amplification efficiency and in the expected ranges for each target. The comparison of average internal positive control (IPC) C_t for sample replicates between the HID NIMBUS Presto QNA System and the manual workflow showed a C_t difference within 1 cycle and did not indicate the presence of any PCR inhibitors. Samples with a high concentration showed the expected IPC C_t shift in response to increasing DNA concentrations (data not shown).

The quantification results for duplicates analyzed on the HID NIMBUS Presto QNA System showed reproducible values based on the small autosomal target. The relative standard deviation for duplicates ranged from 0.4% to 37.1% for the automated workflow and 0.8% to 38.3% for the manually processed samples. The highest variability was an expected result from a nail sample due to stochastic effects (data not shown). Figure 1 shows the average quantity of the duplicates for the manual and automated quantification workflows.

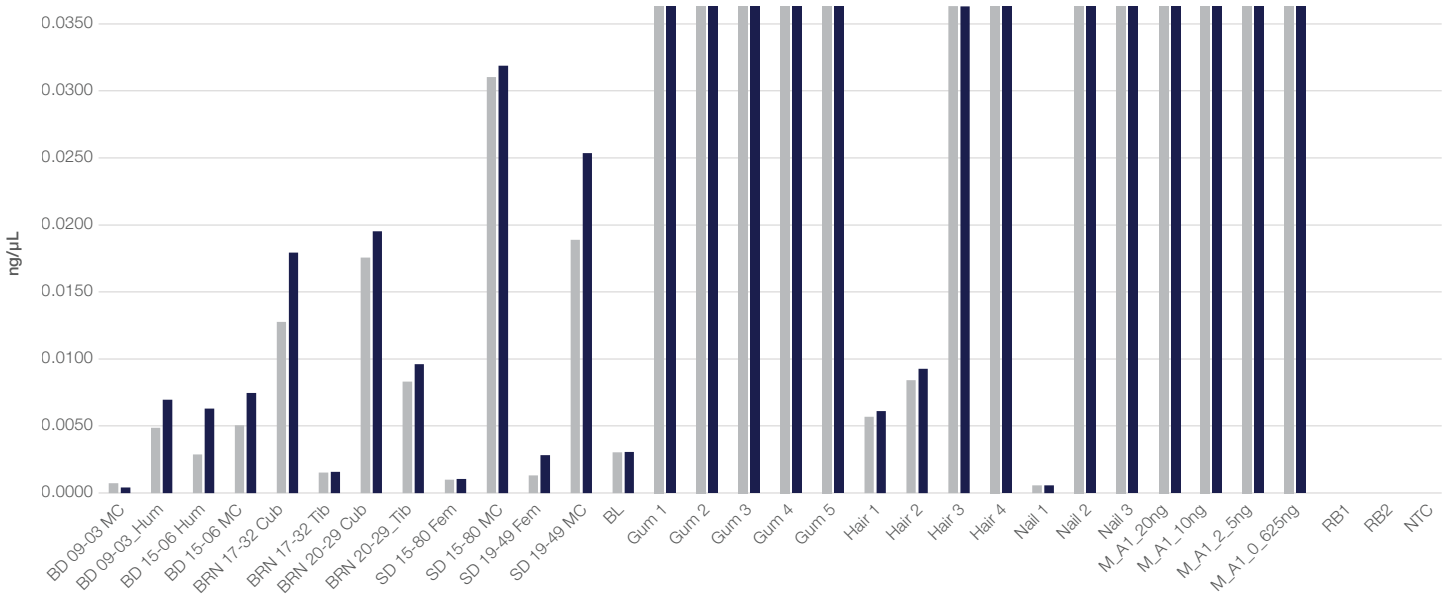

Figure 1. Comparison of the average quantity results for the samples in the manual and automated workflows. Results ranged from 0.0004 to 18.5 ng/µL; note, the y-axis is scaled to 0.0350 ng/µL to focus on the lower-concentration samples. BD = buried bone, BRN = burnt bone, SD = surface decomposed bone, BL = blood, M_A1 = male control 007, RB = reagent blank, NTC = no-template control.

Amplicons from the HID NIMBUS Presto QNA System workflow yielded well-balanced, average heterozygous peak heights above 6,400 RFU for the sensitivity series, as shown in Figures 2 and 3 and Table 2. The peak height ratio (PHR) of sister peaks was consistently above 60% (range of 63–100%) for all 4 DNA input amounts. The peak height ratio results were comparable when processing the samples in the manual workflow (Table 3).

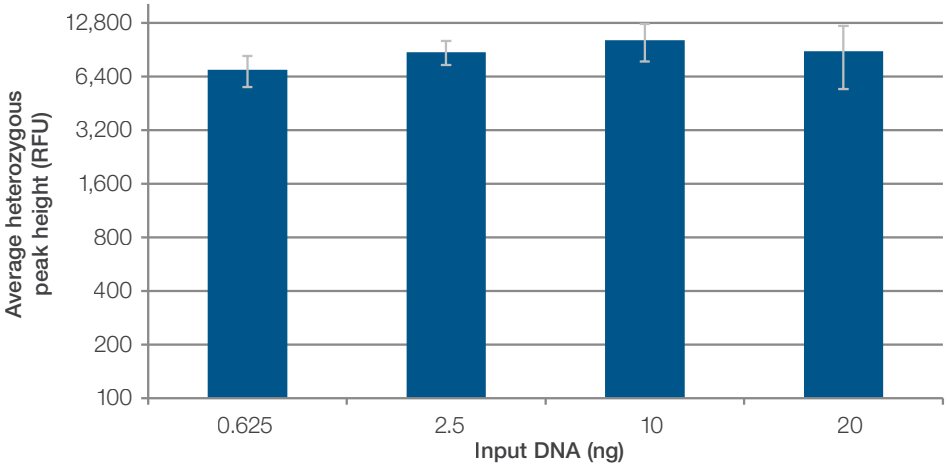

Figure 2. Average heterozygous peak height for the positive control dilutions normalized at 1 ng and prepared using the HID NIMBUS Presto QNA System. The error bars show 1 standard deviation.

Table 2. PHR across markers for the positive control dilutions prepared using the HID NIMBUS Presto QNA System.

Input DNA (ng)	Rep	D3S1358	vWA	D16S539	CSF1PO	AMEL	D8S1179	D21S11	D18S51	D2S441	D19S433	TH01	FGA	D22S1045	D7S820	SE33	D10S1248	D1S1656	D12S391	D2S1338	Minimum PHR	Maximum PHR
20	1	84%	80%	91%	77%	90%	80%	80%	93%	70%	71%	75%	95%	99%	82%	82%	98%	87%	83%	71%	70%	100%
	2	91%	97%	97%	70%	72%	84%	100%	98%	84%	92%	83%	80%	73%	88%	95%	96%	93%	78%	72%		
10	1	100%	96%	94%	90%	63%	80%	97%	96%	99%	94%	88%	89%	89%	81%	100%	78%	89%	88%	74%	63%	100%
	2	100%	78%	96%	79%	73%	95%	90%	100%	91%	89%	66%	74%	98%	77%	96%	96%	94%	83%	87%		
2.5	1	78%	91%	92%	81%	67%	95%	94%	94%	93%	87%	82%	92%	94%	93%	77%	95%	80%	89%	84%	67%	99%
	2	79%	99%	94%	97%	75%	97%	91%	78%	99%	94%	86%	86%	77%	80%	83%	94%	91%	78%	94%		
0.625	1	93%	92%	89%	98%	83%	94%	93%	95%	87%	98%	89%	84%	91%	98%	70%	100%	98%	80%	92%	68%	100%
	2	80%	88%	87%	94%	70%	72%	94%	86%	98%	75%	96%	98%	79%	87%	70%	95%	85%	68%	89%		

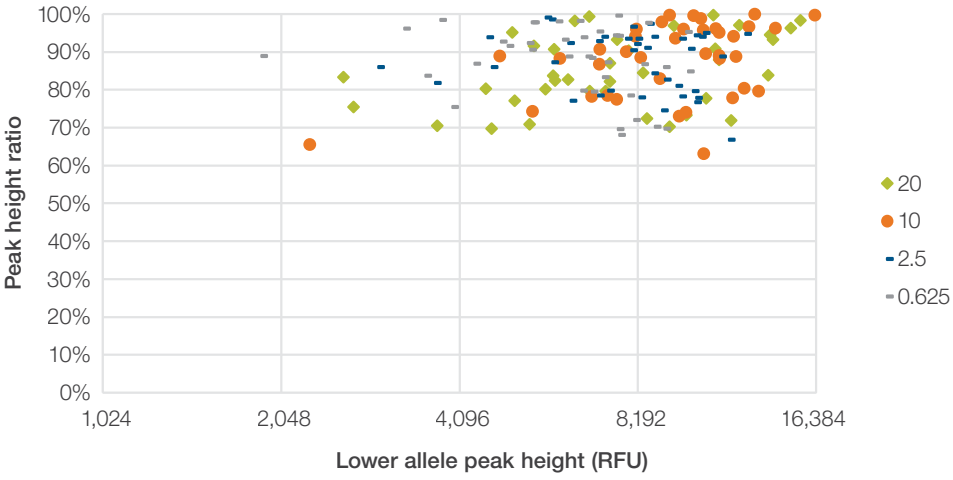

Figure 3. Peak height ratio vs. peak height for the positive control dilution duplicates prepared using the HID NIMBUS Presto QNA System.

The mock casework samples (NP1–25) average peak height results are shown in Figure 4 with the relative total input DNA used in the PCR reaction. The difference in average peak height across samples is due to the sample-to-sample variability, sample type, and stochastic effects responsible for random variations, particularly with low template samples, leading to variability in amplification efficiency.

Conclusions

This study highlights the effectiveness and reliability of the HID NIMBUS Presto QNA System for automated forensic DNA analysis. The system helps enhance efficiency by streamlining DNA quantification and amplification processes, supporting faster turnaround times and larger caseloads. Results achieved with the HID NIMBUS Presto QNA System were better than or comparable to traditional manual methods, demonstrating consistent and reproducible outcomes. The automated workflow delivered high-quality results from diverse sample types, including bone, hair, nail, gum, and blood. Performance quantification metrics within expected ranges and well-balanced profiles with consistent heterozygous peak heights and peak height ratios indicate the ability of the HID NIMBUS Presto QNA System to manage the quantification and amplification setup. The system effectively managed low-template samples, addressing degradation and inhibition. Overall, the HID NIMBUS Presto QNA System can be a valuable tool for modern forensic laboratories, helping to enhance operational efficiency, accuracy, and consistency in DNA analysis.

Table 3. Peak height ratios across markers for the positive control dilutions duplicates prepared with the manual workflow.

Input DNA (ng)	Rep	D3S1358	vWA	D16S539	CSF1PO	AMEL	D8S1179	D21S11	D18S51	D2S441	D19S433	TH01	FGA	D22S1045	D7S820	SE33	D10S1248	D1S1656	D12S391	D2S1338	Minimum PHR	Maximum PHR
20	1	86%	94%	92%	75%	75%	90%	88%	93%	86%	92%	77%	83%	75%	98%	90%	74%	97%	76%	74%	74%	99%
	2	82%	91%	87%	98%	81%	99%	74%	87%	85%	76%	97%	99%	96%	91%	81%	84%	94%	80%	83%		
10	1	91%	90%	83%	82%	77%	98%	99%	99%	83%	93%	93%	78%	84%	91%	89%	95%	79%	97%	71%	65%	100%
	2	87%	99%	85%	100%	95%	81%	82%	99%	95%	84%	73%	95%	82%	98%	93%	80%	85%	65%	99%		
2.5	1	93%	92%	98%	92%	77%	93%	87%	89%	99%	99%	82%	77%	80%	95%	83%	95%	98%	74%	98%	67%	100%
	2	85%	92%	80%	90%	89%	85%	85%	98%	78%	97%	67%	97%	87%	89%	84%	100%	83%	81%	99%		
0.625	1	82%	98%	77%	82%	64%	92%	83%	91%	82%	85%	82%	95%	86%	91%	90%	98%	97%	90%	90%	64%	100%
	2	95%	85%	86%	91%	90%	89%	87%	100%	78%	87%	86%	99%	80%	77%	99%	96%	94%	98%	82%		

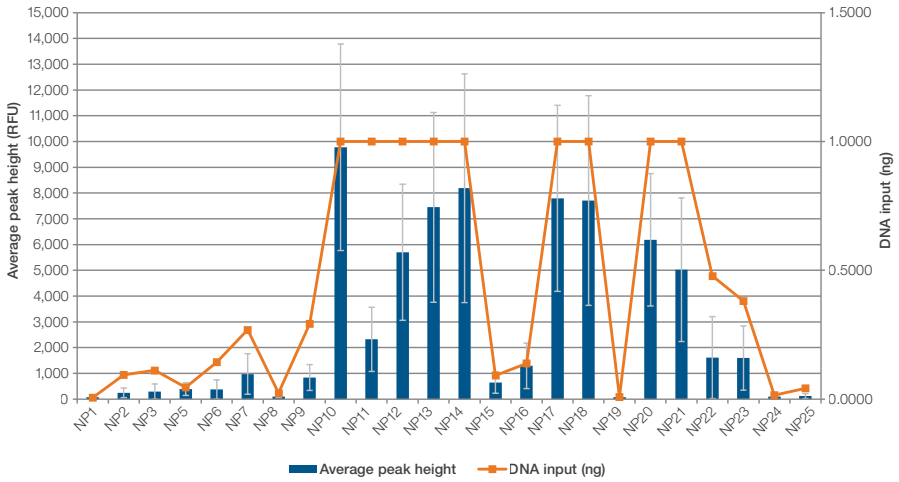

Figure 4. Average peak heights of the mock casework sample duplicates (blue bars) and total PCR input DNA in ng (red line). The NP sample types are: buried bone (NP1–NP3), blood (NP5), burnt bone (NP6–NP9), gum (NP10–NP14), hair (NP15–NP18), nail (NP19–NP21), and surface decomposed bone (NP22–NP25). The error bars represent the standard deviations of the peak heights. NP4 yielded negligible results so it is not shown in this graph.

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