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Rapid DNA

A summary of available Rapid DNA systems

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Summary

This report summarizes information on the current Rapid DNA systems available. The report is based on information collected directly from the suppliers together with information publicly available on the web, scientific publications and information made available on forensic DNA scientific meetings and conferences.

The conventional methods of forensic DNA analysis require trained forensic analysts, advanced instrumentation and data interpretation software as well as facilities with designated pre- and post-PCR laboratory areas. In Rapid DNA analysis all steps of the DNA analysis are performed by a single instrument: cell lysis, extraction, purification, PCR, electrophoretic separation, detection and data analysis. The "sample in – profile out" process is typically performed within two hours.

Rapid DNA systems have the following main application areas; booking stations, military needs, DVI situations and forensic analysis.

For reference buccal samples and DVI samples (which are rich in DNA and where there is enough biological material for reanalysis) Rapid DNA systems can be used to provide DNA profiles. The use of Rapid DNA systems on crime scene samples are recommended only for stains expected to be single sourced and of high amount and quality where there is enough material for a subsequent second analysis using conventional methods. If applied under other conditions there is a non-negligible risk of consuming single traces from a crime scene without results, traces that could have generated DNA profiles if analysed in a forensic DNA laboratory using conventional analysis.

If using Rapid DNA systems of current design on crime scene samples, evaluation of the results and the comparison in the specific case always needs to be performed by trained forensic DNA experts.

In conclusion, Rapid DNA systems entails several advantages: DNA analysis is performed within two hours using a single instrument, there is less need for dedicated laboratory areas and it minimizes the time for transport of the items/samples to be analysed, as the Rapid DNA systems can be installed on several sites or used in a mobile setting. The Rapid DNA concept has in general the following limitations: low throughput, expensive analysis, comparably low success rate and the sample is generally consumed upon a run.

Sammanfattning

Denna rapport sammanfattar information om nu tillgängliga Rapid DNA-system. Rapporten baseras på direktinformation från företagen tillsammans med öppen information på Internet, vetenskapliga publikationer samt information från möten och konferenser inom det forensiska DNA-analysområdet.

För konventionella forensiska DNA-analysmetoder krävs utbildad erfaren personal, avancerad instrumentering och mjukvaror samt särskilda utrymmen för arbete pre- respektive post-PCR. För Rapid DNA-analys sker alla delar i kedjan d.v.s. extraktion, rening, normalisering, PCR, elektrofores och detektion integrerat i ett enda instrument. Processen ”prov in – profil ut” genomförs normalt inom två timmar.

Rapid DNA-system används huvudsakligen inom följande områden; vid gränskontroller eller arrest/häkte, inom det militära, i DVI-situationer och vid forensisk analys.

För personprov (saliv på tops) och prov från DVI-situationer, där det i normalfallet finns mycket biologiskt material att tillgå, kan Rapid DNA-system användas för att ta fram DNA-profiler. Att använda Rapid DNA-system till brottsplatsprov rekommenderas endast för fläckar som bedöms komma från en person, och som förväntas innehålla mycket DNA av god kvalitet och där det finns tillräckligt med material kvar för en efterföljande analys med konventionella metoder. Om tekniken används under andra förutsättningar finns en icke försumbar risk för att analysen förbrukar hela spåret utan att generera resultat, för prov som hade kunnat generera DNA-resultat om de analyserats med konventionell analys vid ett forensiskt DNA-laboratorium.

Om Rapid DNA i dess nuvarande design används på brottsplatsprov måste utvärderingen och värderingen av resultaten i det enskilda ärendet alltid utföras av utbildade, kvalificerade forensiker/DNA-handläggare.

Sammanfattningsvis har Rapid DNA följande fördelar: snabb DNA-analys som utförs i ett instrument, minskar behovet av lokaler, kortar transporttiden för prov/material i de fall systemet installeras lokalt eller används i en mobil enhet. Som nackdel har systemet jämfört med konventionell analys: låg kapacitet, analysen är betydligt dyrare, kräver mer biologiskt material för att generera resultat samt att prov förbrukas vid analys.

Table of contents

1 Introduction

1.1 Aim and methodology

The aim of this project has been to gather and compile information on the Rapid DNA systems available.

In order to collect thorough information regarding the systems a questionnaire was prepared and sent to the two suppliers of Rapid DNA systems; Thermo Fisher Scientific and ANDE Corporation (Appendix 1).

After reviewing the replies (Appendix 2 and 3) separate video meeting were held with each supplier to discuss the information provided and handle further questions.

The information received together with information publicly available on the web, in scientific publications and information made available on forensic DNA scientific meetings and conferences, is summarised in this report.

1.2 A need for fast DNA analysis

Since the introduction of forensic DNA analysis in Sweden in 1991, the number of DNA samples analysed at the Swedish National Forensic Centre (NFC) has steadily increased. 2021 NFC analysed 66,700 crime scene samples and 32,800 reference samples[1]. In parallel to increasing sample numbers the case complexity has increased, including an increased number of complex mixtures and analysis of low levels of DNA as well as an increase of inter-disciplinary cases in which different forensic examinations – often competing ones - are to be performed. At the same time, there is an increasing need and demand for shorter case turnaround times.

At NFC, the introduction of a one-tube DNA extraction method, automated sample transfer and dilution for crime scene samples, together with improved workflow, LIMS development and direct amplification of reference samples have significantly reduced the hands-on-time and the time for performing DNA analysis [1-6]. These improvements have reduced the turnaround time, especially for reference samples. The turnaround time for reference samples is at present 1-2 days and as low as 2.5 hours for highly prioritized samples.

[1] Reference sample: DNA samples taken from individuals such as a suspect or a victim. In general these samples are taken as buccal swab samples using a standardised kit. Depending on the case circumstances blood or tissue samples may be taken.

For cases, in which turnaround times apart from the DNA analysis also include trace search and recovery as well as interpretation and reporting, the case turnaround times are 1-2 months for serious crime/complex cases and 1-2 weeks for high volume crime/less complex cases. In prioritized high profile cases (side stepping the queue) the turnaround time for a limited number of items to be examined and a few samples to be processed and reported can be as low as 7 hours of which the analysis takes about 5-6 hours. Obviously, in the general perspective reduced DNA analysis time has a small impact on cases due to the longer turnaround time as a whole, but for cases with few items to examine or a few swabs to process being the case in many high volume crime cases it could still be of value.

The situation with an increasing workload and a need for shorter turnaround time appears to be similar within the forensic community worldwide. Many laboratories struggle with long turnaround times for both reference and crime scene samples. This, together with immigration, military needs and DVI cases – especially in the US, has pushed the development towards faster DNA analysis both regarding conventional DNA laboratory settings as well as the "sample in – profile out" concept called Rapid DNA.

1.3 The Rapid DNA concept

The conventional methods of forensic DNA analysis require work performed by trained forensic analysts, advanced instrumentation, data interpretation software and facilities with designated pre- and post-PCR laboratory areas. In Rapid DNA analysis all steps of the forensic DNA analysis are performed by a single instrument: cell lysis, extraction, purification, PCR amplification, electrophoretic separation and detection and data analysis. The "sample in – profile out" process is typically performed within two hours [7,8].

Rapid DNA analysis is by the FBI defined as "*a term used to describe the fully automated (hands free) process of developing a DNA profile from a reference sample buccal (cheek) swab without human intervention*". FBI use the term "*Modified Rapid DNA analysis*" to describe when the rapid DNA instrument is used but requires human interpretation and review of the DNA results [7,8]. In this report the term "manual review" is used instead of "*Modified Rapid DNA analysis*".

Rapid DNA systems comprises a set of subsystems: a pneumatic system for driving fluids throughout the cartridges/chip, a thermal cycling system for PCR, a high voltage system for electrophoresis and optical system for exciting and detecting the fluorescently labelled STR fragments separated during electrophoresis. The instrument also has an integrated computer controlling these subsystems, with preinstalled software's to perform data collection and an expert system to handle interpretation of the DNA results [9,10].

The Rapid DNA concept has several advantages:

- DNA analysis is performed within two hours
- No need for dedicated laboratory areas
- No need for a set up with different instrumentation
- Minimizes the time for item/sample transport if used in a mobile setting

The Rapid DNA concept has in general the following limitations:

- Comparably low success rate
- Sample generally consumed upon run
- Low throughput
- Expensive analysis

1.4 Developments in the US

In 2010 FBI established the Rapid DNA Program Office, a development contract for the Department of Defence, the Department of Justice and the Department of Homeland Security, to facilitate the development and integration of Rapid DNA technology for use by law enforcement [7].

The first prototypes of RapidHIT™ 200 System (IntegenX Inc., Pleasanton, CA, US) and DNAscan Rapid DNA Analysis System (GE Healthcare, Waltham, MA, US) were launched in 2012. In 2013, the manufacturers attended a SWGDAM[2] meeting to discuss and receive feedback. Since then, the Rapid DNA systems have continuously been tested and evaluated by NIST[3]. During 2015 to 2017, NIST were also involved in several developmental validations [8].

In August 2017 "*The Rapid DNA Act of 2017*" was signed into US law allowing the FBI director to issue standards and procedures for the use of Rapid DNA systems. SWGDAM has since released guidelines for the Rapid DNA analysis of buccal swab reference samples [11,12]. Based on these guidelines the Forensic Science Regulator in the UK, in 2021 released guidelines for Rapid DNA systems covering the process from sample preparation to profile designation [13].

At present, there are three commercially available Rapid DNA systems:

- ANDE® 6C System[4] (ANDE Corporation, Waltham, MA, US)
- RapidHIT™ ID System (Thermo Fisher Scientific, Waltham, MA, US)
- RapidHIT™ 200 System (Thermo Fisher Scientific)

[2] SWGDAM: Scientific Working Group on DNA Analysis Methods.
[3] NIST: The National Institute of Standards and Technology.
[4] ANDE: Accelerated Nuclear DNA Equipment.

In the US two of the systems, ANDE® 6C System and RapidHIT™ ID System, have received NDIS[5] approval for accredited laboratory use on reference samples. The approval allows accredited NDIS laboratories to process DNA reference samples using the NDIS approved systems and subsequent searches in the FBI's CODIS[6] program, without manual interpretation [7].On February 1st, 2021 a customised version of the ANDE® 6C System (Series G) received NDIS approval for the analysis of reference samples in law enforcement booking stations. A version of RapidHIT™ ID System ("*DNA Booking System v1.0*") received the corresponding approval on July 1st, 2021. These approvals are limited to certain sample cartridges/chips and already approved PCR STR typing kits. Internal validation of all reference sample types (to be analysed) is required before implementation [7].

The FBI clearly states that the system is not authorized for use on crime scene samples for the purposes of uploading or searching in CODIS [7].

1.5 Applications

Rapid DNA systems have the following main application areas:

- **Booking stations** such as custody suites or at a border control where a fast identification of a person is needed [14]. During the last couple of years great efforts have been made in the US as well as in the UK to implement the use of Rapid DNA systems at booking stations[7]. In the US large investments have been made on IT infrastructure to integrate the Rapid DNA systems to enable automatic searches against State and National DNA Databases generating a hit report while the arrestee is still in custody [7].
- **DVI cases** for identification of casualties using various sample/tissue types (e.g. blood, buccal cells, muscle, liver and bone samples), especially when dental records and fingerprints are not available [15,16], or if such information cannot be retrieved from the casualties.
- **Military needs** in field where casualties can be identified similar to DVI situations. For identification purposes a local DNA database including DNA profiles from the personnel is used.
- **Forensic laboratory** for fast analysis of reference samples in prioritized cases.

1.6 Crime scene samples

Rapid DNA systems were initially developed and intended for fast analysis of reference buccal samples e.g. in booking stations by scientists or even non-scientists [17,18]. Due to a growing interest in using Rapid DNA for crime scene samples e.g. in order to generate early investigative leads, the concept has been further developed [16,19].

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[5] NDIS: National DNA Index System.

[6] CODIS: Combined DNA Index System.

[7] ENFSI DNA WG online meeting April 2021. "Real-Time DNA –The Metropolitan Police Journey". Shazia Khan, Head of Secure Operations, Directorate of Forensic Services, Metropolitan Police, London England.

The benefit in time must however be put in relation to the risk of consuming or losing the sample by not receiving useful results. This is because the lower sensitivity of Rapid DNA systems reduces the chance of getting a useful DNA result from for instance compromised samples (due to degradation and/or inhibition) as well as for samples with lower levels of DNA. In addition, a sample processed with a Rapid DNA system is generally considered consumed upon a run. Therefore, the possibility for reanalysis is if not impossible very limited as compared to such possibilities when using conventional DNA analysis techniques. Also, even though these systems could be operated in decentralized environments, they would need to be managed and supervised from an accredited forensic laboratory. In addition, evaluation of the DNA results (both from Rapid DNA and conventional analysis) needs to be performed by trained forensic DNA experts.

The use of Rapid DNA systems for crime scene samples is questioned by experts within the forensic DNA community. In a joint publication from 2020, ENFSI[8], SWGDAM and FBI strongly advice against the use of any Rapid DNA system for the analysis of crime scene samples for automatic submission to CODIS, in their current design. Several areas of concern must be addressed before these instruments are to be considered for forensic crime scene samples [20]. Examples of key features where the technology needs further advances are:

- Improved DNA results with better peak height ratio balance (per locus and between loci) for low quantity samples and mixtures
- Ability to identify samples with low quantity, degradation or inhibition
- On-board approved automated expert system which can handle mixtures - as more than 50% of crime scene samples encountered are mixtures

[8] ENFSI: European Network of Forensic Science Institutes.

2 Available Rapid DNA systems

At present there are three commercially available instruments:

- ANDE® 6C System (ANDE Corporation)
- RapidHIT™ ID System (Thermo Fisher Scientific)
- RapidHIT™ 200 System (Thermo Fisher Scientific)

A summary of key features for the two NDIS approved instruments (ANDE® 6C System and RapidHIT™ ID System) are presented in Table 1. A detailed description of each instrument is presented in section 4 and 5. RapidHIT™ 200 System is briefly described in section 3.

***Table 1**. A summary of key features for NDIS approved Rapid DNA systems.*

	 ***ANDE® 6C System*** *photo: ANDE*	 ***RapidHIT™ ID System*** *photo: Thermo Fisher Scientific*
Supplier	ANDE Corporation	Thermo Fisher Scientific
Size & Weight	75 x 45 x 60 cm, 54 kg	27 x 53 x 48 cm, 25.4 kg
Sample Types & Capacity	Reference buccal samples: 1 to 5 samples Crime scene samples: 1 to 4 samples	Reference buccal samples: 1 sample Crime scene samples: 1 samples
Process time	Reference samples: 94 min Crime scene samples: 106 min	Reference samples: 90 min Crime scene samples: 96 min
Success rate (reference samples)	85-92%	72-94%
STR chemistry	FlexPlex27	AmpFLSTR™ NGM SElect™ Express GlobalFiler™ Express
Software	ANDE Expert System ANDE FAIRS™ Software	RapidLINK™ Software
Maintenance	Annual preventive maintenance	Annual preventive maintenance Change of Primary Cartridge (every 100 runs) Should be run at least once a week to maintain performance
Consumables	ANDE® swab A-Chip (reference samples) I-Chip (crime scene samples)	Primary Cartridge (≥100 runs) RapidHIT™ ID ACE Sample Cartridge (reference sample) RapidINTEL™ Sample Cartridge (crime scene samples)
Operating conditions	10-40 °C Relative humidity 20-80% (non-condensing)	15-30 °C (max fluctuation ±2 °C during an instrument run) Relative humidity 20-80% (non-condensing)

3 RapidHIT™ 200 System

The RapidHIT™ 200, Figure 1, was originally developed by IntegenX (Pleasanton, CA, US), now a subsidiary of Thermo Fisher Scientific. The instrument size is 73 x 71 x 48 cm and its weight 82 kg. The instrument runs with four single use disposable cartridges: two sample cartridges (reagents for extraction, amplification and capillary electrophoresis), one Cartridge A (polymer for capillary electrophoresis) and one Cartridge B (buffer and waste for capillary electrophoresis). The instrument can analyse 1 to 8 samples in parallel, either reference buccal samples or crime scene samples using different performance protocols. The STR chemistries available are AmpFLSTR™ NGM SElect™ Express[9] (17-loci system) and GlobalFiler™ Express[10] (24-loci system) [21].

Figure 1. *RapidHIT™ 200, photo: Thermo Fisher Scientific*

Following a tendering process in 2015 NFC purchased a Rapid HIT™ 200 with the purpose of evaluating the system for analysis of crime scene samples. The system was delivered in late 2015 and the project performed during 2016. NFC encountered numerous issues with the system related to hardware, firmware and software as well as the cartridges. Among issues encountered were the retrieval of an incorrect DNA profile, PCR product or sample leakage and not the least a low success rate. In total, 36% of the runs presented problems or errors effecting two or more samples resulting in a 77% success rate for samples with 1, 2 or 5 µL blood on swabs (corresponding to amounts where complete DNA profiles are the expected outcome when using conventional setups) [22,23].

In conclusion, the poor robustness, contamination risk and low success rate together with high running costs (including investments and maintenance) made the RapidHIT™ 200 System unfit for analysis of crime scene samples. The outcome for NFC was an interrupted evaluation of the RapidHIT™ System for casework [22,23]. For this reason, together with the fact that Thermo Fisher Scientific will end the sale of the Rapid HIT™ 200[11], the instrument will not be described further in this report.

[11] ENFSI DNA WG online meeting September 2021. "Operational examples of the Applied Biosystems™ RapidHIT™ ID System". Stephan Köhnemann, Senior Field Application Specialist, Thermo Fisher Scientific.

4 RapidHIT™ ID System

4.1 General description

The RapidHIT™ ID System is a small and compact one-sample-instrument initially developed for reference samples [24,25]. The system consists of the RapidHIT™ ID System, RapidLINK™ Software and two RFID[12] tagged consumable cartridges: primary cartridge and sample cartridge, Figure 2. The instrument was originally developed by IntegenX and is now provided by Thermo Fisher Scientific.

The instrument has been further developed to also analyse crime scene samples using specific cartridges [19].

Figure 2*. RapidHIT™ ID System instrument, sample cartridge and example view of RapidLINK™. Photos: Thermo Fisher Scientific*

The instrument is a benchtop instrument sized 27 x 53 x 48 cm and weighs 25.4 kg (28.4 kg when loaded with the primary cartridge). It can operate in 15-30 °C with 20-80% relative humidity (non-condensing). The instrument has in-built temperature and humidity sensors that will stop the instrument if the environment is outside the specified ranges. It's powered by 100 to 240 V +/-10% (sine wave AC), 50/60 Hz line power or generator [26].

The approximate run times are 90 minutes for reference samples and 96 minutes for crime scene samples. The extended analysis time for crime scene samples is due to an additional four PCR cycles (32 instead of 28). Besides higher PCR cycle numbers the lysis buffer volume is reduced (300 µL instead of 500 µL) to increase the sensitivity for crime scene samples [19].

On September 1st, 2020, RapidHIT™ ID System (v1.3) received NDIS approval for reference samples for accredited laboratory use in the US. On July 1st, 2021 a customised version of the RapidHIT™ ID System ("*DNA Booking System v1.0*") received NDIS approval for use in

[12] RFID: Radio frequency identification.

law enforcement booking stations for the analysis of reference samples. DNA Booking System v1.0 is optimised for booking stations. The approvals are limited to certain sample cartridges/chips and already approved PCR STR typing kits [7].

4.2 Consumables and chemistries

The primary cartridge contains the lysis buffer and all reagents needed for electrophoresis and supports for 150 runs (to achieve a minimum of 100 sample runs). The cartridge weighs 3 kg and is loaded at the front into the lower part of the instrument [10].

The sample cartridge is a single use, disposable consumable. It contains one sample chamber, in which the sample is loaded, and all reagents for lysis to PCR. There are two different sample cartridges: for reference samples (RapidHIT™ ID ACE) and for crime scene samples (RapidINTEL™). The cartridge type for reference samples is available in two STR-chemistries: AmpFLSTR™ NGM SElect™ Express (17-loci system) and GlobalFiler™ Express (24-loci system) [26].

Both chemistries include the Expanded ESS (European Standard Set) core loci [27] while GlobalFiler™ Express also includes the 20 CODIS core loci [28]. The cartridge type for crime scene samples is only available in the GlobalFiler™ Express STR-chemistry. Since AmpFLSTR™ NGM SElect™ Express is not available for crime scene samples and close to all studies of the RapidHIT™ ID System conducted the latest years has been using GlobalFiler™ Express all studies referred to in this report is from using GlobalFiler™ Express [26].

The shelf life of the sample cartridge and the primary cartridge is 12 months from manufacture (stored at 4-10 °C)[13]. Upon delivery, a minimum shelf life of 2 months is guaranteed. Sample cartridges can also be stored at ambient temperature (15-25 °C) for up to 2 months as long as the expiry date is not exceeded. The quality of the results will not decrease below acceptable levels within the specified shelf life. The cartridges are RFID tagged and will be rejected if the expiry date is exceeded[14].

[13] Personal communication, Thomas Simon, Senior Key Account Manager HID Central, Thermo Fisher Scientific, October 20, 2021.
[14] Personal communication, Louise Hebert, Senior Key Account Manager HID Nordics, Thermo Fisher Scientific, March 26, 2021.

4.3 Expert system

The RapidHIT™ ID System is supplied with the data management software RapidLINK™ Software. The system can operate in a Windows Active Directory and is optimised for Windows™ Server 2012 R2. Besides linking RapidHIT™ systems the RapidLINK™ Software main features are [26]:

- Accessing, reviewing and editing DNA profiles
- Application for database matching, familial search analysis, kinship analysis and staff elimination database
- Monitoring of instrument throughput, health condition and consumable counts (locally or remotely)
- Managing user access
- Export of CMF files for upload to the CODIS DNA database

The RapidLINK™ Software also includes an expert system which uses the GeneMarker™ HID Software (SoftGenetics LLC, State College, PA, US) and rule-based algorithms to analyse the DNA results (e.g. stutter and locus-specific filters, analytical thresholds). Obtained results are flagged as a traffic light: pass (green), requires manually review (yellow), failed ("no DNA profile", red). In order for a sample to pass a set of requirements (e.g. locus peak heights, peak height ratios, alleles per marker) must be met. These requirements have been determined during developmental validations [10,19]. A sample which does not meet all requirements is marked as yellow. Any results with 3 or more detected autosomal alleles per locus are flagged as potential mixed source (yellow) [26]. For GlobalFiler™ Express, mixture detection has been demonstrated down to 1:10 for the RapidHIT™ ID ACE Sample Cartridge and down to 1:8 for the RapidINTEL™ Sample Cartridge (Appendix 2).

In the RapidLINK™ software optical pre-processed raw data can be exported in the file format .fsa, either to the folder on the network or to a connected USB stick (open or encrypted). The .fsa files can be analysed using the software GeneMarker™ HID Software on a stand-alone computer or via the RapidLINK™ software. The .fsa files can also be reviewed using GeneMapper™ ID-X Software (Thermo Fisher Scientific) although this process has not been through developmental validation by Thermo Fisher Scientific. DNA profiles can additionally be exported from the RapidLINK™ Software for use by an external LIMS[15] (Appendix 2).

By installing the RapidLINK™ Software on a separate and centrally located stand-alone computer RapidHIT™ instruments located in different places can be arranged in a network that can be monitored and controlled remotely e.g. from a forensic facility [26].

[15] LIMS: Laboratory Information Management Systems.

4.4 Operating the instrument

4.4.1 Swabs and sample collection

According to the User guide the Puritan 3" Sterile Standard Cotton Swab w/Semi-Flexible Polystyrene Handle (SKU: 25-8032 PC) (Puritan Medical Products Company LLC, Guilford, ME, USA) or Whatman™ OmniSwab 09-923-376 (WB100035) (Qiagen, Hilden, Germany) is recommended for reference buccal sample collection [26]. The system is however not limited to a specific swab, but they must meet certain requirements (Appendix 2):

- Max length 75 mm to fit the sample cartridge container
- Must not be high absorbent ("lollipop swabs")
- Must not contain antimicrobial substances as these could cause inhibition

However, one study has compared the results from the Puritan cotton swab with other swabs: 4N6-FLOQSwabs™ (Copan Flock Technologies), MacroPur™ Swabs (Solon Manufacturing Company, Rhinelander, WI, USA), EasiCollect Swab (GE Healthcare) and with 3 mm punches from FTA® cards (Qiagen). Although the numbers of samples in this study were limited the results obtained indicated lower success rates with the other swabs and with punches from FTA® cards [24].

4.4.2 Processing a sample

The RapidHIT™ ID System process one sample per run. It uses an on-instrument graphical user interface guiding the operator to process a sample in the following steps [26]:

1. The collected sample is placed in the sample cartridge (ACE cartridge for reference samples, INTEL cartridge for crime scene samples)
2. The operator signs into the instrument
3. Sample ID is entered (scan using on-board camera, barcode reader or enter manually)
4. The sample cartridge is inserted (the run protocol is automatically selected depending on the inserted cartridge type)
5. Run completes after 90 minutes (reference sample) to 96 minutes (crime scene samples) and the sample cartridge is removed;
    - Preliminary data interpretations are performed and run success is presented as traffic lights
    - Run data is automatically transferred to the RapidLINK™ software for review by a trained expert in DNA profile interpretation
6. Another sample is ready to be analysed

As the run starts, 300 or 500 µL Prep-N-Go™ Buffer enters the sample chamber from below. The sample is incubated at 75 °C for 8 minutes and the lysate is moved within the cartridge to the PCR chamber and passes through a solid state capture media (a 1.2 mm diameter paper disc) trapping a specific volume of the lysate prior to amplification. The primers, master mix and lysate are pumped into the PCR chamber to a final volume of approximately 12 µL. The PCR program consists of the following steps: 95 °C for 1 minute (enzyme activation) followed by 28 or 32 cycles at 94 °C for 5 seconds (denaturation) and 60 °C for 40 seconds (annealing/extension). The final extension step is performed at 60 °C for 8 minutes. Following PCR, internal lane standard is pumped through the PCR chamber moving the amplified product to a mix chamber. The mix is then moved through a heat denaturation zone (95 °C) and into the primary cartridge for injection into the capillary and the subsequent electrophoresis (injection parameters: 5 kVs for 8 seconds). Post signal processing generates an electropherogram trace file in .fsa file format. The .fsa files are automatically imported into GeneMarker™ HID Software for allele calling. The software employs a library of allelic ladder used for size determination of detected alleles in the electropherograms. During a run an allelic ladder is automatically selected from the pool of ladders. The ladder library for the RapidHIT™ ID System comprises of pre-installed ladders and the allelic ladder derived from the last recorded control cartridge [10].

4.4.3 System maintenance

The instrument should be run at least once a week to maintain performance, either by running a sample or a control cartridge. The primary cartridge needs to be replaced every ~100 runs or if the date expires. Due to the need for gel refrigeration within the primary cartridge the instrument always needs to remain powered. The primary cartridge can be changed by the operator but requires administrator or supervisor login. An annual preventative maintenance of the instrument by a Thermo Fisher scientific field service engineer is recommended [26].

4.5 Reference samples studies

In the developmental validation of RapidHIT™ ID System for reference buccal samples 54 buccal swabs from 51 unique donors collected using cotton-tipped swabs (Puritan) were analysed. When processed using two RapidHIT™ ID Systems the pass rate was 94%. One sample failed due to size standard issues leaving 53 samples for further evaluation [10].

All results from the 53 samples were concordant in comparison to analysis using conventional methods for STR typing. The mean peak height ratios in heterozygote markers ranged between 0.78 and 0.91 with a mean of 0.85. In 8 out of 915 (0.9%) heterozygote pairs the peak height ratios were below 0.5. The intra colour balance ranged between 0.18 and 0.47. The size precision, measured using ten allelic ladders were well below the 0.166 bp size standard deviation (the limit for 99% accurate sizing) for all alleles, indicating reliable size precision with low risk of false allele calling. No run-to-run contamination was observed [10].

In a subsequent internal validation study 50 buccal reference samples were processed at a success rate of 72% [24].

4.6 Studies on crime scene samples

In the developmental validation study of the RapidINTEL™ Sample Cartridge [19] the supplier concludes: "*The RapidINTEL™ Sample Cartridge is recommended for use with blood and saliva samples only*". In the reply to the questionnaire a wider use is suggested, including epithelial cells and tissue on various matrices. Crime scene samples such as blood, saliva, epithelial cells and tissue on various matrices, porous or non-porous substrates, can be analysed using the RapidHIT™ ID System. Internal validation studies are recommended to set the limitations (Appendix 2).

In the developmental validation study, more than 1,200 samples of various sample types were analysed using 6 different RapidHIT™ ID System. In summary:

- 402 blood samples (ranging from 0.0625 µL to 4 µL of blood on cotton swabs) and 417 saliva samples (ranging from 0.25 µL to 12 µL of saliva on cotton swabs) were tested. In summary, at 0.5 µL, 1 µL and 2 µL of blood 5%, 26% and 55%, respectively, generated complete DNA profiles[16] without a need for manual review. The corresponding results for 1 µL, 2 µL and 4 µL of saliva were 6%, 11% and 44%, respectively.
- 69 blood samples (4-15 µL) on various substrates (such as glass, drywall, tarred surface, denim and white cotton) were tested. 19 of 69 (28%) of the samples generated a complete DNA profile without a need for review and another 5 (7%) after manual review.
- Mock crime scene samples containing saliva such as cigarette butts and drinking containers were swabbed and the swabs analysed. Complete DNA profiles without a need of review were generated in zero of 13 cigarette butts, zero of 3 water bottles, zero of 4 soda cans, 3 of 8 drunken coffee mugs/cups, zero of 3 gums (chewed for 30 minutes).
- Blood on fabric (1-2 µL of dried blood on cotton or denim) were swabbed and the swabs analysed. In none of 9 samples complete DNA profiles without a need for manual review were generated.
- Blood on glass (7.5-30 µL of dried blood on glass) were swabbed and the swabs analysed. In 2 of 9 samples complete DNA profiles without a need for review were generated.

[16] The number of markers for a complete DNA profile can differ depending on the chemistry used. For simplicity complete profiles are compared. The volumes of blood and saliva correspond to amounts where complete profiles are expected using conventional analysis.

- Of 81 positive control runs performed, 79 completed successfully while 2 failed with no results. The mean peak height ratios for heterozygote markers for the positive controls ranged from 0.73 to 0.91 with an average across all loci of 0.82. The intra colour balance ranged between 0.32 and 0.42.
- Regarding concordance 1,199 of 1,216 samples (98.6%) were concordant with data from the ABI 3500xL Genetic Analyzer (Thermo Fisher Scientific). In 13 of the 17 occasions allelic drop out caused false homozygote alleles (a single peak at a marker above the stochastic threshold).

DNA samples on swabs are preferable as other sample types must be fixated to the bottom of the cartridge container (e.g. using the backside of a swab or a lancet) as the lysis buffer enters the cartridge container from below and to a height of approximately 10 mm. Any liquid DNA samples to be analysed must first be transferred to a solid matrix[17].

4.7 Sample re-testing

As previously described a sample is generally considered consumed upon a Rapid DNA instrument run. However, if the sample needs to be re-tested after the run has completed, either because it failed to produce satisfying DNA results or because there is a need for the result to be confirmed by conventional DNA analysis methods, the sample may be removed from the sample cartridge and subjected to a second DNA analysis. This has been demonstrated for samples collected on cotton swabs as cotton are prone to absorb DNA and only releases a part of it during a run on the RapidHIT™ ID System. The sample can be removed from the sample cartridge, allowed to dry and sent for re-analysis using conventional DNA analysis methods, potentially leaving enough DNA to produce satisfying DNA results [10]. To our knowledge no data regarding the loss in sensitivity has been described.

4.8 Relocating the instrument

The RapidHIT™ ID System must be operated in a sheltered environment at a stationary location since it is sensitive to shocks, vibrations and temperature fluctuation.

When the primary cartridge is installed the instrument should always be powered. The instrument can be unpowered for up to 20 minutes before the gel in the primary cartridge starts to degrade, leading to reduced performance. Thus, e.g. unexpected power losses of less than 20 minutes will typically cause no issues. For transports up to 4 hours the primary cartridge can be uninstalled (and stored between 4-10 °C) and reinstalled at the new location. Installation takes between 50 minutes and 2 hours, the latter if a new primary cartridge needs to be installed. For transports exceeding 4 hours the instrument must be connected to a mobile power supply [29].

[17] Personal communication, Louise Hebert, Senior Key Account Manager HID Nordics, Thermo Fisher Scientific, March 26, 2021.

Thermo Fisher Scientific has so far not validated the performance of the system in a mobile unit or in a vehicle and does not guarantee system performance for mobile use. The costumer is recommended to validate the instrument performance in their specific mobile environment. When moving the instrument, e.g. using a van it is recommended to house the instrument on a specific shock absorbing platform to protect it from shocks and vibrations. The vibrations must still be limited and transports should only be made on well-paved roads. The mobile unit must be completely stationary and the operating conditions (Table 1) met before a run is commenced. It is recommended that the stable environmental conditions of a typical laboratory should be recreated inside the vehicle for optimal performance (temperature should not fluctuate more than ±2 °C during a run). The RapidHIT™ ID System should always be connected to a continuous power source (power grid, generator, or portable battery power station) producing pure sine wave AC power to maintain refrigeration of the reagents [29].

4.9 Pricing

The 2021 list prices for RapidHIT™ ID System, consumables and service contracts are listed in Table 2[18].

***Table 2**. List prices for RapidHIT™ ID System, consumables and service contracts.*

Product	Price (SEK)
RapidHIT™ ID System	1,322,000
RapidLINK Software v1.0, single license	86,300
RapidLINK Software v1.0 Staff Elimination Database Application	42,580
RapidLINK Software v1.0 Kinship Application	42,580
RapidLINK Software v1.0 Match Application	42,580
RapidLINK Software v1.0 Familial Application	42,580
Computer for the RapidHIT™ ID System	24,542
Service contract including 1 preventive maintenance	144,648
RapidHIT™ ID ACE GlobalFiler Express 50 Sample Kit *50 sample runs, 2 positive controls and 2 negative controls runs*	51,100
RapidHIT™ ID ACE NGMSElect Express 50 Sample Kit *50 sample runs, 2 positive controls and 2 negative controls runs*	51,100
RapidINTEL Sample Cartridge Kit (GlobalFiler Express) *50 sample runs, 2 positive controls and 2 negative controls runs*	57,800
RapidINTEL Sample Cartridge Evaluation Kit (GlobalFiler Express) *10 sample runs, 2 positive controls and 2 negative controls runs*	19,270
RapidHIT™ ID Primary Cartridge GlobalFiler Express 100 Kit *For 100 sample runs, plus additional CNTRL runs and system maintenance under normal operating conditions*	66,600
RapidHIT™ ID Primary Cartridge NGMSElect Express 100 Kit *For 100 sample runs, plus additional CNTRL runs and system maintenance under normal operating conditions*	67,600

[18] Personal communication, Thomas Simon, Senior Key Account Manager HID Central, Thermo Fisher Scientific, October 20, 2021.

5 ANDE® 6C System

5.1 General description

The ANDE® 6C System was originally developed under the name "*DNAscan Rapid DNA Analysis System*" by NetBio (Waltham, MA, USA), which later became ANDE Corporation. The system consists of four components (Figure 3): the ANDE® instrument, ANDE® chip, ANDE® swab and ANDE FAIRS™ Software.

ANDE® 6C System is a benchtop instrument with the size 75 x 45 x 60 cm and a weight of 54 kg. It can operate in 10-40 °C in 20-80% relative humidity (non-condensing) and is powered by 100 to 240 V +/- 10% (sine wave AC), 50/60 Hz by line power or generator [30].

The system uses a single microfluidic BioChipSet cassette (also called ANDE® chip) which can be loaded with up to five ANDE® swabs. The chip is a single use, disposable consumable containing all reagents and components required to perform a complete STR analysis. The run time for reference samples and crime scene samples are approximately 94 minutes and 106 minutes, respectively (Appendix 3).

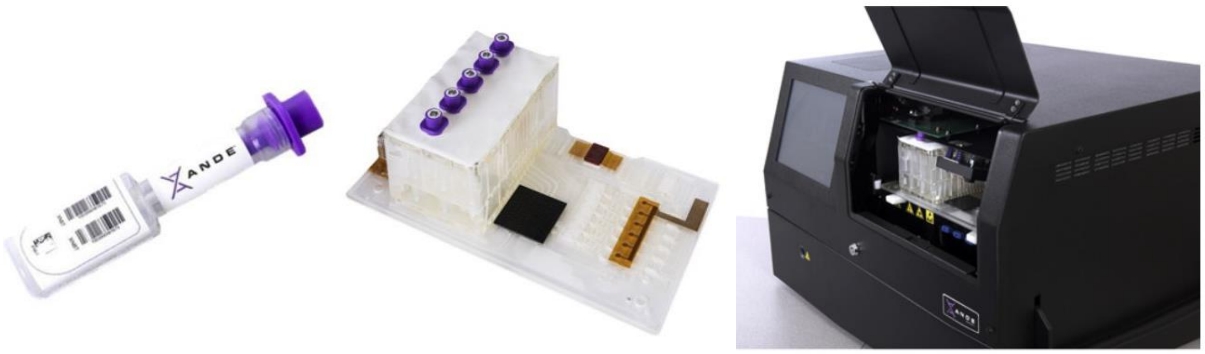

***Figure 3**. ANDE® swab, ANDE® chip (A-Chip) and the ANDE® 6C System. Photos: ANDE*

On June 4th, 2018 ANDE® 6C System received NDIS approval for accredited laboratory use for reference samples in the US. On February 1st, 2021 a customised version of the ANDE® 6C System (Series G) received NDIS approval for the analysis of reference samples in law enforcement booking stations. The Series G is optimised for enrolment, i.e. with reduced operator options and limited presentation of the results. The approvals are limited to certain sample cartridges/chips and already approved PCR STR typing kits [7].

5.2 Consumables and chemistries

ANDE® 6C System uses the FlexPlex27 STR chemistry[19], a 27-loci system containing 23 autosomal loci, Amelogenin and three Y-chromosomal loci. The STR chemistry includes the European expanded ESS core loci as well as the 20 CODIS core loci [30].

There are two types of ANDE® chips: A-Chip, which processes one to five reference buccal samples, and I-Chip, which processes one to four DNA samples with lower DNA amount(s) such as crime scene samples or DVI samples. As the I-Chip is a further development of the A-Chip there are only small differences between the two chips, the main being that the I-Chip contains a concentration module positioned downstream of the purification module, to reach a lower limit of detection. Post-purification, the processing steps of the A- and I-Chips are similar [9,16].

The ANDE® chips are pre-loaded with all reagents (extraction and PCR reagents, buffers, and separation polymer) as well as harbouring the capillaries for electrophoresis to separate and detect the DNA fragments. The reagents are either in liquid or lyophilized form. No other consumables are needed for a run. The chip is closed and each sample is therefore processed through its own sealed processing path, and samples and reagents do not come in contact with the instrument itself. The ANDE® chips have a 6-month shelf life at 5-25 °C. The system will reject the chip if the expiry date is exceeded [30].

The two ANDE® chips consist of four major components [16,17]:

- The “Smart cartridge” contains five (or four) separate purification units which includes the swab chamber into which the swabs are inserted. Each purification unit holds four liquid purification reagents reservoirs. The cartridge also includes one formamide storage reservoir.
- The “Gel smart cartridge” contains the remaining reagents for the electrophoresis (buffers and polymer).
- The “Integrated biochip” is the heart of the chip containing microfluidic channels performing the purification by the transfer of liquids from chamber to chamber of the smart cartridge. The PCR chambers are located on the Integrated biochip and contains PCR-mix, internal lane standard (ILS) and the allelic ladder, all in lyophilized form.
- The “S&D biochip” contains the capillaries and performs the separation of STR fragments by electrophoresis by first receiving the amplified DNA from the Integrated biochip and electrophoresis reagents from the Gel smart cartridge.

There are several critical interfaces between the instrument and the chip: pneumatic interface for driving the fluids throughout the chip, thermal interface for the PCR reaction, a high-

[19] FlexPlex27: D1S1656, D2S1338, D2S441, D3S1358, D5S81, D6S1043, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, FGA, CSF1PO, Penta E, TH01, vWA, TPOX, SE33, Amelogenin, DYS391, DYS576, and DYS570.

voltage interface for the electrophoresis and an optical interface for the fluorescence detection after the electrophoresis [16,17].

5.3 Expert system

The ANDE® 6C System is supplied with ANDE Expert System for raw data processing, allele assignment and automatic interpretation of the DNA results [16].

The ANDE® 6C System is NDIS-approved for reference buccal samples using the A-Chip. The same expert system but with other parameter threshold settings (Table 3) is used for DVI and crime scene samples [16]. These settings have been determined empirically by analysing more than one thousand samples[20].

***Table 3**. Parameter threshold settings for the ANDE Expert System for DVI and crime scene samples using the I-Chip.*

Parameter	Threshold
Analytical threshold	120 rfu
Stochastic threshold	235 rfu
iNTA[21] peak height ratio threshold	Locus-dependent ranging from 0.02 to 0.34
Heterozygote peak height threshold	Locus-dependent and nominally at 0.35
Tri-allele peak height ratio threshold	Third peak must have a peak height ratio of at least 0.37 to be considered as an allele

Directly, subsequent to finishing a run the system completes the steps of raw data processing, peak identification, and spectral separation. A strict set of criteria is then utilised for the evaluation of ILS and the allelic ladder. If the allelic ladder in the run fails, a pre-installed allelic ladder is used. The parameter settings for the alleles are assigned by comparing them to an allele table (a table managed by ANDE and consisting of alleles reported in literature). Only peaks present in the table are labelled. Each locus is determined as homozygote or heterozygote by evaluating peak height, peak height ratio, iNTA ratio, stutter ratio, and peak morphology. Loci that fail are labelled with red warning boxes. After all rules are applied the sample will either be classified as Pass[22], Low signal, High signal, Mixture or Retake. For the version Series G the classifications are limited to pass (green checkmark), requiring manual review (yellow checkmark) and no DNA profile (red cross). For each run a .csv file is

[20] Personal communication, Lewis Shaw, Field Application Scientist, ANDE Corporation, May 10, 2021.
[21] iNTA: formation of -A peaks.
[22] A run is classified as pass if the results meet the threshold for the minimum number of loci called. This threshold is user defined.

generated, as well as individual .fsa and .png files per sample, whereas an .xml file is only generated for each successfully typed sample [16].

The ANDE® 6C Expert System is designed to identify and flag profiles of mixtures from two or more contributors. Profiles are flagged as a mixture for manual review if: i) two or more loci have three alleles or ii) one locus with four or more alleles is present. In two-person mixtures with a minor contributor of less than 1:5, the system will call the major contributor [16].

The ANDE® 6C System is also supplied with ANDE FAIRS™ Software, a Windows-based application for secure file handling, match results and kinship determination. FAIRS™ enables export in the file formats .csv, .png, .xml and .fsa which include electropherograms for review by a qualified DNA analyst as well as optical pre-processed raw data to permit review with GeneMarker™ HID Software or GeneMapper™ ID-X Software [9].

5.4 Operating the instrument

5.4.1 Swabs and sample collection

The ANDE® swab is a key component in the ANDE® 6C System. The swab is a Bode SecureSwab2 (ANDE Corporation) with a RFID tagged cap and a 2D barcode for sample tracking [31]. The barcode sticker has a 4-letter designation that allows the user to visually identify the swab that is in the process of being loaded. The samples are irreversibly locked into the chip during sample loading. All sample positions must be sealed with the RFID tagged cap of an ANDE® swab, e.g. when less than a full run is performed or if the sample has been collected using something else than an ANDE® swab [30].

During sample collection of crime scene or DVI samples (or at least prior to the run) the DNA sample should be collected using an ANDE® swab. A study using a conventional cotton tipped swab (Puritan) on the ANDE system concluded a substantial decrease in success rate compared to using the ANDE® swab [32].

For dry stains/deposits on non-absorbing surfaces it’s suggested to simply swab the object (e.g. a drinking container or chewing gum) using an ANDE® swab pre-wetted with sterile water. Alternatively, a dry stain can first be moistened by adding sterile water, and then collected using a pre-wetted ANDE® swab [16]. For DNA samples on absorbing surfaces such as blood on fabric there are two alternative methods to use. Either use the same procedure described above, moisten the stain with sterile water and then swab the stained area. Or cut a

piece of the stained area, place it directly in the sample chamber and insert an ANDE® swab on top[23].

5.4.2 Processing a sample

The instrument has an integrated touch screen on which a graphical user interface guides the operator throughout a run [30]:

1. The operator signs into the instrument (as Operator, Admin or SuperAdmin)
2. For each sample the operator presents the ANDE® swab to the integrated RFID/bar-code reader (on the front of the instrument), peels of the plastic seal from the sample chamber and loads the sample swab into any of the sample chambers of the A-/I-Chip. Blank swabs are placed into all remaining unused sample chambers
3. The operator then inserts the A-/I-Chip in the instrument. The run initiates as soon as the instrument access lid is closed. The run protocol is automatically selected depending on the inserted chip type
4. A second RFID reader located inside the instrument automatically reads the RFID chips in each of the swab caps to identify where each swab has been placed within the ANDE® chip
5. Run completes after 94 minutes (reference sample) or 106 minutes (DVI and crime scene samples)
    - Data interpretations are performed and run success is presented as traffic light
    - The access door opens automatically to allow removal of the A-/I-chip
6. Another run is ready to be performed

The system uses a DNA extraction including guanidine-based lysis, ethanol-based wash, and TrisEDTA-based elution. All solutions are pneumatically driven across a 5 mm^2 silica membrane binding the DNA. The purified DNA extract is utilized to reconstitute the lyophilized PCR reagents prior to thermal cycling. Parameters such as PCR cycle number, anneal, extend and denature times as well as temperatures cannot be altered [9,30].

To our knowledge, there is no information available regarding PCR volume, PCR cycle numbers or injection parameters.

5.4.3 System maintenance

There is no user maintenance needed on the instrument, but the system requires a preventative maintenance once per year performed by an ANDE trained service engineer [30].

5.5 Reference samples studies

In the developmental validation of the ANDE® 6C System buccal reference samples from 1,387 unique donors were tested using 13 separate ANDE instruments, run at different laboratories by a number of operators. The overall success rate (full 20 CODIS core loci profiles)

[23] Personal communication, Lewis Shaw, Field Application Scientist, ANDE Corporation, October 26, 2021.

was 92%. Concordance was evaluated using 1,338 unique donors. The accuracy allele calling rate was 99.998% for the 20 CODIS core loci due to one observed allelic drop out. The 1,338 samples had a peak height ratio ranging from 0.72 to 0.89, with an average across all loci of 0.81. Based on 402 runs, allele sizing differences were all well within 0.5 base pairs and with acceptable sizing precision. No run-to-run or lane-to-lane contamination was observed. The report concluded that the swabs can be stored in room temperature for at least one week prior to analysis. The ten potential PCR inhibitors tested (mint, beer, bloody swab, cigarette, coffee, gum, mouthwash, tea, tobacco dip, and toothpaste) did not impact negatively on the results [9].

In 2018, NIST organized a study of Rapid DNA maturity assessment involving nine laboratories. In the study 100 reference buccal samples across five different ANDE® 6C systems were analysed (20 samples per instrument) with an average success rate of 85% for 20 CODIS core loci. The observed concordance was 99.98% with one incorrect allele called (in Penta E) by the expert system (compared to results from PowerPlex Fusion 6C and PowerPlex 21) [8].

In a report describing a validation of the ANDE® 6C systems for reference samples 104 reference buccal samples were tested on one instrument. The authors reported that 97% of the samples gave "*interpretable signal*" and a concordance of 99.96% compared to when samples were subjected to conventional DNA analysis [31].

5.6 Studies on crime scene samples

In the developmental validation of the ANDE® 6C System for forensic casework and DVI samples 1,705 mock casework and DVI samples of various sample types were tested using the I-Chip. The swabs were stored in a desiccant-containing protective tube until processing. Outcome in summary [16]:

- The limit of detection was tested using 0.1, 0.5, 1.0, 3.0, 10 and 25 µL of blood from ten different donors, applied on ceramic tiles, allowed to dry at room temperature, and collected using an ANDE® swab. Triplicates per donor were used for 0.1-3.0 µL and duplicate for 10 and 25 µL per donor. Full profiles were generated for all samples containing 1.0 to 25 µL. At 0.5 µL input blood, 21 samples (70%) generated full profiles. One sample had a microfluidic failure, and the remaining 8 generated partial profiles with a mean of 18.0 CODIS core loci called. At 0.1 µL input blood, three samples generated full profiles, one sample generated no called peaks, and the remaining 26 generated partial profiles with a mean of 18.2 CODIS core loci called.
- Allelic concordance for CODIS core loci was 99.996% (with one drop-out and one drop-in observed) for obtained STR profiles from 1,299 samples across several sample types: blood on ceramic tiles, blood on FTA papers, blood on cotton fabrics, blood on denim fabrics, blood on knifes, oral epithelial samples from drinking cups, saliva on non-FTA papers, saliva on FTA papers, neat semen, semen on cotton fabrics,

chewing gums, and DVI samples (bone, brain, liver, kidney, lung, muscle and teeth). Allelic concordance for CODIS core loci was measured to be 99.996%.

- No run-to-run or lane-to-lane contamination was observed.
- The mean peak height ratios for heterozygote markers for the same 1,299 samples ranged from 0.65 ± 0.21 at SE33 to 0.84 ± 0.12 at Amelogenin, with a mean for all autosomal markers of 0.79 ± 0.16.
- For loci where iNTA were observed, the iNTA peak height ratio varied from 0.090 ± 0.025 at D2S441 to 0.26 ± 0.21 at Amelogenin. No iNTA was observed at D10S1248, D13S317, Penta E, D2S1338, CSF1PO, D6S1043, D7S820, D5S818, or TPOX. Mean iNTA were 0.16 ± 0.13.
- Complete profiles were generated from 3 µL blood containing up to 1.0 µg/µL of humic acid, whereas 1.5 µg/µL or higher concentrations did cause inhibition. Complete profiles were also generated from samples containing indigo dye (fabric cuttings from unwashed dark blue denim and cotton fabrics).
- In mixtures 19:1 and 1:19, only alleles from the major contributor were called. Samples with mixes of ratios 1:1, 1:5 and 5:1 were flagged for manual review.

5.7 Sample re-testing

The samples are irreversibly locked into the chip when samples are loaded to prevent interaction(s) potentially having a negative impact on the sample and result. The user is instead encouraged to take two sample swabs per trace, one for back up if required[24].

5.8 Relocating the instrument

The ANDE® 6C instrument has been ruggedized according to US Mil-STD-810G[25] for transportation vibration and shock. The instrument is delivered in a transport case but can withstand a drop from 4 inches (10 cm) outside the transport case as well as the vibration expected during transportation by plane, truck or hand carry. The instrument must, however, reach the operating temperature (10-40 °C) before starting a run. There are no prerequisites other than turning off the instrument before moving it [30].

At the new location the instrument is simply plugged in and the power switch turned on. The system will do an automatic initiation check of the various subsystems and is ready for use in approximately 15 minutes. No parts or consumables require replacement before starting a run at the new location. The instrument can be carried via a two-person lift using the carrying

[24] Personal communication, Lewis Shaw, Field Application Scientist, ANDE Corporation, May 10, 2021.
[25] U.S. Military Standard, Department of Defense test method standard, environmental engineering considerations and laboratory tests, the United States Department of Defense, April 2014, http://everyspec.com/MIL-STD/MIL-STD-0800-0899/MIL-STD-810G_CHG-1_50560/.

handles located on the sides of the instrument [30]. The ANDE® 6C System has successfully been deployed in a van and used for the identification of human remains in DVI cases[26,27].

5.9 Pricing

The 2021 list prices for ANDE® 6C system, consumables and service contracts provided by the supplier T3 Solutions AB are listed in Table 4[28].

***Table 4**. List prices for ANDE® 6C system, consumables and service contracts. Prices were received in USD but is also presented in SEK using the currency rate **8.67 SEK/USD**.*

Product	Price (USD)	Price (SEK)
ANDE® 6C System (including ANDE FAIRS™ Software)	300,000	2,601,000
Transport casing	10,000	86,700
1 year service contract	30,000	260,100
2 year service contract	55,000	476,850
3 year service contract	78,000	676,260
ANDE® A-/I-Chip	1,425	12,355
ANDE® swab (pack of five)	60	520

[26] Paradise lost: inside California's campfire (2018). CBS News, 60 Minutes. https://www.cbsnews.com/news/paradise-lost-inside-california-camp-fire-60-minutes/ (accessed December 10, 2021).

[27] Officials used 'Rapid DNA' tech to identify California boat fire victims (2019). The New York Post. https://nypost.com/2019/09/05/officials-used-rapid-dna-tech-to-identify-california-boat-fire-victims/ (accessed December 10, 2021).

[28] Personal communication, Niclas Asada, Business Development Manager, T3 Solutions AB, August 24, 2021.

6 Discussion

6.1 Reference samples

6.1.1 Success rate

The developmental studies report a success rate of 92-94% for reference buccal samples and a high concordance with conventional DNA analysis. A presentation held by Bode Technology at Promega 2021 International Symposium on Human Identification[29] described the experiences from the implementation of Rapid DNA in border control in the US. They reported a success rate of 94% and small variations between instruments despite having systems situated at different geographical locations. Other studies have shown success rates of 72-92%[30][8,24].

For reference samples the biological material is usually not limited to single samples or one single run, therefore any limitations of the Rapid DNA systems may in the end have less impact since a repeated analysis in most cases will be possible. The same applies to many DVI situations, although the DNA in the samples might be highly degraded.

6.1.2 Searches against the national DNA database

It is important to address that after analysis using a Rapid DNA system, the results would in a Swedish setting need to be transferred to the NFC in order to be compared to other results in the specific case or in order to be uploaded to the national DNA database. Such IT-structure is not in place and would therefor need to be developed. The costs and possible timeline for such developments have not been part of this study.

Both the ANDE® 6C System and RapidHIT™ ID System can, with some adjustments, export data files compliant to the software used at NFC. The transfer of data to current systems and searching a profile in the national DNA database would require several manual steps. Reducing the manual steps is feasible but requires efforts in order to integrate the Rapid DNA systems into current systems.

6.1.3 Highly prioritised reference samples

By using Rapid DNA systems for highly prioritised reference buccal samples DNA profiles can be generated in about 1.5 h without interfering with other processes. By installing Rapid DNA systems on strategical sites or use in a mobile setting the time for transporting samples for analysis could be minimized.

[29] ISHI Conference, September 2021, "Lessons Learned from Implementing and Operating a 24/7/365 Rapid DNA Program Across the United States". Dane Plaza, Director of Federal Operations, Bode Technology.
[30] ENFSI DNA WG online meeting, April 2021, "Real-Time DNA –The Metropolitan Police Journey". Shazia Khan, Head of Secure Operations, Metropolitan Police Service, London England.

At NFC, the turnaround time for the 35,000 reference samples analysed yearly is 1-2 days from sample arrival at the lab. The generally low turnaround time reduces the need for running specific samples with high priority. During the last four years approximately 20 highly prioritized reference samples have been analysed at NFC. Such reference samples can be analysed in about 2.5 hours. These samples briefly block resources in the DNA analysis process, potentially delaying other reference samples with a few hours.

6.2 Crime scene samples

6.2.1 Success rate

The samples collected at a crime scene are often limited and of unknown quality and content. In a forensic DNA laboratory, different optimised DNA extraction methods are chosen depending on sample type and substrate with current techniques able to provide results from samples containing only a few cells. In such cases consensus analysis is applied by performing the amplification at least twice, thereby reducing the stochastic effect from low DNA amounts during amplification.

During quantification issues like degradation or presence of inhibitors can be identified. This information can be used to treat samples differently in order to optimise the chance of generating useful results. DNA quantification is not performed by Rapid DNA systems making it impossible to adjust the analysis based on such information. Quantification is a formal requirement shared by SWGDAM and some of the member countries of ENFSI, although not Sweden, and is therefore part of the key features described in the joint letter [20].

Technical data in available literature reveals that Rapid DNA systems produce DNA results with generally lower quality for crime scene samples compared to conventional DNA analysis. The lower peak height ratio balances (per locus and across loci) and issues with iNTA peaks indicate poorer efficiency from lysis to amplification as compared to conventional analysis. The occasional incorrect profiles in reports, especially for RapidHIT™ ID System (and even from samples with high DNA amounts), seem to be caused by stochastic effects from the amplification due to low DNA input in the PCR in combination with low stochastic threshold settings. In contrast, the electrophoresis and data collection seem to have reached an acceptable level not far from conventional system.

Available data of the sensitivity (limit of detection) is from different samples and sample types and a fair comparison is only possible if replicates from the same sample is analysed, this since the amount of DNA differs between individuals as well as over time for the same individual. There is also loss of DNA, if for example the blood is dried on a surface and thereafter swabbed as a regular recovery step, as compared to if the same amount of blood is placed directly into a tube or pipetted onto a swab for further processing. Still, the available data indicates significant differences in limit of detection between the systems as well as compared to conventional analysis.

The sensitivity of the RapidHIT™ ID System has been tested with blood and saliva pipetted directly onto cotton swabs. From samples of 2 µL of blood and 2 µL of saliva full DNA profiles were generated in 55% and 11%, respectively. The poor sensitivity is likely because the system is developed for reference buccal swabs (being rich in DNA) and the fact that there are only small differences between the protocols for reference buccal samples and crime scene samples.

Regarding sensitivity of the ANDE® System, dried blood on ceramic tiles were swabbed using a pre-wetted ANDE® swab. At 0.5 µL and 1.0 µL of blood 70% and 100%, respectively, of the runs generated a full profile. The higher sensitivity compared to the RapidHIT™ ID System is likely due to the concentration module on the I-chip.

The sensitivity measured as the minimum volume of blood needed to generate a DNA profile has not been determined at NFC. However, verification tests of extraction solutions using 0.015-0.030 µL (about 50 times less blood compared to 1 µL) of high-quality blood pipetted directly in to microfuge tubes are regularly subjected to standard DNA analysis. With few exceptions full DNA profiles are retrieved. If using consensus analysis, it will be possible to generate results of even lower amounts of blood. This data clearly indicates a lower sensitivity for Rapid DNA analysis, and especially RapidHIT™ ID System, compared to conventional DNA analysis.

Samples from crime scenes often contain low levels of DNA. As a comparison, internal studies using 1 µL of blood and 1 µL saliva pipetted directly into tubes gives a DNA concentration of approximately 0.31 ng/µL and 0.15 ng/µL, respectively. Evaluating DNA concentrations from crime scene samples analysed at NFC from January until October 2021, about 90% of the samples were below 0.30 ng/µL. This data indicates from a general perspective that there are a limited number of samples suitable for Rapid DNA analysis.

One major consequence of using current Rapid DNA systems on crime scene samples is the risk of consuming (single) traces that could have generated results if analysed in a forensic DNA laboratory.

6.2.2 Sample types

Rapid DNA systems are developed for samples collected on swabs. The ANDE® 6C System requires their specific ANDE® swab to be used for full functionality. Using the RapidHIT™ ID System it is recommended but not limited to use a cotton-tipped swab. Crime scene stains on non-absorbing and smooth surfaces are collected using a pre-wetted swab. Stains on rough or absorbing surfaces such as blood on fabric are more challenging for the Rapid DNA systems since swabbing might not be the preferred optimal method for recovery.

Using the RapidHIT™ ID System it is suggested to fixate the material to the bottom of the cartridge container (e.g. using the backside of a swab or a lancet). There are limited data available on success rates applying this approach. When NFC tested blood on fabric using a lancet during the evaluation of RapidHIT® 200 the material sometimes shifted position in the sample chamber, which resulted in poor DNA results.

Using the ANDE® 6C System for stains on absorbing surfaces requires several steps of pre-processing. These are steps traditionally performed in a forensic laboratory since there is a need for additional equipment and chemicals as well as dedicated work spaces. In order to perform these steps additional measures has to be taken to minimise the risk for sample loss or mix ups as well as contamination.

6.2.3 Mixtures

Rapid DNA systems lack an on-board approved automated expert system which can handle mixtures. The expert systems only flag potential mixtures for manual review but with limits regarding mixture ratios. The ANDE® 6C Expert System also call the major contributor in assumed two-person mixtures with a minor contributor of below 20%, thereby discarding potentially useful DNA information. As with single source DNA profiles, mixtures need to be identified and subjected to manual review by a trained forensic DNA expert.

6.2.4 Reanalysis

It is inevitable that the DNA analysis will fail to some extent, either due to sample limitations such as low DNA amounts, or technical issues. For conventional DNA analyses subparts of the analysis (e.g. amplification or electrophoresis) can in general be redone or a complementary analysis performed from the extract. Rapid DNA is more of a one shot only. This limitation must be considered if samples with trace amounts are to be analysed using Rapid DNA.

Samples analysed on RapidHIT™ ID System can be re-collected following a run and then subjected to conventional DNA analysis. This can be done since it is anticipated that not all of the DNA on the swab will be released from the swab during the analysis[31]. Obviously the success of such a reanalysis may be doubtful and there is to our knowledge no available data of the success rate during the analysis or impact on the conventional DNA analysis.

Reanalysis of samples upon a run on ANDE® 6C System is not possible since the sample is locked irreversible in the chip. The user is instead encouraged to take two sample swabs, one for back up if required.

[31] ENFSI DNA WG online meeting April 2021. “Sample Retesting Applied Biosystems™ RapidHIT™ ID System”. Stephan Köhnemann, Senior Field Application Specialist, ThermoFisher Scientific.

The possibility of using double swabs, one for the Rapid DNA system and the other for conventional analysis still consumes half the trace. Such an approach obviously bares limitations regarding e.g. small stains and not the least trace DNA in which sufficient amount of DNA cannot be anticipated. Also, regarding the option to perform reanalysis if the RapidHIT™ ID System fails still gives suboptimal conditions to perform conventional analysis with success, this since part of the sample is consumed in the first run.

6.2.5 Highly prioritised crime scene samples

If using Rapid DNA systems for highly prioritised crime scene samples DNA profiles can be generated in about 1.5-2 h without interfering with other processes. By installing Rapid DNA systems on strategical sites or use in a mobile setting the time transporting items or samples for analysis could be shortened.

Apart from samples and sample types not suitable for Rapid DNA, the number of samples per instrument run could be confining in cases where multiple samples are to be analysed. If using RapidHIT™ ID System only one sample can be analysed per run, while using the ANDE® 6C System up to four samples can be analysed per run. At NFC, the most commonly applied DNA extraction methods includes up to 19 samples per batch and several batches can be processed in parallel.

At NFC almost 70,000 crime scene samples are analysed yearly. The mean turnaround time using the fastest and most frequent DNA extraction method is about 2.5 calendar days[32] from sample arriving to the DNA analysis lab until a result is reported to the reporting officer for further handling in the specific case. Highly prioritized crime scene samples can be analysed within 5-6 h. During the last four years samples from 38 highly prioritized cases have been processed at NFC outside of office hours. Two of the cases only contained blood on swabs (of unknown amount), samples that might have been able to process on a Rapid DNA system. One case included blood on a swab plus additional items, the other samples/items in the remaining cases would not have been considered appropriate for Rapid DNA. Even more cases have been prioritized but the requested delivery time has been accomplished using normal office hours. These highly prioritized samples block resources in the DNA analysis process, delaying the analysis of other crime scene samples.

As for reference samples, results generated with a Rapid DNA system would need to be transferred to NFC in order to be evaluated and compared to other results in the specific case or uploaded to the national DNA database. Such IT-structure is not in place.

[32] The statistics is expressed in calendar days which also include weekends and holidays when routine analysis is not performed.

6.3 Relocating the instrument

The ANDE® 6C System can withstand vibration and shocks from transportation. After 15 minutes of automatic initiation check the system is ready for use. The system has successfully been deployed in vans and used for DVI cases.

The RapidHIT™ ID System is for indoor use only. It is sensitive to shocks, vibrations and temperature fluctuation. It can be moved using a specific shock absorbing platform but vibrations must still be limited. The system cannot be unpowered for more than 20 minutes or else the primary cartridge must be uninstalled before the transport and reinstalled at the new location, a process requiring 50 minutes to two hours.

For both systems the operating conditions (Table 1) must be met. In order to maintain acceptable conditions, especially during wintertime when relative humidity often is low in Sweden, the area of deployment (room or van) must be equipped with a system regulating the temperature as well as the relative humidity.

6.4 Costs

The purchase cost for the systems based on the list prices (Table 2 and 4) including the instrument, other hardware and software is about 1,400,000 SEK for RapidHIT™ ID System and about 2,700,000 SEK for the ANDE® 6C System[33].

In figure 4 and 5 the running cost for the systems is estimated based on the list prices for RapidHIT™ ID System and ANDE® 6C System respectively. These figures include the "running costs" such as costs for annual preventive maintenance and consumables (but not including repairs). As the annual preventive maintenance must be performed regardless of the number of samples analysed the cost per sample will be higher when only a few samples are analysed.

For the RapidHIT™ ID System, in which one sample or a control must be run once a week (in order to maintain system performance), the cost efficiency per sample is low if analysing only a few samples. The limited shelf life in combination with kit size of the sample cartridges (50 samples per kit) adds to the costs and cost efficiency. As the sample cartridges could have a shelf life of 2 to 12 months upon delivery the costs are presented as intervals. For example, running 100 samples per year would cost 310,000 to 850,000 SEK and the cost per sample would be 4,300 to 7,500 SEK.

[33] The costs for personal, validation, accreditation, training and implementation as well as for developing an IT-structure needed for integrating the Rapid DNA systems into the current process was outside the scope of this project and has not been estimated.

For the ANDE® 6C System the costs are highly dependent on the number of samples analysed per run. The A-Chip (for references samples) and I-Chip (for DVI and crime scene samples) holds five and four sample positions, respectively. Running samples in all positions in each run is obviously more cost efficient but most likely not a possible reality in many cases (leaving empty sample positions). For this reason, the costs for the ANDE® 6C System is also presented as intervals. The lower line presents the costs when each run is performed with five samples and the upper line when only one sample is used per run. As an example, running 100 samples per year would cost 520,000 to 1,500,000 SEK and the cost per sample would range between 5,200 and 15,000 SEK.

In Table 5 annual running costs and costs per sample for a specified number of samples under certain conditions are stated.

Chemical costs for crime scene samples and reference samples at NFC are 75 SEK and 55 SEK per sample[34] respectively.

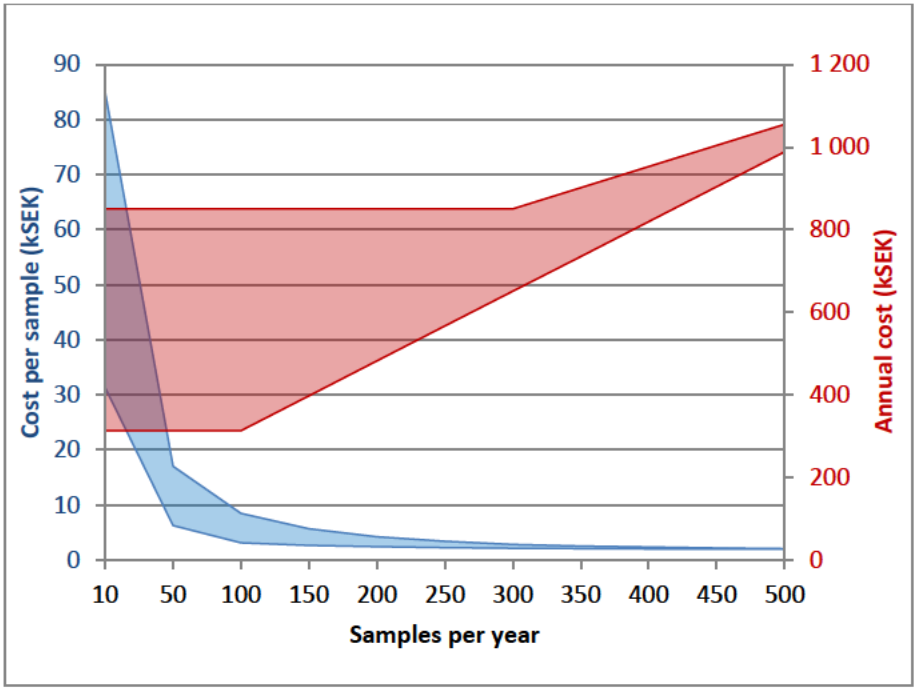

***Figure 4**. Annual running costs (red) and cost per sample (blue) for **RapidHIT™ ID System** when analysing reference buccal samples (the cost per sample for crime scene samples are slightly higher). Costs are from list prices and in kSEK. Upper lines are calculated based on 2 months shelf life upon delivery. Lower lines are calculated based on 12 months shelf life upon delivery.*

[34] Since Rapid DNA systems would be a complement and not replace conventional analysis, it is not relevant to include costs for instrumentation etc.

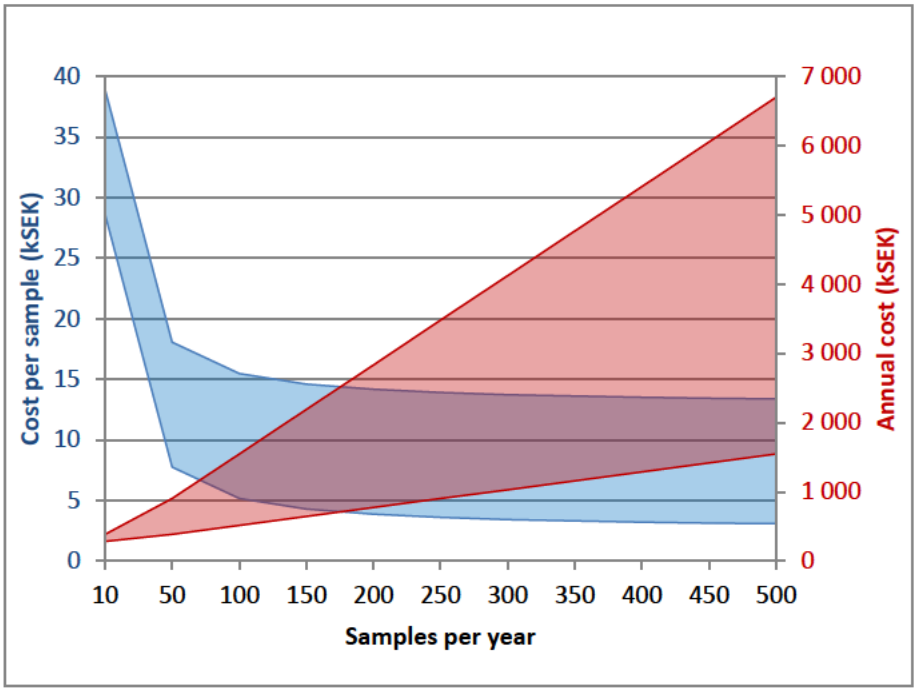

***Figure 5**. Annual running costs (red) and cost per sample (blue) for **ANDE® 6C System** when analysing reference or crime scene samples. Costs are from list prices and in kSEK. Upper lines are calculated based on loading 1 sample per run. Lower lines are calculated based on loading 5 samples per run.*

***Table 5.** Annual running costs and cost per sample for the ANDE® 6C System (when analysing 2 samples per run) and the RapidHIT™ ID System (when assuming 6 months shelf life for the cartridges).*

Samples per year	Annual cost (SEK)		Price per sample (SEK)	
	RapidHIT™ ID System	ANDE® 6C System	RapidHIT™ ID System	ANDE® 6C System
10	380,000	324,000	38,000	32,400
50	380,000	582,000	7,600	11,600
200	482,000	1,550,000	2,410	7,740
500	989,000	3,480,000	1,980	6,960

6.5 Examples of the use of Rapid DNA systems

There are countries (e.g. the US, France and Guinea[35]) using Rapid DNA systems for analysis of reference samples (buccal swabs), this in order to speed up the turnaround time for example in booking stations. In such situations, a Rapid DNA system on site can be of value. Also, the Metropolitan Police in London are planning to pilot the technology for reference samples and have gained accreditation for this application of the device[36,37]. In the presentation from Bode Technologies mentioned earlier it is pointed out that a Rapid DNA program is more than the use of a single instrument. For example, a lot of efforts need to be put into training and support organisation, as well as handling the results from the instruments.

From a DVI perspective there are examples from the US where the ANDE instrument has been used successfully in identifying victims/human remains[38,39,40]. In Sweden DNA analysis related to DVI is not handled by NFC. These analyses are performed at the National Board of Forensic Medicine (RMV).

As described, with present day performances, ENFSI, SWGDAM and FBI strongly advice against the use of Rapid DNA systems for the analysis of crime scene samples for automatic submission to national DNA databases [20]. Rapid DNA can however be used for crime scene samples in order to generate early investigative leads. There are examples of local (e.g. county or state based) databases from the US, where the DNA results are used as investigative leads, but not uploaded to the national database or used in court[41,42]. Also, laboratories in Europe (LKA in Hamburg and Stuttgart) have started to use Rapid DNA, but only when there is a second sample to analyse with conventional methods[43].

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[42] Online presentation "Solving Crime Through Local DNA Databases". Fred Harren, Director, Bensalem Township Police Department Bucks County, Pennsylvania U.S. https://www.bodetech.com/DNA-Forensic-Services/rapid-dna-services (accessed August 14, 2020).

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In the Netherlands there is an ongoing joint project - the LocalDNA pilot - on the use of Rapid DNA with the National Police, Amsterdam University, Public Prosecution and the Netherlands Forensic Institute (NFI). It has been an extended process for many years with several validations due to hardware/software/kit changes, process to get accreditation and adoption of the law. One of the requirements has been to be able to perform contra analysis using conventional methods. This has been accomplished by using special swabs that can be split into two halves – one for each process. Current recommendations are restrictive and limit the use to bloodstains larger than 5 mm and visible stains of saliva spit. The next step of the project is to evaluate the effects of speed in the process and to compare success rate between Rapid DNA and conventional analysis[44].

The French Gendarmerie reports validating RapidHIT™ ID System for samples rich in DNA (saliva, blood, and fresh bones), reference samples and DVI. The instruments can be placed locally (also on local forensic laboratories at their overseas territories) but all data is to be transferred to the central forensic laboratory in Paris for further processing. The analysis as such was accredited in 2021[45].

[44] ENFSI DNA WG, online meeting September 2021, "The Netherlands initiatives on the use of Rapid DNA systems: the LocalDNA pilot". Sander Kneppers, Program Manager Innovation & Technology, Division Biological Traces, Netherlands Forensic Institute, the Hague Netherlands.

[45] ENFSI DNA WG, online meeting September 2021. "Rapid DNA at the French Gendarmerie: a mobile and remote control application for decentralized human identification capabilities". Sylvain Hubac, Head of DNA unit, Forensic Science Laboratory of the French Gendarmerie Paris, France.

7 Conclusions

The aim to gather information on the Rapid DNA systems available is fulfilled with this report.

The most important findings and our conclusions are the following:

- For reference buccal samples and DVI samples (where there is enough biological material available for reanalysis) Rapid DNA systems can be used to provide DNA profiles.
- The time for analysis of reference samples is 1.5 hours using a Rapid DNA system compared to 2.5 hours for urgent cases using conventional analysis at NFC.
- The use of Rapid DNA systems on crime scene samples can only be recommended for stains expected to be single sourced and of high amount and quality where there is enough material for a second analysis using conventional methods.
- If used on other sample types there is an obvious risk of consuming single traces from a crime scene without useful results, samples that could have generated DNA profiles if analysed in a forensic DNA laboratory using conventional analysis.
- The time for analysis of crime scene samples is about 1.5-2 hours using a Rapid DNA system compared to 5-6 hours for urgent cases using conventional analysis at NFC.
- With present day performances, ENFSI, SWGDAM and FBI strongly advice against the use of Rapid DNA systems for the analysis of crime scene samples for automatic submission to national DNA databases.
- If using Rapid DNA systems of current design on crime scene samples, evaluation of the results and the comparison in the specific case always needs to be performed by trained forensic DNA experts.
- If running e.g. 50 samples per year using *one* Rapid DNA system the cost per sample would be approximately 7,500 SEK (RapidHIT™ ID System) or 11,600 SEK (ANDE® 6C System) compared to 55 SEK for reference samples and 75 SEK for crime scene samples on conventional analysis.

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9 Appendices

Appendix 1 Questionnaire sent to suppliers
Appendix 2 Thermo Fisher Scientific – response to questionnaire
Appendix 3 ANDE Corporation – response to questionnaire

Polisen
Swedish Police

Date
2 October 2020

Swedish Police Authority
Christina Forsberg

Questions regarding Rapid DNA instrumentation

1. General

a) What Rapid DNA instruments/models do you offer? If more than one please state which instrument your answers concerns.
b) Specify size, weight and noise level.
c) Do you recommend putting the instrument in a pre-PCR area or post-PCR area?
d) Specify the addressed parameters in the developmental validation of the instrument(s).
e) Specify the expected success rate for reference buccal samples.
f) Has the instrument been validated for reference samples by any forensic laboratory accredited according to ISO 17025? If yes, state which.
g) Has the instrument been validated for crime scene samples by any forensic laboratory accredited according to ISO 17025? If yes, state which.

2. Materials and sample types

a) What sample types can be analysed with the instrument (i.e. reference samples and/or crime scene samples)?
b) What kind of biological materials can be analysed (e.g. blood, saliva, tissue)?
c) What matrices can be analysed (e.g. cotton swabs, fabrics, cigarette butts etc.)
d) Specify all commercially available swabs that have been validated or verified for use with the instrument.
e) Specify the maximum input volume available for the sample (in the sample container).

3. Operating the instrument

a) How does the instrument operate? Please describe all steps included such as sample loading, operating the instrument, handling of consumables.
b) What number of samples that can be analysed in one run?
c) What is the run time(s)?
d) Are there more than one run protocol? Please specify available protocols and all the differences.

e) What technique is used to minimize the risk of sample mix-up?
f) What technique is used to minimize the risk of contamination during sample loading?
g) What maintenance is needed?
h) When is long-term storage of the instrument needed and what maintenance is then required?
i) Which instrument parts (e.g. capillary, motors etc.) needs continuously replacement and with which intervals?
j) If the instrument is relocated to a new facility. What actions are needed before transport and what start up procedure is necessary before running?
k) Is it possible to remove the extract/sample for use in repeated analysis? If so, describe the recommended procedure.

4. System details

a) Describe the analysis process (extraction, quantification /normalization, electrophoresis injection etc).
b) Has the instrument an integrated method of human specific co-amplified internal positive controls used to identify low quantity, degradation and inhibition?
c) What is the dynamic range of DNA load (from limit of detection to overload), i.e. the range of cell numbers that provides complete DNA profiles without bleed through peaks. Please specify the cell type applied in the test and if the cells were in suspension or dried.
d) What is the locus balance (heterozygote balance) for samples within the dynamic range of DNA load and state the recommended limit for accepting two alleles as a true heterozygote pair.
e) What is the instrument settings (e.g. PCR program, capillary electrophoresis (CE) injection time, CE injection voltage, CE run voltage and peak height thresholds)?
f) What measures are taken to minimize the risk for channel-to-channel and run-to-run contamination?
g) Specify the expected failure rate for samples due to other causes than low DNA amount. Please specify the most common causes.

5. Chemistry and consumables

a) Which STR analysis chemistry is recommended for the instrument? Specify STR-markers included in the chemistry if it isn’t a commercial available kit.
b) Are there other STR analysis chemistries available for the instrument(s)? Specify STR-markers included in the chemistry if it isn’t a commercial available kit.

c) Describe how a high reproducibility is ensured between cartridges originating from different reagent lots, e.g. lysis buffer and STR reagents.
d) Specify all molecular PCR inhibitors that have been tested on the instrument, e.g. hematin, humic acid or melanin. State the respective maximum amounts of these inhibitors that the instrument handles without allelic dropouts or major imbalances. Specify the source of DNA and amounts that were used for the tests.
e) State whether the instrument has been tested on materials (commonly encountered in casework) known to contain PCR inhibitors, e.g. blood and denim. If so, describe these tests and specify the results.
f) Specify the shelf-time and the required storage conditions for all included components needed for a run.
g) State the delivery time of consumables.

6. Data transfer

a) Can the instrument operate in the specified network environment, Windows Active Directory?
b) What data can be extracted?
c) How can the data be extracted?
d) Can the data be extracted with encrypted USB?
e) Is it possible to export analysable raw data (optical preprocessed)?
f) What is the file format of raw data?
g) Specify possible software's for analysis of exported raw data.

7. Expert System

a) Describe the Expert System used for data interpretation in detail.
b) How are the detection and analytical thresholds set?
c) How does the instrument's software discriminate true alleles from background noise?
d) How does the instrument's software discriminate true alleles from stutters and artefacts?
e) How does the instrument handle mixtures?
f) What mixture ratios can the instrument and Expert System handle?
g) How does the Expert System handle minor contributor(s) in mixtures (i.e risk for the sample to appear as single source)?
h) Considering the thresholds, would it be possible to analyse samples with low amount of DNA in the same run as samples with high amount of DNA?
i) Specify how DNA profiles can be stored in a local database. Is it possible to import DNA profiles to the database or can you only store profiles generated by the specific instrument?

8. Manufacturing quality control

a) State whether or not the manufacturing processes (instruments and consumables) are accredited/certified. Specify the type of accreditation/certification.
b) State whether or not the manufacturing process of cartridges/consumables is closed, i.e. without human intervention.
c) Specify measures taken to make sure that cartridges/consumables are DNA free.
d) Specify the controls performed to verify that cartridges have not been contaminated with human DNA during the manufacturing process.
e) State whether it is possible for a DNA contamination found at NFC to be compared against DNA profiles from manufacturing staff.

9. Specify list price of

a) Instrument.
b) Consumables (if the consumables are sold in a kit, specify the size and content of the kit and number of samples that can be analysed using the kit).
c) Service contract (including degree of extent, e.g. response time, spare parts).
d) Maintenance (if needed).

Please also attach **manuals** and report of **developmental validation** together with the replies.

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National Forensic Centre, Swedish Police Authority

Response to Questions Regarding Rapid DNA Instrumentation

Thermo Fisher Scientific (Thermo Fisher) are please to provide answers to questions posed on 2, October 2020 by the National Forensic Centre of the Swedish Police regarding our products and solutions for rapid DNA instrumentation for forensic DNA analysis.

Question Categories:

1. General

a) What Rapid DNA instruments/models do you offer? If more than one please state which instrument your answers concerns.

Thermo Fisher has two Rapid DNA instruments. Both can process reference and crime scene type samples.

- Applied Biosystems™ RapidHIT™ ID System – Catalogue Number: A41810
- Applied Biosystems™ RapidHIT™ 200 System – Catalogue Number: 1000010RH

A brochure for the RapidHIT ID instrument is located at annex 1

The associated Applied Biosystems™ RapidLINK™ software is referenced within this document. RapidLINK software is a software which is supplied with RapidHIT instruments. Its main functions are:

- Accessing, reviewing and editing of DNA profiles
- Application of:
  - Database matching
  - Familial Analysis
  - Kinship Analysis
  - Staff Elimination Database
- Monitoring instruments remotely or locally
- Monitoring instrument throughput and health
- Managing enrolled users

The remainder of the document will focus on the Applied Biosystems™ RapidHIT™ ID system only.

Should you require further information regarding the RapidHIT™ 200 this can be provided.

The compact and easy-to-use Applied Biosystems™ RapidHIT™ ID System combines multiple workflows for the forensic DNA process and is the ideal rapid DNA platform for generating lab-quality forensic DNA profiles from reference samples and crime scene samples. With just one minute of hands-on time, the fully automated RapidHIT ID System can generate short tandem repeat (STR) DNA profiles in the lab or in the field. Paired with Applied Biosystems™ RapidLINK™ Software, the system offers full control of DNA results with powerful sample matching, familial search, kinship, and staff elimination database applications.

The RapidHIT ID system has only 2 main consumable parts:

The Primary Cartridge:

The RapidHIT ID System Primary Cartridge contains all components required for Capillary Electrophoresis and supports a minimum of 100 runs. It is user interchangeable. Once installed, the RapidHIT ID instrument must remain powered to ensure the Gel Polymer for Electrophoresis is maintained at the correct temperature conditions.

The Sample Cartridge:

The RapidHIT ID System uses self-contained sample cartridges to transform a multicomponent protocol into a single, user-initiated task. There are two types of sample cartridges:

- The Applied Biosystems™ RapidHIT™ ID ACE Sample Cartridge (for reference samples)
- The Applied Biosystems™ RapidINTEL™ Sample Cartridge (for crime scene samples)

b) Specify size, weight and noise level.

Table 1 below identifies the RapidHIT ID instrument specifications including size and weight:

System	Specification	Description
Performance	Processing time	Less than 90 Minutes
Size and weight	Dimensions (W x D x H)	27cm W x 53cm D x 48cm H
	Weight	28.4Kg – With Primary Cartridge
		25.4Kg – Instrument only
		3.0 Kg – Primary Cartridge
Environmental	Operating Temperature	15 - 30ºC
	Elevation	Altitudes up to 2,000m
	Humidity	20 – 80% Relative Humidity (non-condensing)
	Ingress Protection	Ordinary Equipment: rated IPX0
Electrical	Power input voltage	100 – 240 VAC
	Frequency	50/60 Hz
	Power Cord	Power cord rated for the voltage used and a current minimum of 6A (18 AWG, 0.75mm^2)
	Power	600 W
	Fuse Rating	5A 250 VAC (5x20mm)  250V 5A T
Laser	Wavelength	488 nm

Table 1 – RapidHIT ID instrument specifications

c) Do you recommend putting the instrument in a pre-PCR area or post-PCR area?

The RapidHIT ID instrument can operate within a DNA clean (pre-PCR), an amplified DNA area (post PCR) or a no-DNA area (i.e. an office environment, arrestee book station, or field operations) if the environmental specifications as covered within Table 1 apply.

d) Specify the addressed parameters in the developmental validation of the instrument(s).

Thermo Fisher performed developmental validation experiments in accordance with the DNA Advisory Board Quality Assurance Standards (September 1, 2011) and guidelines from the Scientific Working Group on DNA Analysis Methods (SWGDAM, December 2016) to evaluate the performance of the RapidHIT ID System.

Thermo Fisher has validated the system for use in Human Identification (HID) testing for database applications. It is recommended that each laboratory using the RapidHIT ID System should perform its own appropriate internal validation and verification studies to establish interpretation criteria and demonstrate that the instrument is appropriate and fit for its own HID uses.

Thermo Fisher can support a customer's approach through HID Professional Services (HPS) which can advise and execute on validation and/or verification studies, results analysis and report writing.

e) Specify the expected success rate for reference buccal samples.

In internal validation studies, success rates were 94%. Details can be found at Annex 1.

In customer trials, success rates have been as high as 99. The soon to be published NDIS submission data demonstrates an approximately 87% pass rate with a 99.5% run completion rate upon completion of 880 samples tested by 7 labs.

f) Has the instrument been validated for reference samples by any forensic laboratory accredited according to ISO 17025? If yes, state which.

NDIS approval achieved 2020
https://www.fbi.gov/services/laboratory/biometric-analysis/codis/rapid-dna

Validation studies have been carried out in several European laboratories and are at various stages of completion. Examples from across Europe include:

- Italian Carabinieri – complete
- French Gendarmerie – complete
- Polícia Judiciária Portugal – complete
- La Polizia Scientifica Italy – complete
- Dutch National Police - in progress
- Poland Police Laboratory- in progress
- Key Forensic Services (United Kingdom) - complete

Other customers are in progress/completed however due to confidentiality we cannot disclose further at this point. There are additional users outside of Europe

g) Has the instrument been validated for crime scene samples by any forensic laboratory accredited according to ISO 17025? If yes, state which.

Validation studies have been carried out in several European laboratories and are at various stages of completion Examples from across Europe include:

- Italian Carabinieri – complete
- French Gendarmerie – complete
- Polícia Judiciária Portugal – in progress
- La Polizia Scientifica Italy – complete
- Dutch National Police - in progress
- Poland Police Laboratory- in progress

Other customers are in progress/completed however due to confidentiality we cannot disclose further at this point. There are additional users outside of Europe

2. Materials and sample types

a) What sample types can be analysed with the instrument (i.e. reference samples and/or crime scene samples)?

The RapidHIT ID system can process samples from both reference and crime scene situations where the DNA material resides on a swab, card substrate or other solid matrix. DNA samples in liquid form can be processed when a swab of the sample is taken, or an aliquot is transferred to a card substrate or other solid matrix.

Internal and customer studies have demonstrated results with the following sample types.

- Reference Samples:
  - Buccal swab
  - FTA Card
  - Purified DNA

- Crime Scene samples
  - Blood
  - Saliva
  - Cigarette Butts
  - Chewing gum
  - Hair
  - Bone
  - Touch samples

b) What kind of biological materials can be analysed (e.g. blood, saliva, tissue)?

Many types of samples can be processed, including blood, hair, saliva and tissue. We recommend performing your own appropriate internal validation studies.

c) What matrices can be analysed (e.g. cotton swabs, fabrics, cigarette butts etc.)

Many matrices can be analysed using for analysis, including cotton, fabric, cigarette butts and various porous and non-porous substrates. We recommend performing your own appropriate internal validation studies.

d) Specify all commercially available swabs that have been validated or verified for use with the instrument.

A variety of swabs have used with success on the RapidHIT ID instrument.

Internal developmental validation data was generated using Puritan® cotton swabs: *Puritan 3" Sterile Standard Cotton Swab w/Semi-Flexible Polystyrene Handle (SKU: 25-803 2PC).*

There is no requirement to use a Thermo Fisher supplied swab on the RapidHIT ID instrument.

Customers who have used COPAN 4N6FLOQswabs® have encountered processing. The antimicrobial agent has been demonstrated to cause inhibition in multiple systems including RapidHIT ID.

Other products from the COPAN FLOQSwab range have not shown these issues with the INTEL and ACE sample cartridges.

https://www.copanusa.com/forensic-and-genetic/

All results will depend upon the sample, age, storage conditions etc.

Other swab types used include:

- Whatman™ OmniSwab™
- Generic cotton swabs

Many other swabs may be suitable for use on the RapidHIT ID cartridges. The main limitations are:

- The chamber size:
  - max length of 75mm
- Absorption of lysis reagent:
  - Large foam paddle swabs (sometimes referred to as “lollipop swabs”) are not recommended due to the volume of liquid the foam absorbs. This affects the performance of the workflow due to absorption of the lysis buffer and can result in a decreased amount of DNA being available for amplification and/or instrument run errors due to insufficient lysis volume detection.

We recommend performing your own appropriate internal validation studies.

e) Specify the maximum input volume available for the sample (in the sample container).

As per response to question 2.a), DNA samples in liquid form can be processed when a swab of the sample is taken, or an aliquot is transferred to a card substrate or other solid matrix.

For maximum sample size/volume, the amount of sample is only restricted by the sample chamber dimensions. DNA recovery extends to a height of ~10mm of the sample (maximum lysis solution depth).

DNA-rich liquid and cell pellets are compatible.

3. Operating the instrument

a) How does the instrument operate? Please describe all steps included such as sample loading, operating the instrument, handling of consumables.

The RapidHIT ID instrument is a simple to use platform that can be operated by both scientists and non-scientists following a short training exercise. The on-instrument Graphical User Interface guides the operator in the simple steps to follow to process a sample.

The following are the steps for processing a sample the instrument:

- Obtain a sample and select the appropriate Sample Cartridge:
  - Reference (ACE cartridge recommended – see 3d below)
  - Crime scene (INTEL cartridge recommended – see 3d below)

- Sign into the instrument (any one of the following):
  - Facial recognition
  - Fingerprint
  - 6-digit PIN

- Enter sample ID (any one of the following):
  - Use touchscreen
  - Use on-board camera to scan a barcode
  - Use USB barcode reader

- Insert Sample Cartridge into the instrument

- [Sample processing starts automatically]

- Return after approximately 90 minutes and remove the Sample Cartridge

- During the run process, the instrument software will carry out preliminary data interpretation and present a traffic light indication of the run success:

- On completion of the run, the run data is automatically transferred to the RapidLINK software for review (as required) by a trained expert in DNA profile interpretation.

Any pre-run sample collection or preparation must be carried out by a trained and competent person, and any post-run interpretation of DNA profiles results must be carried out by a trained DNA Expert where required.

Additionally, the Primary Cartridge must be changed by the operator when either of the following conditions are met:

- The maximum number of runs is reached (guaranteed minimum of 100 samples)
- The expiry date has been reached (eight months from manufacture)

The change of the Primary Cartridge is simple and quick (< 5 minutes hands on) and can be performed by the user following a short training program. The on-instrument Graphical User Interface guides the operator in the simple steps to follow to change a Primary Cartridge.

b) What number of samples that can be analysed in one run?

The RapidHIT ID instrument can process one sample per run in approximately 90 minutes.

c) What is the run time(s)?

The run time for reference samples using the ACE sample cartridge (either GlobalFiler™ Express or AmpFLSTR™ NGM SElect™ Express) is approximately 90 minutes.

The run time for samples using the INTEL sample cartridge (GlobalFiler™ Express) is approximately 96 minutes (additional time is due to additional PCR cycle numbers.)

d) Are there more than one run protocol? Please specify available protocols and all the differences.

There are three sample run protocols available:

- ACE GlobalFiler™ Express:
  - recommended for the processing of reference samples
- ACE AmpFLSTR™ NGM SElect™ Express:
  - recommended for the processing of reference samples
- INTEL GlobalFiler™ Express:
  - recommended for the processing of crime stain samples

The recommendations provided above should be tested and validated in the customer environment to determine if the results obtained meet the requirements of local and national jurisdictions.

The chosen Sample Cartridge has an RFID tag that is pre-programmed so the instrument automatically runs the appropriate protocol based on the user's cartridge choice. Insertion of the Sample Cartridge invokes the appropriate protocol to operate – no additional user selection is required.

Additionally, there are Allelic Ladder, Positive and Negative control cartridges for each protocol.

All of the cartridges above use the same instrument and Primary Cartridge configuration, i.e. the only customer action is to choose the appropriate Sample Cartridge type. There is no need to change or configure the instrument for a specific Sample Cartridge type.

Each of the run protocols have optimized System Thresholds. A summary comparison is shown in Table 2:

System Threashold	RapidHIT™ ID ACE GlobalFiler™ Express Sample Cartridge	RapidINTEL™ Sample Cartridge	RapidHIT™ ID ACE NGM SElect™ Express Sample Cartridge
Analytical threshold	35 RFU	50 RFU	All loci 50, except: SE33 = 35
Stochastic threshold (inconclusive Homozygote (IHO flag) threshold)	All loci 91 RFU, except: * TPOX - 105 * Y indel = 35 * DYS391 = 35 * TH01 = 140 * SE33 = 105 * D12S391 = 105 * D2S1338 = 105	1,600 RFU 50 RFU for Y indel and DYS391	All loci 150, except: * D22S1045 = 200 * TH01 = 200 * D2S441 = 100 * D1S1658 = 100 * SE33 = 105
Minimum peak height ratio threshold (Heterozygote Imbalance (IMB flag) threshold)	40%	40% 99% for Y indel and DYS391	40%
Minimum heterozygous peak intensity threshold (inconclusive Heterozygous (IHE flag) threshold)	-	640 RFU	-
Stutter filters	20%	Locus Specific	20%
Locus-specific filter	20%	21% 30% for the positive control	20%
Ploidy (PL flag) threshold (maximum number of expected peaks)	2	2	2
Global filter (between loci)	20%	21% 30% for the positive control	20%
Minimum off ladder (OL) intensity	30 RFU	30 RFU	30 RFU

Table 2 – comparison of each RapidHIT ID run protocol thresholds

e) What technique is used to minimize the risk of sample mix-up?

The instrument can process only one sample at a time, therefore there virtually zero risk of mixing up samples at the point of sample processing.

Samples can be collected and prepared in the vicinity of the instrument or can be collected and prepared elsewhere depending upon customer procedures.

Once the sample is added to the Sample Cartridge, the lid can be closed and sealed ready for use.

Thermo Fisher recommend that appropriate Standard Operating Procedures are put in place by the customer to prevent sample mix up during sample preparation and pre-run storage.

During the operation of the instrument, sample identification is required when the Sample Cartridge is loaded, and this identification can be entered by keyboard or barcode reader.

There is an on-screen touch screen keyboard and a built-in camera, which can be used to read barcodes.

Alternatively, an external barcode reader or keyboard could be used via USB. These are not supplied with the instrument but can be added.

In all cases, there is an option of double entry, where the user would be required to enter sample identification details twice for added sample identification integrity.

f) What technique is used to minimize the risk of contamination during sample loading?

The sample can be collected in the vicinity of the instrument or elsewhere. The sample is loaded into the Sample Cartridge and the lid closed/sealed. Again, this can be in the vicinity of the instrument or elsewhere.

During the loading process the Sample Cartridge should be closed/sealed to prevent inadvertant contamination events occurring.

g) What maintenance is needed?

General Maintenance:

- The instrument should be run at least once a week (one sample of any type) to maintain performance.
- The instrument needs to remain powered due to the need for gel refrigeration within the Primary Cartridge.
- It is recommended that the touch screen on the instrument is clean periodically.

Preventative Maintenance:
We recommend an annual maintenance of the instrument by a Thermo Fisher Field Service Engineer.

h) When is long-term storage of the instrument needed and what maintenance is then required?

Should a customer choose to power down and take an instrument into long term dormancy, it is recommended that the installed Primary Cartridge is removed, and a Transport Primary Cartridge inserted (supplied with each instrument).

Following a period of planned dormancy, re-activation can be supported by Thermo Fisher.

i) Which instrument parts (e.g. capillary, motors etc.) needs continuously replacement and with which intervals?

The Primary Cartridge contains polymer, bulk reagents, waste and the capillary. Thermo Fisher guarantees a minimum of 100 runs before a change is required (subject to expiry date). No other parts need to be replaced by the customer.

Each Sample Cartridge is single use only.

j) If the instrument is relocated to a new facility. What actions are needed before transport and what start up procedure is necessary before running?

A guidance document is available and can be found at Annex 1. A summary is provided here:

For a short move, perhaps to another room or building on the same site (< 4 hours) then no specific action needs to take place.

For a longer move (>4 hours e.g. to a different site)

a) the Primary Cartridge should be removed from the instrument and kept at 4°C during the time of transportation.
b) a transport Primary Cartridge must be installed to the instrument for the duration of the move

Once the instrument is relocated, the existing Primary Cartridge might be re-installed or a new Primary Cartridge inserted.

k) Is it possible to remove the extract/sample for use in repeated analysis? If so, describe the recommended procedure.

The swab can be removed from the Sample Cartridge and re-processed:

- The swab can be re-processed using standard chemistry methods. A document covering a study into the can be found in Annex 1.
- The swab can be re-processed using a RapidHIT instrument

4. System details

a) Describe the analysis process (extraction, quantification /normalization, electrophoresis injection etc).

After the run is initialized, lysis occurs in the sample lysis chamber within the Sample Cartridge. The DNA-rich lysate passes through solid state capture media in the PCR chamber prior to amplification.

Following PCR, internal lane standard is added, and the mixture is transferred from the Sample Cartridge to the Primary Cartridge for injection into the capillary and subsequent electrophoresis (injection parameters: 5kVs for 8s).

The Instrument employs Applied Biosystems™ Prep-N-Go™ Buffer chemistry. There is no quantification or normalization as part of the process. Instead, a specified volume of lysate is retained for PCR.

b) Has the instrument an integrated method of human specific co-amplified internal positive controls used to identify low quantity, degradation and inhibition?

The STR chemistry used within the Sample Cartridge is either the standard GlobalFiler™ Express or NGM SElect™ Express STR chemistries identical to non-RapidHIT chemistries. These do not contain any internal quality control (IQC).

c) What is the dynamic range of DNA load (from limit of detection to overload), i.e. the range of cell numbers that provides complete DNA profiles without bleed through peaks. Please specify the cell type applied in the test and if the cells were in suspension or dried.

For the ACE NGMSE run protocol, samples containing between 200,000 cells and 3,125 cells from the 1000M cell line were processed. Assuming that the DNA content in a diploid cell is 6 pg, the number of cells applied to the swabs is equivalent to 1.2 µg–18.75 ng of DNA. Complete profiles were obtained down to 25,000 cells (or 150 ng of DNA). At 12,500 cells (or 75ng of DNA) 98% of the expected alleles were called. Sensitivity samples containing 6,250 cells and 3,125 cells resulted in partial profiles with <80% of the expected alleles being called.

The ACE GFE and RapidINTEL run protocol studies did not focus on known levels of DNA input, but rather focused on sample level input as, at the point of collection, the DNA quantity would be unknown.

d) What is the locus balance (heterozygote balance) for samples within the dynamic range of DNA load and state the recommended limit for accepting two alleles as a true heterozygote pair.

The minimum recommended limit of Peak Height Ratio (PHR) is 40%. Table 3 shows the average PHR for each of the sample cartridge types.

Average PHR / Marker	RapidHIT™ ID ACE GlobalFiler™ Express Sample Cartridge ACE	RapidINTEL™ Sample Cartridge	RapidHIT™ ID ACE NGM SElect™ Express Sample Cartridge ACE
AMEL	0.87	0.83	0.84
CSF1PO	0.84	0.85	NA
D10S1248	0.87	0.86	0.86
D10S1248	0.87	0.86	NA
D12S391	0.85	0.84	0.85
D16S539	0.85	0.87	0.82
D18S51	0.87	0.88	0.87
D19S433	0.89	0.84	0.87
D1S1656	0.85	0.83	0.88
D21S11	0.87	0.79	0.87
D22S1045	0.83	0.73	0.80
D2S1338	0.82	0.86	0.82
D2S441	0.90	0.87	0.88
D3S1358	0.86	0.91	0.88
D7S820	0.85	0.79	NA
D8S1179	0.85	0.85	0.86
FGA	0.89	0.89	0.87
SE33	0.80	0.79	0.80
TH01	0.86	0.85	0.82
vWA	0.83	0.75	0.84

Table 3 – Average PHR for each locus against RapidHIT ID run protocol

e) What is the instrument settings (e.g. PCR program, capillary electrophoresis (CE) injection time, CE injection voltage, CE run voltage and peak height thresholds)?

Table 4 shows the RapidHIT ID instrument settings:

	RapidHIT™ ID ACE GlobalFiler™ Express Sample Cartridge ACE		RapidINTEL™ Sample Cartridge		RapidHIT™ ID ACE NGM SElect™ Express Sample Cartridge ACE	
PCR Stage	Temperature (°C)	Time (Seconds)	Temperature (°C)	Time (Seconds)	Temperature (°C)	Time (Seconds)
Activation	95	60	95	60	95	60
Denaturing	94	3	94	3	94	3
Annealing	61.5	30	61.5	30	59	20
Extension	61.5	30	61.5	30	65	29
Final Extension	60	480	60	480	60	300
PCR Cycles	28		32		29	
Ramp Rate (Heating & Cooling)	2°C/s		2°C/s		3.5°C/s	

Table 4 – RapidHIT ID instrument settings

f) What measures are taken to minimize the risk for channel-to-channel and run-to-run contamination?

Channel-to-channel contamination:
The RapidHIT ID is designed to process single samples, therefore there is no risk of channel-to-channel contamination.

Run-to-run carry over:
As with any capillary electrophoresis-based platform, run to run carry over is an identified risk.

The sample run protocols have built-in steps at appropriate stages of the run workflow to carry out line flushes to minimize the risk of run-to-run carry over, the same as would be found in traditional capillary electrophoresis platforms.

Contamination studies were a component of the Thermo Fisher Developmental Validation procedure.

Negative samples were run immediately after high-yield positive samples.

In summary, it was concluded that: 'Negative controls that are run after high-concentration samples show no reproducible contamination.'

Full details can be found in the following document that is located at Annex 1: RapidINTEL™ Sample Cartridge for blood and saliva samples RapidHIT™ ID System v1.1.3, Publication Number MAN0018979 Revision A.0, Pages 14, 16, 49.

g) Specify the expected failure rate for samples due to other causes than low DNA amount. Please specify the most common causes.

Aside from low DNA input, other possible failure modes include Sample Cartridge issues or capillary electro-kinetic injection issues. These failure modes generally do not exceed 1%.

5. Chemistry and consumables

a) Which STR analysis chemistry is recommended for the instrument? Specify STR-markers included in the chemistry if it isn't a commercial available kit.

The RapidHIT ID instrument has a choice of two STR chemistries for sample processing:

- GlobalFiler™ Express (different Sample Cartridge types recommended for Reference and Casework samples)
- AmpFLSTR™ NGM SElect™ Express.

Each of the available chemistries has additional cartridges types which are included in each kit.

- Allelic ladder,
- Positive Control
- Negative Control

The chemistry contained within all sample types is identical to the standard commercial products. All are manufactured in the same manufacturing facilities and under identical conditions and to the same standards.

b) Are there other STR analysis chemistries available for the instrument(s)? Specify STR-markers included in the chemistry if it isn't a commercial available kit.

At this stage no additional STR analysis chemistries are available on the RapidHIT ID instrument

c) Describe how a high reproducibility is ensured between cartridges originating from different reagent lots, e.g. lysis buffer and STR reagents.

The manufacturing process was validated to ensure performance to the product specifications. This included testing of multiple lots as well as stability testing.

d) Specify all molecular PCR inhibitors that have been tested on the instrument, e.g. hematin, humic acid or melanin. State the respective maximum amounts of these inhibitors that the instrument handles without allelic dropouts or major imbalances. Specify the source of DNA and amounts that were used for the tests.

In addition to the inhibitor performance demonstrated in the GlobalFiler Express and NGM SElect Express developmental validations, testing was completed in the presence of coffee and tobacco. Both were applied in volumes of 2, 10, 50 and 100ul directly on to a sample swab containing 50,000 1000M cells. Expected profiles, peak heights, and peak height ratios were obtained for all samples with the exception of 100ul tobacco sample. While the expected DNA profile was obtained, lower peak heights and peak height ratios were observed.

e) State whether the instrument has been tested on materials (commonly encountered in casework) known to contain PCR inhibitors, e.g. blood and denim. If so, describe these tests and specify the results.

Thermo Fisher developmental validation did not demonstrate any pattern of inhibition for blood samples using the RapidINTEL Sample Cartridge and instrument protocol. Performance on blood

samples was extensively evaluated in the RapidINTEL developmental validation referenced at the end of this document. While some mock casework samples were tested internally, it is recommended that each laboratory using the RapidHIT ID System should perform its own appropriate internal validation and verification studies to determine performance on select substrates.

f) Specify the shelf-time and the required storage conditions for all included components needed for a run.

Sample Cartridges:
The shelf life of the GlobalFiler™ Express and AmpFLSTR™ NGM SElect™ Express Kit Sample Cartridges is 8 months from manufacture if the stored in refrigerated conditions (4°C to 10°C).

The Sample Cartridges can also be stored at ambient temperature (15°C to 25°C) for up to 2 months at any point before the expiry date as long as the expiry date is not exceeded.

The expiry date is prominently visible on the Sample Cartridge and additionally, the expiry date is contained within the Radio Frequency Identification (RFID) tag embedded into each Sample Cartridge.

The instrument will reject any attempt to run a Sample Cartridge after the expiry date shown.

Primary Cartridge:
The shelf life of the Primary Cartridge is 8 months from manufacture at room temperature.
This shelf life of the gel polymer component is 8 months from manufacture if stored at 4°C to 10°C.

Once the gel polymer is installed to the Primary Cartridge and the Primary Cartridge then installed in the RapidHIT ID instrument, gel refrigeration is automated if the instrument is connected to power (either turned on or in stand-by mode.)

The RFID tags on the Primary Cartridge contain the expiry date and will prevent the instrument from processing a sample run should the expiry date be reached.

The expiry date of the Gel/Primary Cartridge (whichever is sooner) is permanently displayed on the user interface.

g) State the delivery time of consumables.

Standard policy for the delivery of consumables is 24 to 48 hours from order receipt if order is placed prior to 4pm CET.

6. Data transfer

a) Can the instrument operate in the specified network environment, Windows Active Directory?

Yes, the RapidHIT ID instrument can operate in Windows Active Directory. Support on IT network configurations for RapidHIT ID and RapidLINK can be offered by Thermo Fisher HID Professional Services (IT).

b) What data can be extracted?

The run folder contents including the .fsa data file as well as associated log files is unrestricted for download and viewing.

c) How can the data be extracted?

No special software tools are required.

d) Can the data be extracted with encrypted USB?

The Windows Operating Software (Win 10) supports mounting encrypted USB devices

e) Is it possible to export analysable raw data (optical preprocessed)?

Yes, it is an .fsa file

f) What is the file format of raw data?

The file format for the raw data of DNA sample profiles is *.fsa

Prior to .fsa file generation additional steps such as spectral separation occur that require proprietary algorithms and software.

g) Specify possible software's for analysis of exported raw data.

The data exported from the RapidHIT ID instrument is analysed using SoftGenetics® GeneMarker® software v2.9.5.

GeneMarker software can be run standalone or via RapidLINK* software.

The *.fsa files generated by the GeneMarker software can be extracted and reviewed in Applied Biosystems™ GeneMapper™ ID-X Software, however this is process has not been through developmental validation by Thermo Fisher.

Allele bins, size standard settings and panel files can be provided to support any such customer development and validation exercise for the use of GeneMapper™ ID-X Software

7. Expert System

a) Describe the Expert System used for data interpretation in detail.

It is a rule-based system requiring single source DNA profiles in order to be considered passing

b) How are the detection and analytical thresholds set?

The threshold values are fixed as was determined during developmental validation.

c) How does the instrument's software discriminate true alleles from background noise?

In the case of the RapidHIT ID, static detection thresholds were developmentally validated and implemented.

d) How does the instrument's software discriminate true alleles from stutters and artefacts?

A rule-based criteria is used as defined in 3.d) above.

e) How does the instrument handle mixtures?

Any autosomal locus with at least 3 detected alleles results in the sample being flagged as originating from a potential mixed source.

f) What mixture ratios can the instrument and Expert System handle?

Mixture detection was demonstrated down to the following levels:
ACE NGM - 1:9
ACE GFE - 1:10
RapidINTEL – 1:8

g) How does the Expert System handle minor contributor(s) in mixtures (i.e risk for the sample to appear as single source)?

Any autosomal locus with at least 3 detected alleles results in the sample being flagged as originating from a potential mixed source.

h) Considering the thresholds, would it be possible to analyse samples with low amount of DNA in the same run as samples with high amount of DNA?

The RapidHIT ID instrument is designed for single source samples. Mixtures will be identified and flagged, but no mixture interpretation will be performed automatically by the system and will required separate customer development/validation.

i) Specify how DNA profiles can be stored in a local database. Is it possible to import DNA profiles to the database or can you only store profiles generated by the specific instrument?

All data generated from the instrument is automatically transferred to the computer operating the RapidLINK software application. The run data can be optionally deleted automatically from the instrument after successful transfer.

The RapidLINK software can be installed on a computer (can be supplied) local to the instrument or remotely connected by network. RapidLINK software is installed on the supplied computer which has a number of database applications available.

- Database matching
- Familial Analysis
- Kinship Analysis
- Staff Elimination Database

DNA Profiles contained within the RapidLINK software can additionally be exported for use by external Laboratory Information Management Systems (LIMS), local, national and international DNA Databases as required.

To enable the use of RapidHIT ID results together with non-Rapid results, we support third party software solutions.

Support on IT network configurations and data export for use on data platforms outside of the RapidLINK software can be offered by Thermo Fisher HID Professional Services (IT).

8. Manufacturing quality control

a) State whether or not the manufacturing processes (instruments and consumables) are accredited/certified. Specify the type of accreditation/certification.

ISO 9001:2015 registered (Certificate Number FM 601077)

b) State whether or not the manufacturing process of cartridges/consumables is closed, i.e. without human intervention.

There is human interaction with Sample and Primary Cartridges during the manufacturing process in a controlled environment using PPE and a validated and monitored process for environment.

c) Specify measures taken to make sure that cartridges/consumables are DNA free.

We maintain a robust Quality System, having obtained ISO 9001-certification in 2014. We have manufacturing capabilities and facilities specifically designed for Human Identity products. In addition to the quality standards required for compliance with our quality system, our Human Identity products are manufactured in dedicated manufacturing facilities designed to minimize the risk of human DNA contamination.

We have Specialized Staff for Forensic Products who participate in continuous training to maintain a high level of expertise and skill. Personnel are always required to wear prescribed Personal Protective Equipment in our controlled ISO7 HEPA filtered positive pressure controlled facility.

We operate to a strict environmental monitoring program of our human DNA free facility with routine testing for residual DNA, particulate monitoring and bioburden.

For product manufacturing we control key raw materials and testing/ traceability from components through production. Final functional testing includes detection of any human DNA contamination which we certify on or Certificates of Analysis.

Allelic ladder is manufactured in a separate facility from other kit components. Allelic Ladder Cartridges are packaged within a heat-sealed, tamper-evident pouch prior to final kit packaging, greatly reducing the risk of product to product contamination.

d) Specify the controls performed to verify that cartridges have not been contaminated with human DNA during the manufacturing process.

All lots of product are functionally tested to ensure no allele peaks are present due to human DNA contamination.

e) State whether it is possible for a DNA contamination found at NFC to be compared against DNA profiles from manufacturing staff.

Thermo Fisher maintain a manufacturing staff elimination database

9. Specify list price of

a) Instrument.

Table 5 lays out the instrument and software list prices (2020):

Catalogue Number	Product	SEK List Price (Ex VAT)
A41810	RapidHIT ID System	1.299.000
A41813	RapidLINK Software v1.0, single license	84.600
A41816	RapidLINK Software v1.0 Staff Elimination Database Application	42.580
A41817	RapidLINK Software v1.0 Kinship Application	42.580
A41818	RapidLINK Software v1.0 Match Application	42.580
A41819	RapidLINK Software v1.0 Familial Application	42.580
A48503	Computer for the RapidHIT ID System	21.788

Table 5 – List pricing and description of RapidHIT ID instrument and RapidLINK

In addition to instruments and software, HID Professional Services can be offered to include IT and Workflow consultation and Validation/Verification Services. Pricing can be discussion in accordance with the needs of the customer.

b) Consumables (if the consumables are sold in a kit, specify the size and content of the kit and number of samples that can be analysed using the kit).

Table 6 lays out the RapidHIT ID Consumables list prices (2020) and kit content:

Catalogue Number	Product	SEK List Price (Ex VAT)	Kit Content
A41831	RapidHIT ID ACE GlobalFiler Express 50 Sample Kit *Allows 50 sample runs in addition to 2 positive controls and 2 negative controls*	50.300	**GlobalFiler Express:** 50 Sample Cartridges 2 POS CNTRL Cartridges 2 NEG CNTRL Cartridges
A41841	RapidHIT ID Primary Cartridge GlobalFiler Express 100 Kit *Suitable for 100 sample runs, plus additional CNTRL runs and system maintenance under normal operating conditions*	67.100	1 Primary Cartridge Housing 1 Gel Polymer Pack 1 Utility Cartridge (Installation) 1 NEG CNTRL Cartridge **GlobalFiler Express:** 1 Allelic Ladder Cartridge 1 POS CNTRL Cartridge
A41838	RapidHIT ID ACE NGMSElect Express 50 Sample Kit *Allows 50 sample runs in addition to 2 positive controls and 2 negative controls*	50.300	**NGMSElect Express:** 50 Sample Cartridges 2 POS CNTRL Cartridges 2 NEG CNTRL Cartridges
A41847	RapidHIT ID Primary Cartridge NGMSElect Express 100 Kit *Suitable for 100 sample runs, plus additional CNTRL runs and system maintenance under normal operating conditions*	67.100	1 Primary Cartridge Housing 1 Gel Polymer Pack 1 Utility Cartridge (Installation) 1 NEG CNTRL Cartridge **NGMSElect Express:** 1 Allelic Ladder Cartridge 1 POS CNTRL Cartridge

A43942	RapidINTEL Sample Cartridge Kit *Allows 50 sample runs in addition to 2 positive controls and 2 negative controls*	56.940	**GlobalFiler Express:** 50 Sample Cartridges 2 POS CNTRL Cartridges 2 NEG CNTRL Cartridges
A43941	RapidINTEL Sample Cartridge Evaluation Kit *Allows 10 sample runs in addition to 2 positive controls and 2 negative controls*	18.980	**GlobalFiler Express:** 10 Sample Cartridges 1 POS CNTRL Cartridge 1 NEG CNTRL Cartridge

Table 6 – List pricing and description of RapidHIT ID Consumables

c) Service contract (including degree of extent, e.g. response time, spare parts).

Table 7 provides the list pricing for standard service contracts:

Catalogue Number	Product	SEK List Price (Ex VAT)
ZG11SCRHID	Service contract including 1 preventive maintenance	144.648

Table 7 – List pricing and description of service contract

d) Maintenance (if needed).

There is no user maintenance except the requirement to run the instrument at least once per week. Occasional cleaning of the touchscreen may be required.

Please also attach manuals and report of developmental validation together with the replies.

See Annex 1

Annex 1 – Additional Supporting Documents

RapidHIT™ ID Brochure

COL012921-RapidLab-Brochure.pdf

RapidLINK Brochure

rapidlink-brochure.pdf

Processing of samples on RapidHIT ID and convention DNA Methods:

nfstc-swab-test-flyer.pdf

RapidINTEL™ Sample Cartridge for blood and saliva samples RapidHIT™ ID System v1.1.3, Publication Number MAN0018979 Revision A.0

MAN0018979A.0_RapidINTEL RHIT v1.1.3

RapidHIT ID User Guide

MAN0018039_RapidHIT_ID_1_0_UG.pdf

RapidHIT ID Release Note (latest version)

rapidhit-id-system-software-v1-1-3-relea

RapidLINK User Guide

MAN0018038_RapidLinkSW1_UG.pdf

RapidLINK Release Notes (latest version)

rapidlink-software-v1-1-5-release-notes

Appendix 3

An Overview of ANDE Rapid DNA for the Swedish Police Authority

The ANDE 6C Series L is the standard ANDE System for Law Enforcement, Military, and Government users. The instrument size is 75(W) x 45(H) x 60(D) cm and it weighs 53kg. The measured noise level is described as 70 dB(A) or lower with fast Lmax ≤60 dB(A), and average Leq ≤ 54 dB(A).

When operating ANDE within a laboratory, we recommend following the FBI's Quality Assurance Standards for instrument location, which states: "A Rapid DNA instrument/System shall be maintained separate from areas used for evidence examination or sample accessioning. Rapid DNA instrument(s)/System(s) can go in an extraction room, amp setup room, or other area so long as the area where the instrument is maintained is not used for evidence examination or sample accessioning. A Rapid DNA instrument/System shall not be used in rooms containing amplified DNA with the exception of the amplified DNA generated in the Rapid DNA cartridge."

As part of the NDIS approval process, ANDE completed a peer reviewed development validation on buccal samples which achieved a 92% first pass success rate using the A-Chip (see attached: *Developmental validation of the ANDE rapid DNA system with FlexPlex assay for arrestee and reference buccal swab processing and database searching,* https://doi.org/10.1016/j.fsigen.2019.02.016). The Tor Vergata University of Rome, in Italy achieved accreditation including the use of ANDE Rapid DNA within an ISO17025 facility as well as expanding it to an outdoor setting (see attached: *Comparative Analysis of ANDE 6C Rapid DNA Analysis System and Traditional Methods, Genes 2020, 11, 582; doi:10.3390/genes11050582*).

The ANDE Rapid DNA System has demonstrated strong success with reference sampling, crime scene work and disaster victim identification (DVI) scenarios. All forensic samples can be analysed on ANDE, including Blood, Saliva, Semen, Hair, Tissue, Bone and Teeth. ANDE has tested multiple biological sample types on many matrices and has simple to follow procedures that all users can replicate. The recent peer reviewed developmental validations published on Crime Scene samples and DVI samples highlight the range and successes (see attached: *Identification of human remains using Rapid DNA analysis, International Journal of Legal Medicine, https://doi.org/10.1007/s00414-019-02186-y* and; *Developmental Validation of the ANDE 6C System for Rapid DNA Analysis of Forensic Casework and DVI Samples, J Forensic Sci, 2020 doi: 10.1111/1556-4029.14286)*

To operate the ANDE System, simply login to the instrument and select perform a run. Type in the name or scan the barcode on the swab. Proceed to scan the RFID in the top of that swab. Peel the blue cover off of the chip chamber of choice and place the swab into that chamber (the swab will lock in place). Proceed to load the remaining samples into the remaining chambers. Once all the samples have been loaded, open the door, insert the chip and close the door. The processing time will appear on the screen. The ANDE A-Chip can analyse 5 buccal swabs concurrently and takes ~94mins. The ANDE I-Chip can analyse 4 Crime/DVI samples concurrently and takes ~106mins. There are not multiple run protocols for the user to select, as each chip has its own RFID that communicates with the ANDE instrument automatically once loaded. When each swab is loaded into the chamber, they lock into place helping to prevent interference with the sample and protect the samples integrity. Each sample chamber has its own dedicated reagents and microfluidic channels which prevents contamination between samples and runs. There is no user maintenance required on the instrument, only a periodic maintenance once per year by an ANDE Service Engineer. The instrument can be powered on or off at any

time for use. If there is a requirement to move the instrument to a new location, there is no prerequisites other than turn it off. No parts or consumables require constant replacement, before or after a run.

ANDE uses a chemistry called FlexPlex™. It interrogates 27 loci: D1S1656, D2S1338, D2S441, D3S1358, D5S818, D6S1043, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, FGA, CSF1PO, Penta E, TH01, vWA, TPOX, SE33, DYS391, DYS576, DYS570, and Amelogenin. Further details can be found within our developmental validations on buccal samples, crime samples and DVI samples (see attached). Both the A and I Chip types have a 6-month shelf life and are room temperature stable (5-25°C). There is no requirement to refrigerate them.

ANDE FAIRS™ is a Windows-based application for the secure management of DNA IDs generated by the ANDE Rapid DNA Instrument as well as those federated from other sources. Following the import of DNA IDs from the ANDE Rapid DNA instrument, FAIRS allows the user to perform an immediate, one-button Search and Match against the database, returning actionable results. The user can then click through the summary results to access a printable PDF version of the Match results, including Match, Possible Match and No Match. ANDE's FAIRS data model is open to allow the federation of DNA IDs and Run Data with a multitude of sources. The sharing of data with other database instances is accomplished through the scheduled or ad-hoc export in industry standard formats. The import of DNA IDs from other sources is accomplished via industry standard formats, including CODIS Common Message Format (xml), Allele Table Format (csv or dat) or from other instances of FAIRS. A .fsa file and a .png file is available for each sample lane. A .xml file is available for each expert system passed lane, and a .csv file is available for all samples on a single run.

See attached:
ANDE Technical Specifications document

Developmental validation of the ANDE rapid DNA system with FlexPlex™ assay for arrestee and reference buccal swab processing and database searching,
https://doi.org/10.1016/j.fsigen.2019.02.016

Comparative Analysis of ANDE 6C Rapid DNA Analysis System and Traditional Methods,
Genes 2020, 11, 582; doi:10.3390/genes11050582

Identification of human remains using Rapid DNA analysis,
International Journal of Legal Medicine, https://doi.org/10.1007/s00414-019-02186-y

Developmental Validation of the ANDE 6C System for Rapid DNA Analysis of Forensic Casework and DVI Samples,
J Forensic Sci, 2020 doi: 10.1111/1556-4029.14286

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