RAPID DNA PROJECT – PHASE 2

Detailed Technical Evaluation Report for the DNAscan System

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TECHNICAL SUMMARY

The GE Healthcare and NetBio DNAscan system was evaluated as part of the Rapid DNA End-to-end project. .

The DNAscan system is a rapid DNA instrument capable of analysing forensic samples from sample-in to profile-out within two hours. The DNAscan system tested in the present study had two BioChipSet Cassettes types available to perform DNA profiling: A High DNA Content Powerplex 16 (HDC PP16) BioChipSet capable of processing samples that contain high amounts of DNA, and a Low DNA Content PowerPlex 16 (LDC PP16) BioChipSet able to process samples with low quantities of starting DNA such as crime scene samples. This Phase 2 project was a re-evaluation of the DNAscan system for the laboratory samples tested in Phase 1 of the project. This was necessary as the biochip sets used in phase 1 were either nearing the expiry date or had expired (due to delays in the project outside the control of any of the partners). Additionally, the LDC PP16 BiochipSet was not available for Phase 1 of the project.

Overall, the DNAscan instrument provided good quality DNA profiles with high accuracy and success. The HDC BioChipSet cassette, which was designed for processing reference buccal samples, provided very high accuracy (100 %) and success rates (95 %) in this study. These rates were comparable to conventional DNA typing methods. While the lane failure rate (5 %) for the reference samples in the current study was comparable to the manufacturer's experience, it is an area for improvement which the manufacturers have stated they intend to work on.

The present study is the first time the Low DNA Content (LDC) BioChipSet Cassette has been tested in the Australian context. The LDC BioChipSet is a modified version of the HDC BioChipSet which was specifically developed to be able to provide rapid DNA profiles for low level DNA case type samples. In general, the results from the samples processed using the LDC BioChipSet were good quality and accurate. The success rate at 81 % was lower for the casework type samples processed using the LDC BioChipSet compared to the reference samples run using the HDC BioChipSet protocol. This may be expected given the inherent variability of case type samples. The accuracy in processing casework type samples was also lower (96%) with the LDC BioChipSet and significantly there were observed to be two samples in which alleles were mistyped and the alleles were not flagged by the system. For one of these samples, the alleles were not flagged but the sample was flagged yellow and upon review the mistyping may have been identified. The other sample was not flagged by the system and the locus was called a homozygote when in fact there was a missing heterozygote allele. These mistypings were only observed for weak touch samples in which stochastic effects are commonly observed. The mistyped loci were observed to be below 1,000 RFU.

Given the detection of these mistypings, it would not be advisable at this stage to upload all profiles using the LDC BioChipSet protocol without a trained DNA analyst to be available to review the samples. The protocol did, however, show promise in being able to successfully process a number of different case type samples. With further optimisation of a stochastic threshold, the accuracy for typing very low level samples could be improved for this new BioChipSet. In addition, the Company has announced HDC and LDC BioChipSets with 27 loci and have stated that they have worked to optimise stochastic thresholds in these settings. As this assay includes the NCIDD core loci, it may be an option to assess in the future.

ABBREVIATIONS

ANZPAA NIFS	Australia New Zealand Policing Advisory Agency National Institute of Forensic Science
CODIS	Combined DNA Index System (USA National DNA database system)
DNA	Deoxyribonucleic Acid
DNAscan	Instrument from GE Healthcare/NetBio
DVI	Disaster Victim Identification
GeneMapper	GeneMapper ID-X (Version 1.4) – Profile analysis software used in this study
HDC	High DNA Content
LDC	Low DNA Content
NCIDD	National Criminal Investigation DNA Database
NSW FASS	New South Wales Forensic & Analytical Science Service
PP16	PowerPlex 16 Amplification System
PP21	PowerPlex 21 Amplification System
PCR	Polymerase Chain Reaction
RFU	Relative Fluorescent Units
STR	Short Tandem Repeat
VPFSC	Victoria Police Forensic Science Centre

INTRODUCTION

The Rapid DNA end-to-end evaluation project was undertaken to investigate the implications of implementing rapid DNA technology within laboratory and law enforcement environments. The first phase of the study involved the evaluation of the performance of three different rapid DNA systems; the IntegenX RapidHIT 200 system, the GE Healthcare and NetBio DNAscan system and the ParaDNA system from LGC. This Phase 2 project was required as the biochip sets used in Phase 1 were either nearing the expiry date or had expired (due to delays in the project outside the control of any of the partners). Additionally, the LDC PP16 BiochipSet was not available for Phase 1 of the project.

The objective of the present study was to re-evaluate the DNAscan instrument using BioChipSet Cassettes with protocols specifically designed for the sample types being tested. This evaluation provides a greater understanding of the capabilities of the DNAscan system using new methodology not available for Phase 1. The retesting of the DNAscan system was carried out at the Victoria Police Forensic Science Centre (VPFSC) and facilitated by the Australia New Zealand Policing Advisory Agency – National Institute of Forensic Science (ANZPAA NIFS). Testing of the instrument in a non-laboratory setting was not performed in this study.

Despite automated DNA laboratories having the capability to process samples in as little as a few days, there are always situations in which an even faster processing time frame would be desired. Rapid DNA instruments such as the DNAscan system can address this issue as the system has the ability to obtain a searchable DNA profile in a very short time frame (within two hours). One of the other major benefits is the system's ability to generate a DNA profile while being operated by non-scientific individuals in non-laboratory settings. To enable this, the system must possess a high first pass rate and the system must be able to reliably and correctly report the DNA profiles generated.

GE Healthcare and NetBio: DNAscan

The GE Healthcare and NetBio DNAscan system performs all DNA analytical processes in a single use, disposable BioChipSet Cassette. The BioChipSet or 'lab-on-a-chip' contains all the reagents necessary to perform STR analysis and uses microfluidics to move solutions through the analytical processes on the BioChipSet. The BioChipSet Cassette is loaded into the benchtop DNAscan instrument for processing and samples are typically collected using specialised swabs that are inserted into the dedicated sample chambers of the BioChipSet Cassette. The system performs fully automated

processing of samples including extraction, amplification, electrophoresis and data analysis to provide an STR profile in under two hours.

Extraction on the DNAscan instrument is performed using a guanidinium-based lysis and silica membrane purification. The DNAscan system utilises the PowerPlex 16 (PP16) amplification system targeting 16 loci (15 STR markers and amelogenin) and amplification consists of 31 PCR cycles. The amplification reagents are lyophilised which enables the BioChipSet cassettes to be stored at room temperature (Tan et al, 2013). Separation of the STR amplicons is also carried out via electrophoresis through specialised channels containing a sieving matrix and laser excitation and detection of the labelled fragments occurs through a detection window incorporated into the BioChipSet cassette. At the completion of separation and detection, signal processing is automatically initiated following which automated allele calling is performed by the expert system based on rules that were developed to mimic the processes used by a forensic analyst (Tan et al, 2013).

At the time of Phase 1 testing, the system was able to process only the High DNA Content (HDC) BioChipSet Cassette, however, for Phase 2 testing the DNAscan instrument is capable of using a second BioChipSet type which has been specifically designed for Low DNA Content (LDC) samples. The HDC BioChipSet Cassette was designed for the analysis of reference mouth swab samples and can process up to five samples plus a ladder in a single run. The LDC BioChipSet Cassette was designed for the processing of crime scene type samples and can process up to four samples plus a ladder in a single run. The instrument automatically detects the type of BioChipSet Cassette that has been loaded and selects the appropriate protocol for processing. Both the High and the Low DNA Content BioChipSet Cassettes were used in this evaluation.

The HDC BioChipSet Cassette was designed to process buccal swabs which can contain very high quantities of DNA (microgram amounts of DNA). Due to this high starting amount of DNA, the BioChipSet Cassette for HDC samples was developed to reduce the quantity of DNA recovered at each process such that the resulting profile would not be overloaded (Tan et al, 2013). The opposite was necessary when developing a BioChipSet Cassette to process low level DNA samples which often contain minute quantities of DNA (often picogram amounts). Thus, the LDC Cassette was designed to maximise the DNA recovered. The changes made included: increasing, to approximately 100%, the percentage of DNA lysate volume put forward for DNA purification, increasing the DNA binding to the purification filter, and concentrating the purified DNA before the amplification step (Turingan et al, 2016).

The inclusion of the concentration module and waste chambers took up more physical space in the LDC BioChipSet resulting in enough room for the processing of only four samples (and a ladder) compared to the five samples for the HDC BioChipSet. Additionally, the modifications to the LDC BioChipSet Cassette raised the processing time to approximately 102 minutes as opposed to the 84 minutes processing time for the HDC BioChipSet Cassette (Turingan et al, 2016).

The sample chamber of the BioChipSet was originally designed for specialised swabs (NetBio BioChipSet swabs) which incorporate an RFID chip in the cap for sample tracking. For case type samples, the substrate can be inserted into the bottom of the sample chamber using forceps and held in place with a clean NetBio BioChipSet swab.

The STR Multiplex PP16 Assay used in the present study is not fully compatible with NCIDD core loci. An expanded STR 27plex locus set (comprising 26 STR loci and amelogenin) has also been applied to the BioChipSet cassettes which may be more beneficial to the processing of degraded samples since there are a larger number of smaller molecular weight loci in the expanded marker set (Turingan et al, 2016).

Flagging system of results

There are three flag options in the DNAscan system: A green checkmark against a sample indicates the sample successfully passed the Expert System rules for all 13 CODIS core loci and also met the success criteria preconfigured (minimum number of CODIS core loci) by the expert system. An .xml (databasing) file is generated with the allele calls for all loci.

A yellow checkmark indicates that the sample has at least one CODIS core locus that did not meet an Expert System Software rule, but the profile did contain passing results for the minimum number of CODIS core loci preconfigured by the system. The purpose of the yellow sample flag is to alert the user that a trained DNA analyst may need to examine the profile. An .xml (databasing) file is generated for samples assigned a yellow checkmark, but the file only contains the loci that passed all Expert System rules.

A red "X" indicates that the sample did not generate a profile that passed the Expert System rules for the minimum number of required CODIS core loci preconfigured by the system and an .xml (databasing) file is not generated for samples assigned a red "X".

The expert system generates an electropherogram in a bitmap file format (.bmp). Allele calls in grey boxes in the electropherogram represent alleles that have met the expert system calling rules. While alleles in red boxes represent alleles that are questionable and require a review by a trained analyst.

EXPERIMENTAL RESULTS

All experiments were performed in a laboratory environment at VPFSC and the instrument operated by DNA analysts.

Success and Accuracy

Success

To assess the success and accuracy of the instrument, 10 buccal swabs were taken from 10 individual donors. The swabs were taken every two hours, excluding times half an hour after eating or drinking. The buccal swabs were processed using the HDC BioChipSet Cassette.

Single source profiles were considered successful while failed and negative samples were considered unsuccessful. A 70% partial profile was thought to possess a sufficient number of alleles for comparison purposes to a crime scene profile as well as enabling upload to a database, if appropriate. Since different laboratories have varying policies for upload to NCIDD, 70% was chosen to reflect a very conservative approach. For the DNAscan system, using the 16 loci, 21 or more alleles were required to be present to be considered at the 70% level.

Accuracy

Accuracy was determined as the number of samples that gave the correct allele calls as a percentage of the number of samples that resulted in a profile. Sample profiles containing loci that were not flagged and contained an incorrect allele call, which were included in the exported allele call table, were deemed incorrect. Severe heterozygosity imbalance resulting in the loss of an allele (i.e. presenting as a homozygote) was considered incorrect. Sample profiles with an incorrect allele call that were flagged by the system as requiring a review were not deemed to be incorrect as a scientist would review them or not included in an exported allele call table (i.e. used to upload and match to a database).

Results

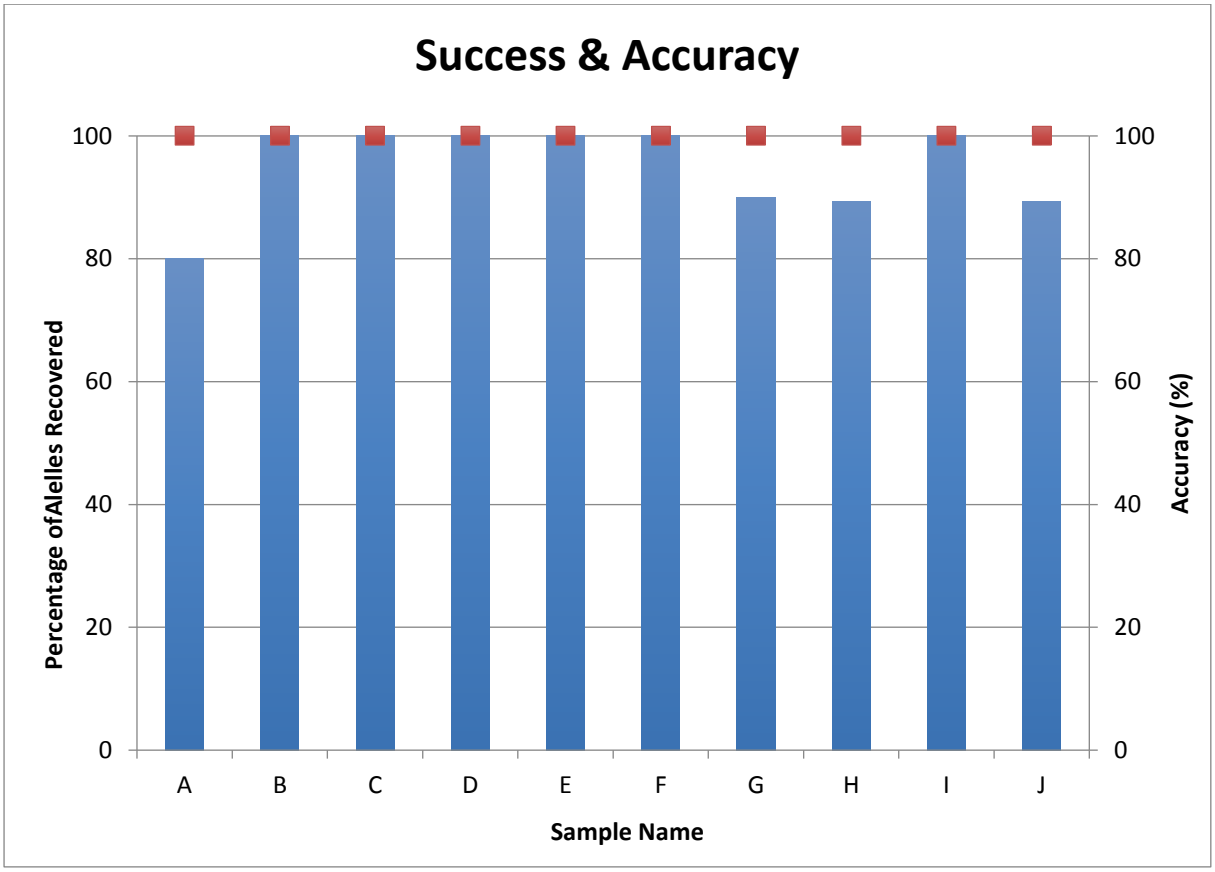


Figure. 1 Percentage of alleles recovered and accuracy for the 100 buccal swabs tested (i.e. 10 swabs from 10 individuals).

The percentage of alleles is based on all successful profiles (including full and partial profiles obtained) out of the 10 samples from each donor processed. A full profile contains 32 alleles. Figure 1 illustrates that for six of the donors all 10 samples processed on the DNAscan instrument were full profiles. Donor H had a partial profile for one sample with unlabelled alleles (flagged red) at one locus and this was due to a large running artefact at that locus. Similarly, Donor J also had one partial profile with one locus in which two alleles were unlabelled due to peak height imbalance. These unreported alleles, that were flagged red, were typed correctly.

Five samples, two for donor A and one for donors G, H & J failed due to poor running of the internal lane standard in the BioChipSet Cassette. In total, four BioChipSet cassettes were associated with these lane failures. Lane failures are thought to be the result of blockages in channels preventing amplification or electrophoresis and the subsequent generation of an STR profile (Tan et al, 2013). GE is continuing to work on improvements to the BioChipSet cassettes to reduce the number of lane failures that occur (J. French 2016, personal communication).

Table 1: Success rates for the buccal samples processed with the HDC BioChipSet Cassette.

Interpretation	Percentage
Full profile	93
Partial profile >70%	2
Failed sample	5*
Negative	0
Mixture	0
Samples with mistyping	0
Total	100

*Five samples failed due to internal lane standard failure.

Assessment of Results

The DNAscan instrument results for success and accuracy showed that full or uploadable profiles were obtained for 95% of the samples tested. Of this figure, 93% were full profiles and two samples were partial profiles with 30/32 alleles reported. The two partial profile samples had only one locus with alleles flagged red. In one case, this was due to a large artefact present at that locus (clearly not a true peak) and for the second instance there was peak height imbalance at that locus. In both cases, it is likely a DNA analyst could have uploaded the samples as full profiles upon review of the profiles.

The observed success rate of 95% is consistent with what is currently reported as the first pass rate using standard DNA profiling technology at the NSW Forensic & Analytical Science Service using the PowerPlex 21 (PP21) system of 95% (S Neville 2016, personal communication). These results are also consistent with the 88-92% lane success rate reported by GE (J. French 2016, personal communication).

In general, the buccal profiles recovered using the DNAscan instrument were of good quality with reasonable peak heights and good peak height balance. This is not unexpected given the relatively high amount of input DNA present on buccal swabs.

Significantly, all buccal samples processed using the DNAscan instrument were concordant with profiles generated from the same donor using the conventional STR typing methods and the PP21 amplification system. In the present study, there were no samples with mistypings for any of the success and accuracy samples, or any of the other samples processed using the HDC BioChipSet Cassette.

Assessment of auto-uploading of profiles without laboratory intervention

One of the potential uses of a Rapid DNA instrument, such as the DNAscan, is the possibility of non-laboratory staff uploading profiles to the DNA database from charge stations or other Police manned areas. The buccal profiles from the DNAscan instrument were assessed to determine how many profiles could have theoretically been automatically uploaded without being reviewed by a trained analyst. Samples that contained red flagged alleles requiring review by a trained scientist were expected to be flagged by the expert system and were considered to be non-uploadable.

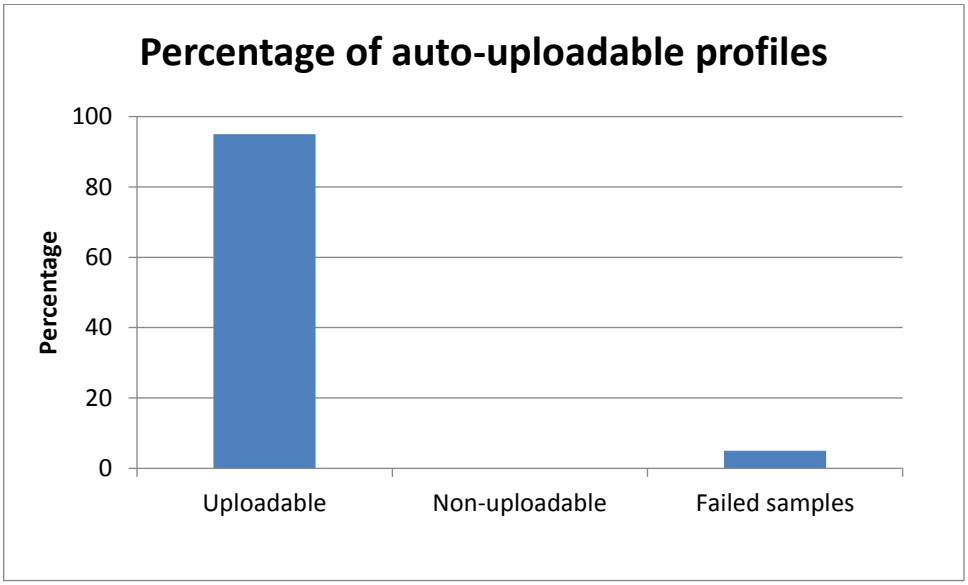


Figure 2. Percentage of the reference samples appropriate for auto-upload.

The results from this study demonstrated that 95% of the profiles from the reference samples were appropriate for automatic upload. Five samples failed due to internal lane standard running issues. This is a significant improvement on the Phase 1 DNAscan testing in which 79% of samples could have been auto-uploaded and demonstrates the potential for DNAscan to be utilised for reference samples in a non-laboratory environment.

Reproducibility

Results

Liquid saliva samples in the following volumes were pipetted onto swabs in triplicate; 5, 25, 100 and 200 µl volumes. Two sets of saliva samples were processed for the reproducibility testing using Low DNA Content BioChipSet Cassettes. The results for reproducibility are presented in Figure 3 and Table 2 below.

The LDC BioChipSet Cassette has been specifically designed for use with samples that may contain lower quantities of DNA such as casework type samples. Since the samples for reproducibility were processed using this LDC BioChipSet Cassette, the success for this testing was measured based on the alleles that passed the expert system (i.e. the allele calls reported by the system in the .xml file).

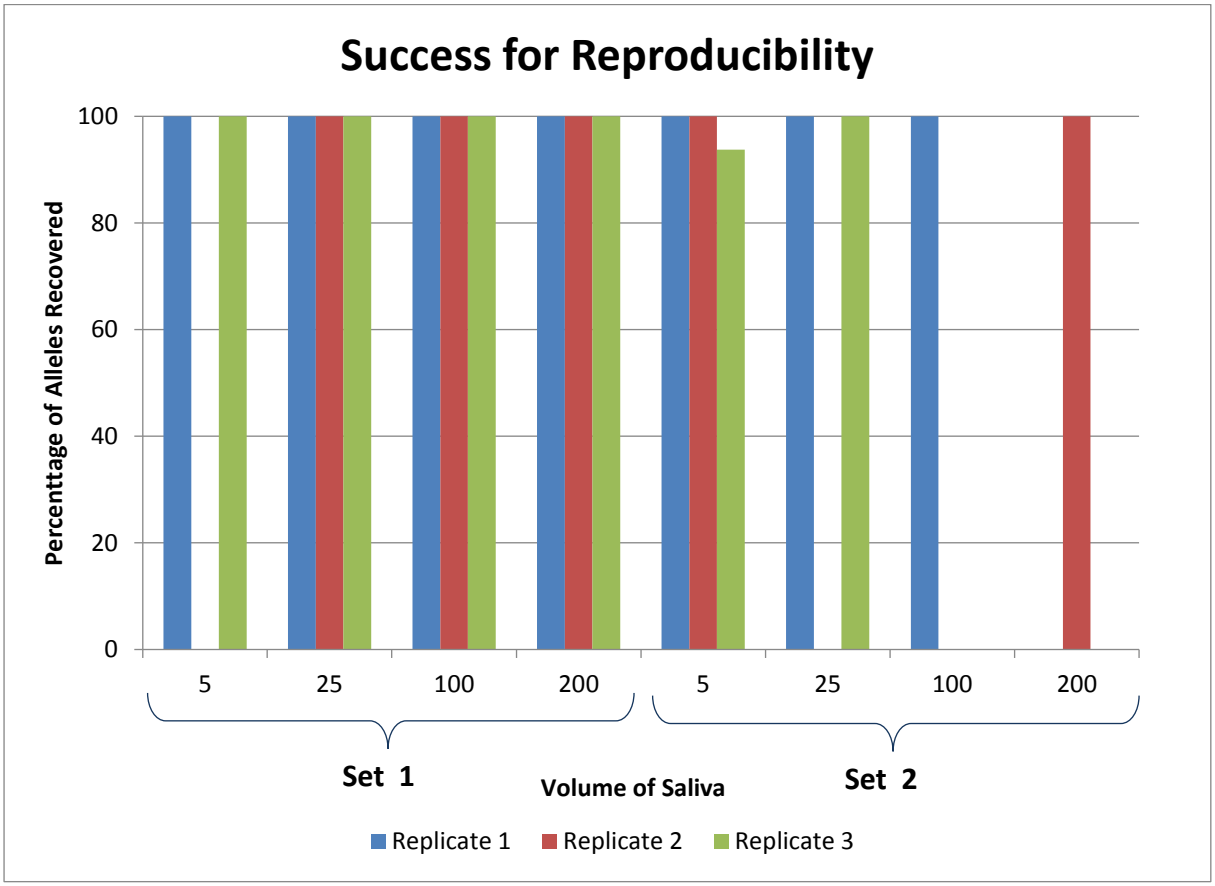


Figure 3. Success rate for reproducibility samples displayed as percentage of alleles recovered out of the total number of alleles possible. (Note: Only alleles that passed the expert system and were reported in the .xml output file were included in the graph.)

Table 2: Summary of results for the reproducibility test.

Sample Type	Set No. and Volume	Comments
Saliva	Set 1, 5 µl	• Two out of the three replicates resulted in full profiles. All alleles above ~1,000 RFU. • One replicate flagged red with 13 red flagged alleles. All peaks in this profile were < ~4,000 RFU.
	Set 1, 25 µl	• All replicates returned full profiles. • All profiles reasonably strong with all alleles >~2,000 RFU and the highest alleles above ~45,000 RFU.
	Set 1, 100 µl	• All replicates yielded full profiles. • All profiles were strong with all alleles >~6,000 RFU and the highest allele above ~130,000 RFU.
	Set 1, 200 µl	• All replicates resulted in full profiles. • All profiles were strong with all alleles >~6,000 RFU and the highest allele above ~145,000 RFU.
	Set 2, 5 µl	• Two replicates yielded full profiles. All alleles above ~800 RFU. • One replicate was flagged yellow due to one locus displaying heterozygote imbalance. The profile could have been considered full upon review.
	Set 2, 25 µl	• Two replicates resulted in full profiles. All alleles above ~4,000 RFU. • One replicate was flagged red with no profile. This was due to a lane failure caused by a poorly run ILS (peak 475 bp was missing from the ILS).
	Set 2, 100 µl	• One replicate returned a full profile. Strong profile with all alleles above ~14,000 RFU. • The other two replicates were flagged red. One of these had no alleles in the profile due to a failed ILS as reported by the system. The other had a nearly full profile but was flagged red due to two loci possessing >2 alleles. These extra alleles were actually quenched peaks being labelled as true peaks.
	Set 2, 200 µl	• One replicate resulted in a full profile. Alleles above ~3,000 RFU. • The two other replicates were flagged red and both appeared to have insufficient DNA to detect peaks (the ILS appeared normal). Profiles were expected from these samples.

Assessment of Results

As expected, the peak heights increased with the increased DNA input volume. However, the 100 and 200 µl samples in Set 2 were reasonably expected to provide a full profile but resulted in no profile. Given that these are the strongest concentrations processed, it may indicate that the samples were overloaded resulting in no profile generation. However, this seems unlikely given that the same volume of saliva from the same stock was successfully profiled in the first set. The saliva

was pipetted directly onto the swab rather than being pipetted onto a surface and swabbed off the surface. The saliva may have been absorbed into the matrix of the swab and not able to be released during the lysis/extraction process. This was the case for blood pipetted directly onto a swabs in this study (see Sensitivity and Accuracy Section of this document).

The number of replicates processed in this study meant that some samples for some of the volumes were run on different BioChipSets. There was, however, no correlation between the BioChipSet used and the samples that did not produce a profile.

The reproducibility study results indicate that allele sizing was reproducible from run-to-run as there was no evidence of mistyping of alleles for any of the reproducibility samples. However, at this stage, the system is not providing consistent results for the same sample input from run-to-run. In this study, the inconsistencies appeared to be due to instrument dependent issues such as lane failures and the expert system mistyping quenched peaks as true peaks, but possibly a sample dependent problem for the 200 µl samples failing to return a profile.

Substrate testing

Results

The substrate study involved testing both blood and saliva on various substrates. 50 µl of either saliva or blood was placed onto the following substrates; denim, leather, wood, paper, acrylic painted surface, carpet (wool) and tile. The liquid was allowed to dry overnight and indirectly sampled by swabbing the surfaces. These swabs were then processed on the DNAscan instrument using Low DNA Content BioChipSet Cassettes. The results are shown in Figure 4.

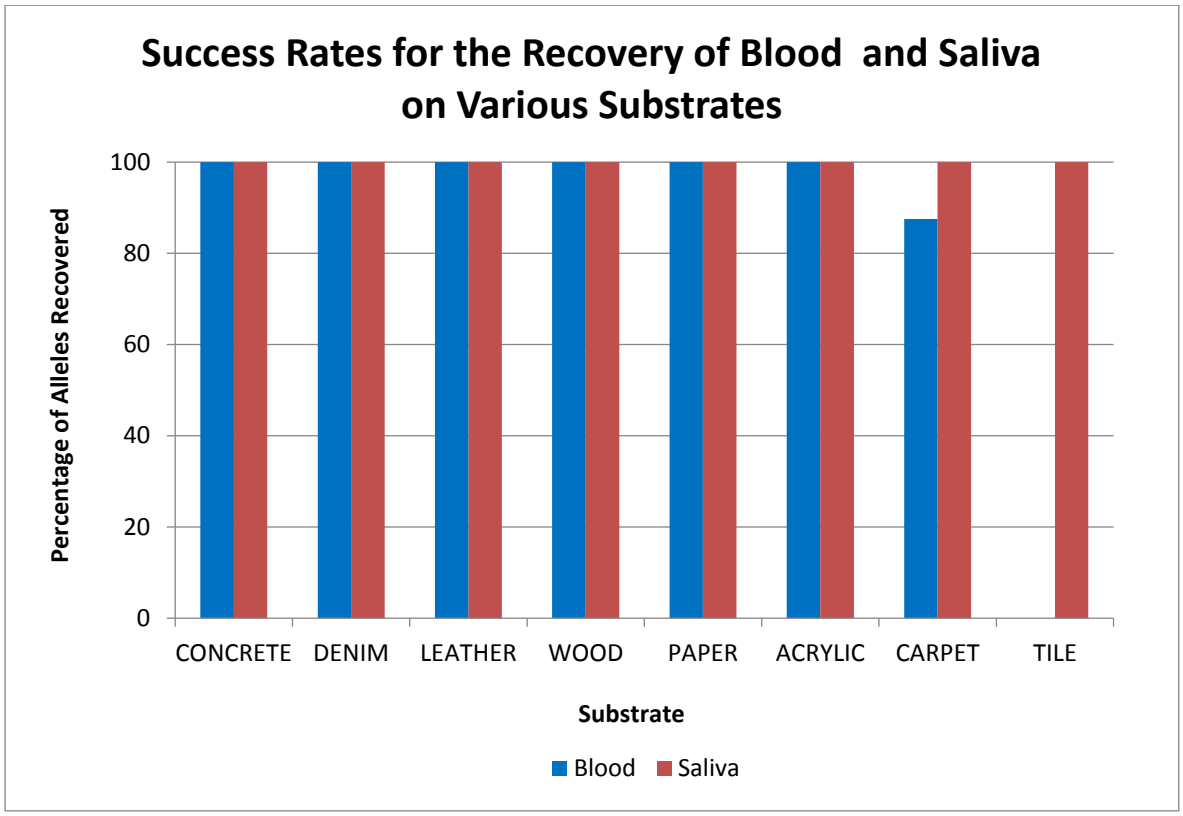


Figure 4. Percentage of alleles recovered for blood and saliva on different substrates.

Assessment of Results

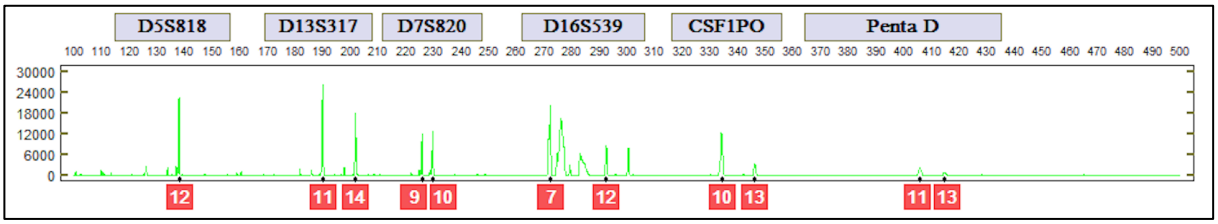
Full profiles were generated for all the saliva samples deposited on the various substrates, and six of the eight blood samples resulted in full profiles. All samples, with the exception of one, were considered uploadable profiles. Full profile details for each sample can be found in Appendix I.

For the blood on carpet sample, 28/32 alleles were called but two of the loci were flagged red. One of these loci had three alleles called at one locus and the other locus had one heterozygote peak below the calling threshold. The locus with three alleles appeared to have a running artefact in the DNAscan electropherogram and this was confirmed when the sample was analysed using the GeneMapper ID-X (Version 1.4) software. Upon review, the running artefact could have been identified and the alleles reported for this sample.

The blood on tile sample was flagged red by the system due to running artefacts present between the 270-285 bp size range in all dyes. The running artefacts were mistyped as true alleles for two loci. For one locus one of the artefacts was typed rather than the true allele (see D16 in Figure 6, A). Upon analysis by a trained scientist, most of the red flagged loci, excluding the two affected by the artefacts, would have been uploadable. It is not clear whether these artefacts are chip related or something introduced along with the sample.

When there were multiple alleles at a locus, it was observed in a few profiles that one of the peaks would not be labelled by the expert system. In some cases the peak not labelled was the true heterozygote partner (see Figure 5, A & B). This indicates that the system has trouble interpreting mixture samples. This is probably not surprising given that the system has not been designed for the analysis of mixtures. Prepared mixture samples were not tested in this study.

A



B

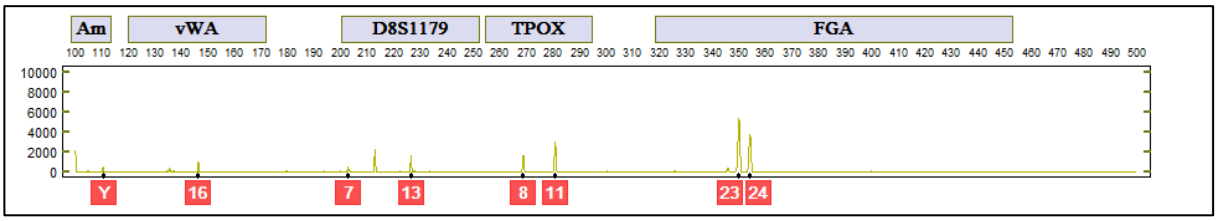


Figure 5. (A) The JOE dye channel for the blood on tile sample (VD2061). Locus D16 shows two alleles being typed, allele 7 is a running artefact and allele 14 has not been typed. (B) TMR dye channel for the 5 µl blood sample (VD2001). Example of three alleles present at D8 but only two alleles were called by the DNAscan expert system.

It should be noted that only one sample for each substrate type was tested here and therefore further testing with more replicates would be required to draw any conclusions regarding the consistency of profiling from particular substrate types.

Accuracy and Sensitivity

Various casework type samples were tested for this section of the evaluation. The LDC BioChipSet Cassette was used to process the casework type samples unless stated otherwise.

Liquid Blood

The following volumes of liquid blood were pipetted directly onto individual swabs, 5, 25, 100 and 200 µl. The results from this experiment were poor. GE representatives were consulted about the most effective approach for the presentation of blood samples for processing in the system. GE recommended that the liquid blood be applied onto a clean glass slide, allowed to dry, before being indirectly sampled by swabbing the dried blood off the slide. Consequently, the blood samples for the second blood experiment were prepared in this way using the same volumes as stated above.

Seminal Liquid

The following volumes of liquid semen sample were pipetted onto individual swabs; 0.5, 10, 25 and 100 µl.

Cigarette Butt

Three cigarette butts were collected from one individual and processed using the LDC BioChipSet Cassette. Another individual provided four cigarette butts which were processed using the HDC BioChipSet Cassette.

Touched Item

One individual's phone was swabbed at the end of the day on three different days.

Drink container

One individual donated three cans from which they had consumed the entire contents.

DVI Samples – Toe Nails

Four toe nails were collected from one individual and placed directly into the sample chamber with a BioChipSet swab inserted to hold them in place.

FTA card

For one sample, one piece of 3 x 3 mm FTA card was excised and placed directly into the sample chamber. This represented the typical amount of FTA card processed through the conventional STR typing system at VPFSC. The second FTA sample contained four pieces of 3 x 3 mm FTA card to investigate processing an excess amount of card.

Hair

Two hair samples were plucked from a single donor. Both hair samples had a root. The hair samples were placed directly into the sample chamber and secured with a BioChipSet Swab.

Soiled Buccal Swabs

Buccal swabs were collected from donors just after having consumed food to investigate whether this would have any impact on obtaining a full profile. These samples were processed shortly after collection (within 30-60 min).

Samples considered to have mistypings were those that would have been uploaded without prior review. Alleles that were mistyped but flagged red and therefore flagged for review were not considered true mistypings but were instead considered non-uploadable loci within the profile.

Results

The results for accuracy and sensitivity are presented in Figure 6 and Table 3.

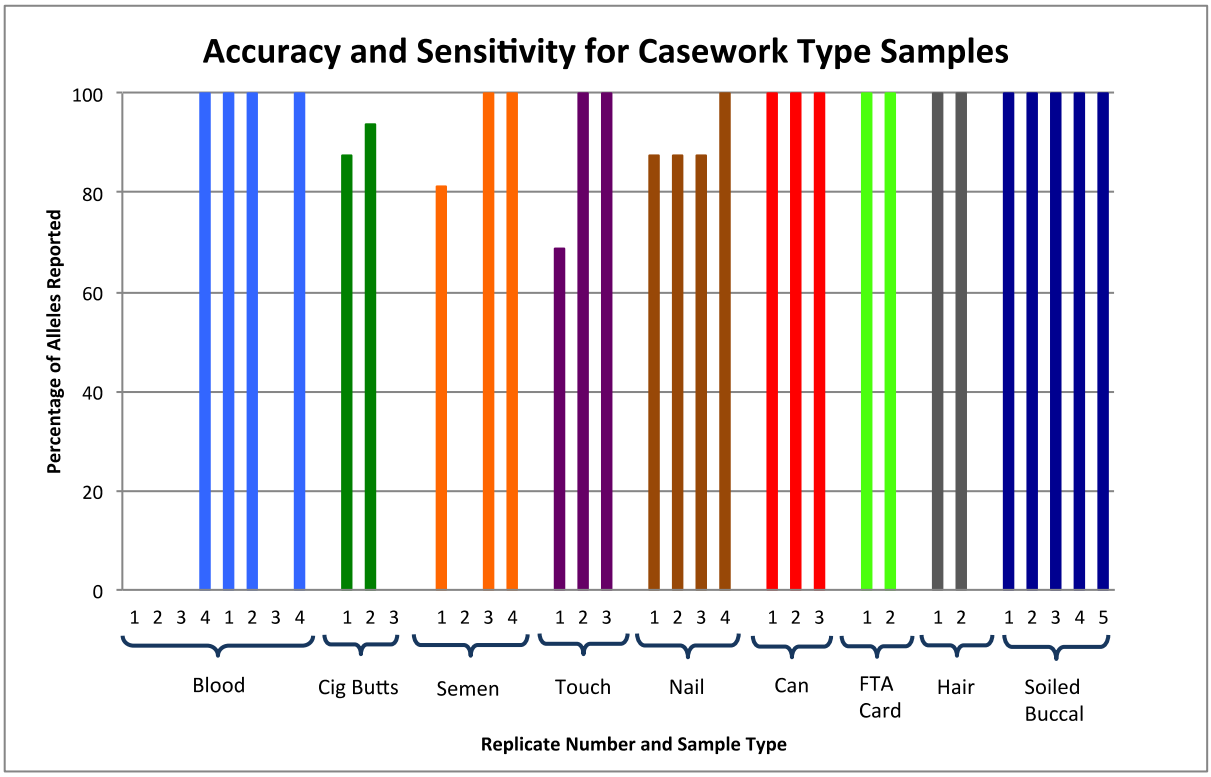


Figure 6. Number of alleles/total number of alleles (as a percentage) reported by the DNAscan system for each casework sample type. Note: For the blood samples, 1 = 5 µl, 2 = 25 µl, 3 = 100 µl, 4 = 200 µl. For the semen samples 1 = 0.5 µl, 2 = 10 µl, 3 = 25 µl, 4 = 100 µl. (Note: Soiled Buccal samples were processed with the HDC BioChipSet Cassette.)

Table 3: Summary of Accuracy and Sensitivity Results for casework type samples.

Sample Type	Number of samples tested	Comments
Blood	8	The first set had the blood pipetted directly onto the swab. This set of blood samples resulted in a full profile for the 200 µl volume and no profile for the 5, 25 and 100 µl volumes. The second set had the blood applied to a slide and swabbed after drying. This set of blood samples returned full profiles for 5, 25 and 200 µl volumes but no profile for the 100 µl.
Semen	4	The 25 and 100 µl volumes yielded full profiles. The 0.5 µl volume of semen resulted in a yellow flagged partial profile with 2 loci (D16 & TPOX) with red flagged alleles and no alleles at D18. A running artefact affected all 3 loci. The 10 µl was a failed sample due to ILS failure.
Cig Butt	3	Two of the samples were flagged yellow with partial profiles. 1 had two loci flagged red due to below threshold alleles and the other sample had 1 red locus due to the presence of 3 alleles – peak looked real, possible second contributor. The third sample was flagged red with no alleles reported. Out of interest, 4 more samples were processed using the HDC BioChipSet Cassette all of which resulted in no profile. This was likely due to not enough DNA being extracted using this BioChipSet Cassette type.
Touched phone	3	Two samples were passed by the system and reported as full profiles and 1 sample was a partial profile flagged yellow. The alleles of one full profile matched the reference sample, however, the other full profile had one locus mistyped as a homozygote (no evidence of a second peak in the DNAscan profile). The sample that was flagged yellow had 3 mistypings. At 2 of these loci one allele was incorrect (possible minor contributor) and a third locus was incorrectly typed as a homozygote. The incorrect loci were all equal to or below 1,000 RFU.
Drink can	3	Full profiles obtained.
Toe nails	4	One full profile and 3 partial profiles were generated. All single source profiles. All alleles flagged red matched the reference sample.
Hair	2	Full profiles obtained.
FTA card	2	Full profiles obtained.
Soiled Buccal*	5	Full profiles obtained.

*Note: The Soiled Buccal samples were processed using a High DNA Content BioChipSet Cassette.

Assessment of Results

The first sensitivity test using blood, in which the various blood volumes were pipetted directly onto the swab, resulted in a full profile being generated for only the 200 µl volume. A partial profile, flagged red by the system, was generated for both the 5 and 25 µl volumes with 16 and 15 loci flagged red, respectively. The partial profiles had peak heights all below 6,000 RFU, with the lowest peaks less than 1,000 RFU, indicating there was a low quantity of DNA extracted from the swabs. This

result was thought to be due to the blood volume being absorbed into the swab and subsequently an insufficient quantity able to be extracted in the BioChipSet Cassette.

GE representatives were consulted at the time of processing and it was recommended to apply the blood volumes to a clean slide, allow the blood to dry, and then swab the slide using a moistened swab. The results of this testing indicated that full and accurate DNA profiles could be generated from as little as 5 µl of blood. All volumes except the 100 µl volume yielded full and accurate profiles. Given that full profiles were obtained for the 5, 25 and 200 µl volumes, it was expected that the 100 µl volume would also result in a full profile, however, the 100 µl had no alleles. There were no anomalies regarding the running of the sample indicating that there was insufficient blood extracted during the process. It is possible that a channel was blocked during the processing interfering with the extraction process thus generating no data.

Two full profiles were obtained from the semen samples with the largest input of DNA (25 and 100 µl). A partial profile was generated for the 5 µl input amount, which was due to obvious running artefacts present in the profile that affected the typing of two loci. Upon review this sample would have been uploadable as a nearly full profile. The 10 µl sample was expected to provide a full profile, given the 5 µl results, however, this sample failed due to an internal lane standard failure.

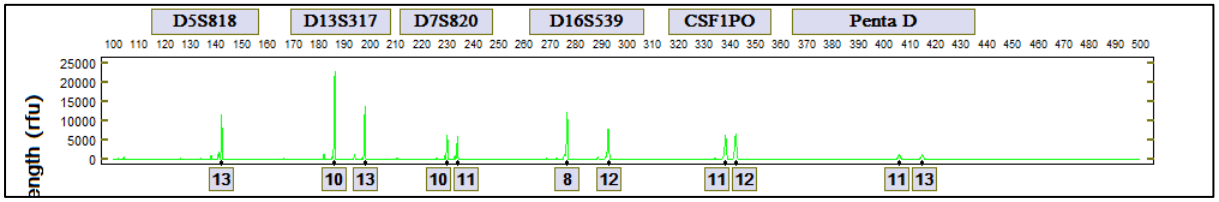
Partial profiles were obtained for two of the cigarette butts analysed. One of these partial profiles was weak with an allele below threshold at two loci. The two loci had peaks below 1,000 RFU. For the other partial profile, three alleles were detected at locus D8. Upon review, using the GeneMapper ID-X software, there was evidence of a second contributor at a second locus (vWA). The third sample was flagged red with no alleles reported, however, the red flagged alleles in the profile were all typed correctly.

Three touch samples were tested in this study. One sample was a full profile and concordant with the reference sample. Another sample was flagged yellow with a couple of loci possessing a below threshold heterozygous peak. Three loci reported for this sample were no concordant with the donor. Two loci had an allele that was incorrect and one locus was mistyped as a homozygote. Given that these samples are touch samples, it is possible that there was a second contributor in the profile and these incorrect alleles belong to that contributor. In fact, upon re-analysis using the GeneMapper software there appeared to be evidence of a second contributor at D5 and THO1. The D16 locus was mistyped as a homozygote when the reference sample was a heterozygote (see Figure 7). Since this sample had been flagged for review, there was a chance that the mistypings could have been identified as there was evidence of the missing allele at D16 in both the DNAscan profile and when the sample was analysed with GeneMapper. The missing allele peak was, however, similar in size to the baseline noise peaks.

The third touch sample was more concerning as one locus (D18) was confidently called and reported by the system to be a homozygote but was in fact a heterozygote with a missing allele (Figure 8). The sample was not flagged for review and potentially could have been uploaded to a database with an incorrect allele. There was no evidence of a second allele in the DNAscan profile and this was confirmed when the sample data was analysed using GeneMapper. This mistyping indicates that the stochastic threshold for this locus should be re-assessed in order to avoid this type of error and provide greater confidence in the results for low level samples. The Company has announced HDC and LDC BioChipSets with 27 loci and have stated that they have worked to optimise stochastic thresholds in these settings. As this assay includes the NCIDD core loci, it is may be an option to assess in future.

Both samples containing mistypings were on the weaker side with the lowest peaks equal to or less than 1,000 RFU.

A



B

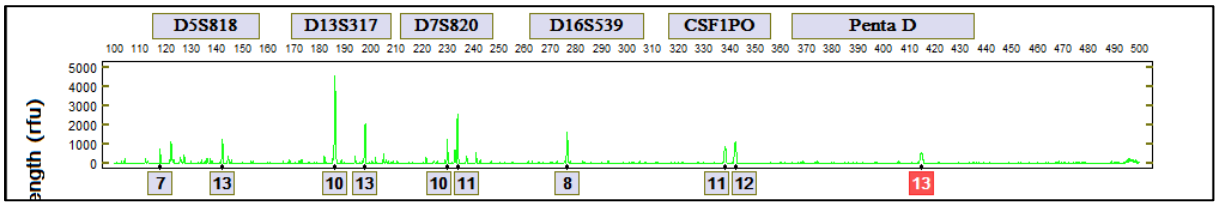
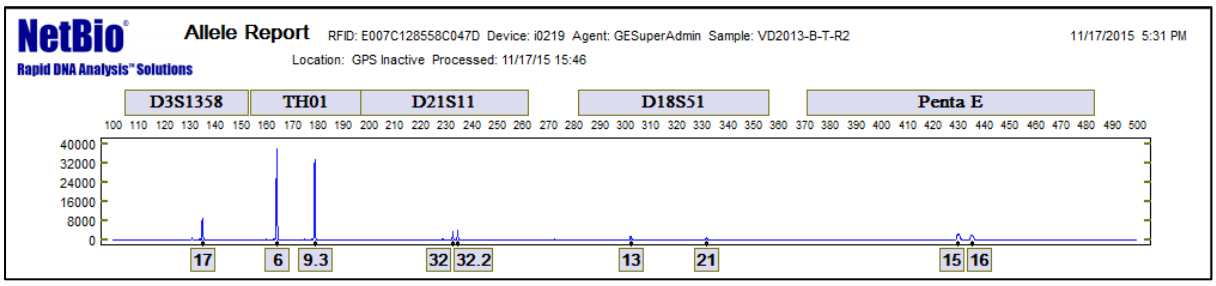


Figure 7. Alleles in the JOE dye channel (A) Correct allele calls at D16 for one of the touched samples (VD2013) and (B) Mistyping at D16 for a touched sample VD2012 for the same donor. Note D5 also has one incorrect allele call (allele 7) and Penta D has one allele flagged red as the system has detected a second allele at the locus which is below threshold.

A



B

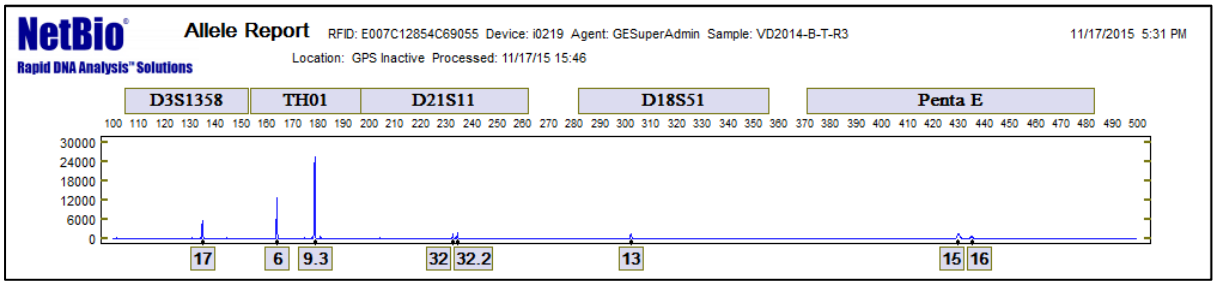


Figure 8. The fluorescein dye channel with (A) Correct allele calls at D18 for one of the touched samples (VD2013) and (B) Mistyping at D18 for the same donor for touched sample, VD2014.

Full profiles matching the reference sample were obtained for the swabbed drink cans. This is not surprising given that saliva was collected from the cans and high quantities of DNA are usually extracted from saliva. Similarly, full profiles were obtained from the FTA card and the soiled buccal swabs which also had saliva as the DNA source.

The hair samples resulted in full profiles and the toe nail samples resulted in one full profile and two uploadable partial profiles. These findings indicate that the LDC BioChipSet has a potential application for the processing of DVI samples. Further testing could investigate the processing of various tissue types using this new LDC BioChipSet but this sample type was not tested in the present study.

Overall, the processing of case type samples using the LDC BioChipSet resulted in a success rate of 81% with accuracy of 96%. Despite these promising results, the mistypings detected indicate that care would be required if low level samples were to be processed on a DNAscan instrument using the LDC BioChipSet, in its current form, in a non-laboratory setting. It should be noted that a limited number of replicates were processed in this study and further testing would be required.

Table 4: Success rates for all samples processed with the LDC BioChipSet Cassette.

Interpretation	Percentage
Full profile	66.7
Partial profile >70%	11.6
Failed sample	4.3*
Negative – alleles present	8.7#
Negative – no alleles present	5.8
Samples with mistyping	2
Total	100

*Samples failed due to internal lane standard failure.

#Alleles were present but flagged red due to various issues and no alleles were reported by the system.

Observations on DNAscan profiles

The DNAscan instrument has been designed to use the ladder of the run unless the ladder fails the expert system rules, in which case a virtual ladder will be applied to the samples in the run. This has been implemented so that profiles will always be generated for any given run, even when the ladder in the BioChipSet fails. If a virtual ladder is applied, this will be noted at the top of the electropherogram. In the present study, all ladders passed for all 41 runs demonstrating the robustness of the ladder processing in the DNAscan system. Since there were no runs in which a virtual ladder was used, the impact of typing under this condition was not assessed.

GE has stated that the balance between the loci has been altered in order to optimise the reagents to be stored in a lyophilised form in the BioChipSet cassette (J. French 2016, personal communication). This appears to be evident in the resulting DNA profiles generated as some loci consistently displayed greater peak heights compared to neighbouring smaller molecular weight loci, which would typically be higher using conventional laboratory typing methods. For example, THO1 had consistently higher peaks than D3 (see Figure 8) and D13 is often greater than D5. This is something that GE has noted they are continuing to work on improving.

The inter locus imbalance was more pronounced for the case type samples run using the LDC BioChipSet cassette compared to the buccal samples processed using the HDC BioChipSet. This may indicate differences between the processing of the two chip types or caused by the samples themselves. It may be an indication that inhibitors are present in the LDC samples causing the imbalance between loci. The classic ski-slope effect seen in traditional typing was not evident in the DNAscan profiles which made it difficult to assess whether a profile was inhibited. When the profiles were re-analysed using GeneMapper the same inter locus imbalance was observed suggesting that the imbalance is due to preferential amplification of some loci. It is possible that the observed imbalance is what inhibited DNAscan profiles look like. Degraded and inhibited samples were not

tested in this study and consequently it is not clear what profile characteristics would be present for degraded or inhibited samples processed using the DNAscan system.

The allele typing for a number of samples was affected by the presence of running artefacts. It is not clear from this study what caused these artefacts. It is possible bubbles were introduced into the channels which can be the result of tilting the BioChipSet Cassette with the preloaded reagents or potentially dirt in the system, possibly introduced with a sample. The Company reports they have since been nearly eliminated in the PP16 cassettes as well as their new 27 locus assay cassettes.

CONCLUSION

The DNAscan instrument did not have any technical issues during the processing of the samples for this study and there was no evidence of cross contamination between samples. Overall, the DNAscan instrument delivered accurate results with a high success rate. The success rate was higher with the buccal swabs using the HDC BioChipSet compared to the case type samples using the LDC BioChipSet. Perhaps this is to be expected given that case type samples often possess contaminants and inhibitors than cause greater variability in DNA profile results.

The success and accuracy results indicate that the DNAscan instrument is able to accurately provide DNA profiles for buccal swabs using the HDC BioChipSet. A high success rate was achieved and all successful samples would have been uploadable to a database. The expert system rules appear to be sufficiently conservative in order to report only the loci that have been accurately typed. Samples with loci in question were flagged by the system and the alleles were not automatically reported. These results indicate that the HDC BioChipSet protocol on the DNAscan system may be suitable for operation by non-scientific staff in a non-laboratory environment.

The results from the present study demonstrate the utility of the newer LDC BioChipSet which has been optimised for the profiling of low level DNA samples such as crime scene samples. Generally this protocol performed well on most samples types. Mistypings only occurred for the weaker samples and when the loci were at or below 1,000 RFU. Further evaluation of the LDC BioChipSet protocol would be required for use with very low level DNA samples i.e. touched samples. Some further optimisation of the homozygote analytical thresholds for each locus could be undertaken. The Company has announced HDC and LDC BioChipSets with 27 loci and have stated that they have worked to optimise stochastic thresholds in these settings. As this assay includes the NCIDD core loci, it is may be an option to assess in future.

For the processing of casework type samples, however, it would be advisable for the profiles generated on the DNAscan system and the LDC BioChipSet to be reviewed by a trained DNA analyst. Case type samples are highly variable in DNA content and quality and some very weak samples are likely to be affected by stochastic effects such as allele drop out and or severe heterozygosity resulting in an incorrect determination of homozygosity such as that observed in this study.

A further reduction in the number of failed samples would be beneficial for processing reference samples as failed runs would require repeat processing. This is particularly important for processing crime scene samples where there is often just one sample and therefore one chance at obtaining a DNA profile. Further work could include an assessment of re-extracting substrates using conventional methods if a profile is not obtained via the DNAscan instrument. Re-extracting of the substrate using conventional typing methods was not assessed in this study.

REFERENCES

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2. Turingan, R.S., Vasantgadkar S., Palombo, L., Hogan, C., Jiang, H., Tan, E., Selden, R.F., Rapid DNA analysis for automated processing and interpretation of low DNA content samples, *Investigative Genetics* (2016) 7:2.

APPENDIX I: DETAILED PROFILE RESULTS

Table 5. Description of failed samples and partial profiles for the success and accuracy samples.

Sample ID	Number of alleles/comments
VD2101-A-5	Sample flagged red. No alleles called but peaks are visible in the profile. ILS failure - 350 bp ILS peak very low (similar to size of baseline noise peaks).
VD2112-A-6	Sample flagged red. No alleles called but peaks are visible in the profile. ILS failure - 350 bp ILS peak very low (similar to size of baseline noise peaks).
VD2108-G-5	Sample flagged red. No alleles called. ILS failure - very abnormal run.
VD2099-H-4	Sample flagged red. No alleles called. ILS failure – no ILS peaks after 240 bp peak.
VD2121-J-6	Sample flagged red. No alleles called. ILS failure – no ILS peaks after 360 bp peak.
VD2119-H-6	30/32 Alleles – Sample flagged yellow. Alleles at D18 flagged red due to large running artefact present at that locus (artefact present only in the fluorescein dye channel). All alleles match reference sample.
VD2101-J-4	30/32 Alleles – Sample flagged green (G-13) meaning that the 13 CODIS loci were reported. Alleles at Penta E flagged red due to heterozygote peak height imbalance. All alleles match reference sample.

Table 6. Detailed profile results for the reproducibility study samples.

Set Number	Saliva Volume Deposited (ul)	Replicate Number	Number of alleles/comments
Set 1	5	1	32/32 alleles. Full profile. All alleles >~1,000 RFU, highest peak ~14,000 RFU.
	5	2	0/32 alleles reported. Sample flagged red. 10 loci with red flagged alleles, 3 loci with no alleles. Red flagged alleles match reference. Weak sample, all alleles <~4,000 RFU, lowest peak <~1,000 RFU.
	5	3	32/32 alleles. Full profile. All alleles >~1,000 RFU, highest peak ~22,000 RFU.
	25	1	32/32 alleles. Full profile. All alleles >~4,000 RFU, highest peak ~82,000 RFU.
	25	2	32/32 alleles. Full profile. All alleles >~2,000 RFU, highest peak ~52,000 RFU.
	25	3	32/32 alleles. Full profile. All alleles >~2,000 RFU, highest peak ~45,000 RFU.
	100	1	32/32 alleles. Full profile. All alleles >~7,000 RFU, highest peak ~150,000 RFU.

	100	2	32/32 alleles. Full profile. All alleles >~10,000 RFU, highest peak ~165,000 RFU.
	100	3	32/32 alleles. Full profile. All alleles >~6,000 RFU, highest peak ~130,000 RFU.
	200	1	32/32 alleles. Full profile. All alleles >~7,000 RFU, highest peak ~145,000 RFU.
	200	2	32/32 alleles. Full profile. All alleles >~6,000 RFU, highest peak ~165,000 RFU.
	200	2	32/32 alleles. Full profile. All alleles >~12,000 RFU, highest peak ~165,000 RFU.
Set 2	5	1	32/32 alleles. Full profile. All alleles >~800 RFU, highest peak ~20,000 RFU.
	5	2	32/32 alleles. Full profile. All alleles >~2,000 RFU, highest peak ~63,000 RFU.
	5	3	30/32 alleles. Sample flagged yellow, D2 alleles flagged red (peak height imbalance). All alleles >~2,000 RFU, highest peak ~40,000 RFU.
	25	1	32/32 alleles. Full profile. All alleles >~4,000 RFU, highest peak ~60,000 RFU.
	25	2	0/32 alleles. Sample flagged red. No alleles reported. ILS failure – 470 bp ILS peak missing.
	25	3	32/32 alleles. Full profile. All alleles >~4,000 RFU, highest peak ~62,000 RFU.
	100	1	32/32 alleles. Full profile. All alleles >~14,000 RFU, highest peak ~165,000 RFU.
	100	2	0/32 alleles. Sample flagged red. No alleles reported. ILS failure – no ILS peaks after 190 bp size.
	100	3	0/32 alleles. Sample flagged red. No alleles reported. All alleles in profile flagged red because D7 has 4 alleles labelled and D8 has 3 alleles labeled – running issue causing split peaks 1 bp apart at these 2 loci. All alleles >~4,000 RFU, highest peak ~165,000 RFU. Alleles match reference, except those 3 labelled split peaks.
	200	1	0/32 alleles. Sample flagged red. No allele peaks but ILS looks normal. Looks like not enough DNA is present.
	200	2	32/32 alleles. Full profile. All alleles >~3,000 RFU, highest peak ~140,000 RFU.
	200	3	0/32 alleles. Sample flagged red. No allele peaks but ILS looks normal. Looks like not enough DNA is present.

Table 7. Detailed profile information for the substrate testing samples.

Sample Type	Substrate	Number of Alleles	Comments
Blood	Concrete	32/32	Full profile. All alleles > ~2,000 RFU.
	Denim	32/32	Full profile. All alleles > ~4,000 RFU except Penta D ~2,000 RFU.
	Leather	32/32	Full profile. All alleles > ~3,000 RFU.
	Wood	32/32	Full profile. All alleles > ~6,000 RFU, except Penta D ~4,000 RFU.
	Paper	32/32	Full profile. All alleles > ~2,500 RFU, except Penta D ~1,500 RFU.
	Acrylic	32/32	Full profile. All alleles ~3,000 RFU, except Penta E and Penta D ~1,500 RFU.
	Carpet	28/32	Sample flagged yellow - 2 loci flagged red. 3 labelled alleles at D3 appear to be a running artifact, and drop out of 1 allele at Penta D. All alleles >~2000 RFU except Penta D and Penta E ~1,000 RFU.
	Tile	0/32	No alleles were called for this profile due to large running artifacts present in all three dyes. All alleles match reference except 2 alleles at D16 and TPOX which were mistyped (artifacts labelled as a true peaks). All alleles > ~6,000 RFU except CSF and Penta D ~2,500 RFU.
Saliva	Concrete	32/32	Full profile. All alleles > ~2,000 RFU.
	Denim	32/32	Full profile. All alleles > ~2,000 RFU.
	Leather	32/32	Full profile. All alleles > ~4,000 RFU.
	Wood	32/32	Full profile. All alleles > ~4,000 RFU.
	Paper	32/32	Full profile. All alleles >~2,000 RFU. Some running artefacts present between 100 -130 bp size but did not interfere with profile interpretation by the expert system.
	Acrylic	32/32	Full profile. All alleles > ~4,000 RFU, except Penta D ~2,000 RFU.
	Carpet	32/32	Full profile. All alleles > ~7,000 RFU.
	Tile	32/32	Full profile. All alleles > ~7,000 RFU.

Table 8. Detailed profile information for the sensitivity and accuracy samples.

Set Number	Volume Deposited (ul) or Replicate Number	Number of Alleles	Profile Comments
Blood (Set 1)	5	0/32	No alleles reported. Sample flagged red. 16 loci with red flagged alleles, D7 had 4 alleles, D8 had three alleles but 1 was not labelled. Possible mixture. 2 alleles at D7 and 1 allele at D8 do not match reference. Weak sample, all alleles <~6,000 RFU, lowest peak <~1,000 RFU.
	25	0/32	No alleles reported. Sample flagged red. 15 loci with red flagged alleles, THO had 3 alleles, appears to be a mixture. All alleles except one at THO match reference. Weak sample, all alleles <~2,000 RFU, lowest peak <~1,000 RFU.
	100	0/32	No alleles reported. Sample flagged red. Peaks present in profile but none labelled. All peaks <~6,000 RFU. Probable running issue.
	200	32/32	Full profile. All alleles >~3,000 RFU, highest peak ~20,000 RFU.
Blood (Set 2)	5	32/32	Full profile. All alleles >~3,000 RFU, highest peak ~24,000 RFU.
	25	32/32	Full profile. All alleles >~2,000 RFU, highest peak ~25,000 RFU.
	100	0/32	No alleles reported. Sample flagged red. No peaks present in profile, no DNA detected.
	200	32/32	Full profile. All alleles >~8,000 RFU, highest peak ~80,000 RFU.
Semen	1	26/32	Partial profile. Sample flagged yellow. D16 and TPOX flagged red, no alleles at D18, all 3 loci affected by running artefacts. All alleles >~2,000 RFU, highest allele ~35,000 RFU.
	2	0/32	No profile. Sample flagged red. Failed ILS. Allele peaks present but in incorrect positions.
	3	32/32	Full profile. All alleles match reference. All alleles >~4,000 RFU, highest allele ~135,000 RFU.
	4	32/32	Full profile. All alleles match reference. All alleles >~10,000 RFU, highest allele ~165,000 RFU.
Cigarette Butts (Set 1)	1	28/32	Partial profile. Sample flagged yellow. Loci D18 & Penta E flagged red, both have 1 allele drop out, both loci <~1,000 RFU. All labelled alleles match reference. Highest allele ~15,000 RFU.
	2	30/32	Partial profile. Sample flagged yellow. D8 flagged red due to 3 alleles labelled, possible mixture. All alleles, except 1 at D8, match reference. All alleles >~1,000 RFU, highest peak ~20,000 RFU.

	3	0/32	No alleles reported. Sample flagged red. 13 loci flagged red. No alleles at D3, vWA and D8. All labelled alleles match reference. Weak sample, all alleles <~9,000 RFU, lowest peak <~1,000 RFU.
Cigarette Butts (Set 2)	1	0/32*	No profile. Sample flagged red. No DNA detected.
	2	0/32*	No profile. Sample flagged red. No DNA detected.
	3	0/32*	No profile. Sample flagged red. No DNA detected.
	4	0/32*	No profile. Sample flagged red. No DNA detected.
Touch (Mobile phone)	1	22/32#	Partial profile. Sample flagged yellow. **Incorrect alleles at D21, D5 and D16.** One incorrect allele at D21 and D5 may be alleles from a second contributor. Allele drop out at D16 such that appears to be homozygote. All other alleles match reference. Weak sample, all alleles <~6,000 RFU, lowest alleles <~1,000 RFU.
	2	32/32	Full profile. All alleles match reference. All alleles >~2,000 RFU, highest allele ~38,000 RFU.
	3	32/32#	Full profile. Sample flagged green - passed the expert system. **Incorrect allele call at D18**, allele drop out, appears to be homozygote. All other alleles match reference. Weaker sample. Highest allele ~27,000 RFU but all other alleles below ~12,000 RFU, lowest alleles <~1,000 RFU.
Can	1	32/32	Full profile. All alleles match reference. All alleles >~1,000 RFU, highest allele ~30,000 RFU.
	2	32/32	Full profile. All alleles match reference. All alleles >~2,000 RFU, highest allele ~28,000 RFU.
	3	32/32	Full profile. All alleles match reference. All alleles >~3,000 RFU, highest allele ~45,000 RFU.
Nail	1	28/32	Partial profile. Sample flagged yellow. Penta D and D8 both had red flagged alleles, likely due to peak height imbalance. All alleles match reference. All alleles >~1,000 RFU, highest peak ~20,000 RFU.
	2	28/32	Partial profile. Sample flagged yellow. Red flagged alleles at TPOX (peak height imbalance), alleles match reference. No alleles at Penta D. All alleles >~1,000 RFU, highest peak ~23,000 RFU.
	3	28/32	Partial profile. Sample flagged G – C13 (all CODIS loci passed). Drop out of both alleles at Penta E and Penta D. All called alleles match reference. All alleles >~1,000 RFU, highest allele ~13,000 RFU.
	4	32/32	Full profile. All alleles match reference. All alleles >~4,000 RFU, highest allele ~90,000 RFU.
Hair	1	32/32	Full profile. All alleles match reference. All alleles >~2,000 RFU, highest allele ~35,000 RFU.
	2	32/32	Full profile. All alleles match reference. All alleles >~5,000

			RFU, highest allele ~60,000 RFU.
FTA Card	1	32/32	Full profile. All alleles match reference. All alleles >~4,000 RFU, highest allele ~45,000 RFU.
	2	32/32	Full profile. All alleles match reference. All alleles >~2,000 RFU, highest allele ~22,000 RFU.
Soiled Buccal	1	32/32*	Full profile. All alleles match reference. All alleles >~2,000 RFU, highest allele ~18,000 RFU.
	2	32/32*	Full profile. All alleles match reference. All alleles >~3,000 RFU, highest allele ~12,000 RFU.
	3	32/32*	Full profile. All alleles match reference. All alleles >~1,000 RFU, highest allele ~8,000 RFU.
	4	32/32*	Full profile. All alleles match reference. All alleles >~2,000 RFU, highest allele ~24,000 RFU.
	5	32/32*	Full profile. All alleles match reference. All alleles >~1,000 RFU, highest allele ~5,000 RFU.

Samples with incorrect allele calls.

* Samples processed using the High DNA Content BioChipSet Cassette.