Advancements and Foresight of Accelerated Nuclear DNA Equipment for Rapid DNA Analysis

ABSTRACT

The 6C Rapid Deoxyribonucleic Acid (DNA) Analysis System marks a pivotal advancement in forensic technology, facilitating rapid DNA profiling directly at crime scenes or other pertinent locations. This innovative system produces human DNA identifications compatible with global DNA databases in under two hours. Its automated design significantly reduces the complexity of analytical processes and the need for extensive user intervention in field-forward biometric and forensic applications. Thus, this short review highlights the recent advancements, advantages, and limitations of the 6C accelerated nuclear DNA equipment, particularly regarding its application in forensic casework. The swift turnaround time it offers allows law enforcement to make timely decisions, enabling the quick identification or exclusion of suspects, linking of cases, and prompt actions. Its portability and user-friendly interface enhance accessibility for non-specialists, thereby expanding its applicability across various operational scenarios. However, despite these strengths, the system faces challenges, including contamination risks, a limited capacity to process complex DNA mixtures, and a dependence on high-quality DNA samples, all of which can affect result accuracy and reliability. Additionally, the current costs and necessity for specialized consumables may hinder broader implementation, especially in resource-limited settings. Future developments in the 6C system should focus on improving sample processing, enhancing sensitivity, optimizing software capabilities, refining electropherogram outputs, and reducing costs to maximize its utility in forensic investigations.

Keywords: forensic DNA, electropherogram. ANDE 6C (Accelerated Nuclear DNA Equipment)

1. INTRODUCTION

1.1.Background

In recent decades, there has been a considerable increase in the application of Deoxyribonucleic Acid (DNA) analysis within criminal investigations and prosecutions, with this trend continually rising. This growing reliance on forensic evidence demands 'rapid' solutions, particularly during the investigative phase, where such evidence increasingly guides the investigation's direction and overall effectiveness [1]. The use of forensic DNA typing was initiated in investigative contexts in 1986, and since that time, there have been notable advancements in the speed and sensitivity of testing procedures. These improvements have solidified DNA analysis as the premier method in forensic science. Currently, Rapid DNA technology enables the completion of testing in less than two hours[2].

The impetus for developing Rapid DNA technologies stems from technological progress and a heightened demand for quicker and more efficient methods of DNA analysis[3, 4]. This innovative technology operates in a hands-free manner, generating a DNA profile from a cheek swab sample within a two-hour timeframe. It is particularly beneficial for the FBI, as it connects commercial instruments to the Combined DNA Index System (CODIS), facilitating rapid searches for unresolved cases and expediting the processing of arrestees[5]. Rapid DNA instruments are designed to execute the complete forensic DNA workflow while utilizing disposable consumables for short tandem repeats (STR) analysis. These instruments are compact and versatile, making them suitable for deployment in various settings, such as laboratories, police booking facilities, disaster areas, and border checkpoints. The most recent models are tailored for decentralized use outside traditional laboratory environments and can be operated by individuals without a scientific background[6]. In 2018, a technology maturity assessment evaluated three Rapid DNA instruments, including the ANDE 6C System developed by ANDE (Longmont), which has since been acquired by ThermoFisher Scientific[7].

The ANDE 6C system is a sophisticated Rapid DNA Identification tool that incorporates a fully automated instrument, single-use microfluidic chips, and a comprehensive Expert System analysis software. Two distinct types of chips are employed within this system: the I-Chips, which are designed for Unidentified Human Remains (UHR) samples, and the A-Chips, intended for Family Reference Samples (FRS) obtained from buccal swabs. Both chip types facilitate automated processes for DNA purification and the PCR amplification of 27

STR markers[7]. The ANDE 6C device is capable of analyzing these 27 loci through the FlexPlex27 Amplification kit (ANDE Corporation, Longmont, USA), which broadens the range of core STR loci that can be utilized. This analytical microchip offers several benefits over traditional technologies, including rapid execution times, enhanced portability of the instrument, reduced risk of contamination, and streamlined analysis processes [8]. The ANDE 6C system was used in tents strategically set up near mock disaster sites[7, 8]. Initially developed by Net Bio, the system integrates all essential steps, including DNA extraction, STR amplification, electrophoretic separation, and detection, completing the entire process within a mere 90 minutes[9].

The ANDE 6C Rapid DNA Act of 2017, enacted in the United States, facilitates the implementation of rapid DNA technology in police departments, pending approval from the National DNA Index System (NDIS). This technology allows for the rapid generation of DNA profiles, which can be automatically matched against the FBI's DNA database using the Rapid DNA Index System (RDI). Recently, advancements have led to the creation of fully automated rapid DNA systems, and the accreditation results concerning the ANDE 6C Rapid DNA Identification System for typing reference samples have been documented[10]. The FBI Laboratory has granted NDIS approval for accredited forensic DNA laboratories to utilize the ANDE 6C System [11]. The findings endorse the launch of a developmental validation study for the system, which will eventually be submitted to NDIS. The ANDE 6C system, along with its Expert System and A-Chip components, is capable of producing STR profiles that comply with the SWGDAM Validation Guidelines, addressing key factors such as reproducibility, sensitivity, accuracy, concordance, precision, resolution, peak height ratio, species specificity, handling of mixtures, inhibitors, stability, and contamination concerns[12].

In practical applications, the ANDE 6C Rapid DNA Analysis system has shown impressive results, successfully analyzing 97% of biological samples, regardless of the varying conditions encountered, while reliably generating accurate DNA profiles from reference samples[10]. When buccal samples were analyzed under precise conditions, they yielded 83-98% complete DNA profiles, contingent upon the accurate typing of allelic ladders[13].

2. ADVANCEMENT OF ANDE 6C SYSTEM

The initial system launched by ANDE was known as DNA Scan, which was later rebranded to ANDE 4C (4-color). This system was utilized to analyze reference samples, as well as samples derived from both controlled and uncontrolled environments[9] In 2016, it became the first and only Rapid DNA System to receive NDIS Approval[14]. The ANDE 6C instrument was developed to accommodate the detection of six different fluorescent dyes. The primary objectives of this internal study focused on the ANDE 6C system and the FlexPlex assay were twofold [12].

The ANDE 6C instrument represents an advanced iteration of the ANDE 4C. It is capable of performing STR analysis using either four or six fluorescent dye labels, features a 2-D barcode scanner for effective sample tracking, and incorporates enhanced ruggedness for operations in mobile and outdoor settings. This upgraded system allows for the detection of two additional dye colors, purple and orange, thus increasing the total number of detectable colors to six[6, 15].

The software associated with the ANDE 6C processes raw data, assigns allele designations, and applies specific rules to interpret DNA profiles. The Expert System software, crafted specifically for analyzing ANDE data, is fully integrated and requires no user intervention. Following the analysis, the ANDE Expert System produces several outputs: an allele table detailing all successful allele calls for each sample, a file (electropherogram) for quick result visualization, a file prepared for upload to the Combined DNA Index System (CODIS), and a file for review with traditional software packages[12, 16].

3. OVERVIEW OF ANDE 6C RAPID DNA ANALYSIS SYSTEM

The ANDE 6C Rapid DNA Analysis System consists of the ANDE instrument, ANDE chips, and ANDE swabs. The ANDE chip operates as a fully integrated lab-on-a-chip, capable of completing all stages necessary for STR analysis in under 90 minutes [8]. The forensic workflow within the chip is divided into three modules: Module 1 handles DNA purification, Module 2 performs STR amplification, and Module 3 is responsible for electrophoretic separation and detection[10].

The ANDE 6C System comes equipped with integrated software for controlling the instrument, collecting data, and interpreting STR profiles using the Expert System. Once the sample is loaded and the door is closed, the system begins processing automatically. The Expert System software processes and interprets the resulting data, offering quick feedback on the usability of the STR profiles. The output files are securely encrypted and can be examined by a qualified DNA analyst through the FAIRS software[4].

Table 1: components of ANDE 6C rapid DNA analysis system

Component	**Name**	**Version number**
Rapid DNA instrument	**ANDE 6C**	**A0120001003**
Typing kit	**Flex plex27**	**Flexplex27**
Cartridge	**ANDE A chip**	**A0210001057**
System software	**ANDE system software (FAIRS)**	**2.0.6**
System expert software	**Expert software**	**2.0.5**

3.1. WORKING PROCEDURE OVERVIEW OF ANDE 6C

The ANDE 6C Rapid DNA Analysis System is designed for fast and fully automated DNA profiling, providing an efficient workflow from sample collection to result generation. The process begins with the collection of a biological sample, such as a buccal swab, blood, or other tissue types. Once collected, the sample is loaded into a specialized cartridge that contains all the necessary reagents for DNA extraction, amplification, separation, and detection. This cartridge is then inserted into the ANDE 6C instrument, which is designed to

be user-friendly, requiring minimal training for operation. Once the sample is inserted, the ANDE 6C system performs a fully automated series of steps. It first lyses the cells in the sample to release DNA. The DNA is then amplified using polymerase chain reaction (PCR) with STR markers, which are highly variable regions of DNA used for identification. Following amplification, the DNA fragments are separated based on size using capillary electrophoresis, and the system detects and records the STR profiles. The ANDE 6C analyzes the detected STR profiles to generate a complete DNA profile, a process that takes approximately 90 minutes. The results are displayed on the system's interface and can be printed or exported as needed [17].

The ANDE 6C instrument is comprised of a set of subsystems, including a pneumatic subsystem for driving fluids throughout the chip, a thermal subsystem for performing multiplexed amplification, a high voltage subsystem for electrophoresis, a 6-color optical subsystem for exciting and detecting fluorescently labeled STR fragments during electrophoresis, and a ruggedization subsystem to allow transport and field forward operation without recalibration or optical realignment. The instrument-integrated touchscreen features a graphical user interface with workflow-driven instructions. After the A Chip containing swab samples has been inserted into the instrument and the door closed, sample processing starts automatically [12].

The A-Chip is a single-use, fully automated consumable designed for STR analysis, containing all the necessary reagents, materials, and waste containment for DNA processing. It includes pre-loaded DNA purification reagents, FlexPlex STR PCR reagents, buffers, and separation polymer, ensuring consistent and accurate results. Its closed system minimizes the risk of cross-contamination, and the FlexPlex component targets 23 autosomal STR loci, offering high precision[12, 14].

The ANDE 6C Swabs are equipped with radio frequency identification (RFID) tags and barcodes for traceability, reducing the chance of sample mix-ups during processing. The integration of RFID technology enhances the accuracy, security, and efficiency of DNA analysis. These swabs are designed to securely lock into the A-Chip, minimizing the possibility of cross-contamination. They also include a built-in desiccant, allowing them to

preserve various sample types such as buccal swabs, bloodstains, firearms evidence, and more, even during extended transport [18].

The Forensic Automated Identification and Retrieval System (FAIRS) is a Windows-based software for securely managing the DNA IDs generated by the ANDE Rapid DNA system[19]. FAIRS™ is the trademarked software that stores DNA profiles, passing internal quality checks with System Software version 2.0.6. It matches profiles, produces reports on matching outcomes, and indicates which loci matched or mismatched between pairs[20]. FAIRS also integrates functionality for database generation, profile search and match, and kinship determination[4]. The ANDE 6C Expert System Software, version 2.0.5, applies a set of rules to interpret DNA profiles. These rules evaluate alleles, loci, peaks, and other quality metrics, labeling failed or questionable data with caution symbols. After processing, profiles are classified as passed, failed, or needing further evaluation. The Expert System also identifies mixed DNA samples by flagging profiles that contain multiple contributors, marking these for analyst review if two or more loci with three alleles or one locus with four or more alleles are detected)[12].

4. PREVIOUS STUDIES CONDUCTED ON ANDE RAPID 6C INSTRUMENT

Numerous tests have been performed on the ANDE 6C Rapid DNA system to assess the accuracy of its expert system, reagent chip system, and overall performance in terms of concordance, repeatability, and reproducibility when compared to traditional DNA methods. Research by Ragazzo et al. found that only two of 2800 markers analyzed were inconsistent with the correct profile, and 97% of the 208 samples were evaluated correctly. Heterozygous markers exhibited acceptable peak height ratios [17]. Furthermore, testing under three different run conditions yielded no notable impact on the nine profiles generated[10].

A comparison study using 150 saliva and blood samples of varying substrates and concentrations showed that the ANDE 6C system performed comparably to the Rapid Human Identification Technology (HIT) instrument, producing full DNA profiles from as little as 5-10 ng of DNA. However, deviations from the manufacturer's suggested sample preparation procedures negatively affected profile generation success[3].

Early validation of the first ANDE device, referred to as the DNA Scan, was conducted in New York City with the assistance of eight laboratories, testing 2300 swabs from 1400 individuals. The study reported an allele call accuracy rate of 99.998%, demonstrating the

system’s precision, repeatability, and dependability in generating full DNA profiles, including the 13 core CODIS loci [17]. Carney et al., (2019) validated the ANDE 6C system across five laboratories, testing 2045 swabs from 1387 individuals with 13 devices. The study showed concordance among laboratories and 92% success in generating complete profiles for all 20 CODIS core loci during the first run[12, 14].

In field deployment studies, Hinton and colleagues tested the ANDE 6C system on controlled samples designed to mimic evidence from improvised explosive devices. Of 12 samples, seven yielded full single-source profiles, while two provided partial profiles. One sample lost power mid-run, preventing data recovery. Another study using 44 uncontrolled samples showed varied success: 25 yielded no profiles, nine provided partial profiles, three were mixtures, and seven yielded single-source profiles, showcasing the expert system’s capability to flag and identify mixture samples for further review[17, 21].

The readiness of the ANDE 6C for deployment in Disaster Victim Identification (DVI) scenarios has also been evaluated. The Australian Facility for Taphonomic Experimental Research and Watherson et al. (2022) tested the system on samples collected from cadavers during simulated mass disasters. The samples included diverse tissue types, such as bone marrow, teeth, and hair. While some samples produced partial or full profiles, others, particularly from DVI scenarios involving decomposition, did not yield results[13]. Similarly, studies following Hurricane Dorian in 2019 encountered difficulties with creating profiles from weather-exposed DVI samples, with only a subset of teeth and rib samples meeting the ANDE system's criteria for a complete run[22].

A study comparing rapid DNA techniques to traditional methods for generating profiles from compromised bone samples revealed that the ANDE 6C had lower sensitivity. Adjusting the bone input amount or extending incubation times did little to improve the profiles generated, indicating that the system's sensitivity is not on par with traditional DNA methods when dealing with such degraded materials[23]. Further research by Turingan et al. (2020a) examined DVI samples stored in different conditions, including refrigeration and outdoor exposure. Results showed that samples collected within 2-3 days after exposure generally produced complete profiles, while prolonged decomposition led to a decline in profile

quality. Of the bone samples tested over a one-year period, 83 out of 87 produced full DNA profiles, while the remainder failed due to chip malfunctions or poor sample quality[7, 8].

The practical application of the ANDE 6C in real-world DVI scenarios was demonstrated by Gin et al. during the 2018 California wildfires. The system analyzed 69 samples, generating 62 DNA profiles that were matched to 255 family reference samples, successfully identifying 58 victims. The majority of full profiles were obtained from dried blood and clots, while tissue and bone samples also contributed. A success rate of 95.2% for finding 20 or 19 CODIS loci was achieved despite the harsh environmental conditions[17].

5. ADVANTAGES OF ANDE 6C IN FORENSIC CASES

In December 2015, FBI Director James Comey proposed the deployment of ANDE 6C rapid DNA technology at booking stations across the U.S. This technology holds potential not only for criminal justice but also for humanitarian applications, such as reuniting families separated by mass migrations, combatting human trafficking, and helping authorities identify victims of mass casualties during natural disasters or conflicts. Since its introduction in 2015, ANDE 6C has assisted law enforcement in solving various crimes, including burglaries, sexual assaults, and violent offenses[24, 25].

The ANDE 6C system is frequently used to generate robust forensic DNA profiles, comprising up to 27 loci, which enables highly reliable and universally compatible identification. This system has been effective in reducing terrorism risks and has a proven history of success. Privacy concerns are addressed by ensuring that the DNA profiles exclude race, ethnicity, and genetic medical information, focusing only on the identification process. Since the method requires minimal personal data to verify identities, it is well-suited for use in government services, including criminal investigations, military operations, disaster victim identification, and other situations that demand rapid DNA analysis[19].

6. LIMITATION OF ANDE 6C

Despite the many benefits of rapid DNA analysis, several challenges persist in the field. Although the range of sample types compatible with current technology is increasing, some samples still require pre-processing, and not all types are fully supported. The yield rate referring to the proportion of samples that yield usable results is not yet at 100%. Furthermore, while rapid DNA analysis tends to be more affordable than traditional methods, it remains prohibitively expensive for some, particularly resource-constrained police departments. The ANDE 6C rapid DNA system has been lauded by lawmakers and law enforcement agencies for its potential to address the backlog of rape kits. However, its effectiveness in processing these kits could be limited by the extent of degradation in the samples, which may occur due to the age of the kits. Nevertheless, the system's ability to provide fast testing platforms at facilities and booking stations could allow other resources to focus on more complex forensic cases and help alleviate the rape kit backlog [20].

7. COMPARISON OF ANDE 6C WITH THE TRADITIONAL METHOD

Rapid DNA systems offer several advantages over traditional DNA testing, such as being more compact, quicker, and easier to operate. However, conventional DNA testing remains more cost-effective, sensitive, and capable of processing a wider variety of sample types[26] .

7.1 Sample Collection

Both rapid and conventional DNA methods require sample collection. Some rapid DNA instruments are optimized for use with specialized swabs, while others can use standard swabs [22, 27]. One particular system utilizes swabs with RFID-tagged lids that seal within the biochip to prevent sample swapping during the analysis. While these RFID swabs enhance the user experience with rapid DNA instruments, they increase costs and reduce the flexibility of transitioning samples between rapid and traditional DNA processes when needed[28].

7.2 Extraction

During extraction, the lysis buffer is combined with mechanical processes like heating and agitation, with the duration depending on the sample's DNA content. Samples with low DNA

content may require incubation for up to 24 hours, whereas high-content samples need significantly less time. After the lysis step, cellular components other than DNA may pass through the system. Due to differences in lysis and purification, traditional DNA extraction methods can often produce higher DNA yields compared to the ANDE 6C rapid DNA system[26, 29, 30].

7.3 Quantification

Traditional DNA testing includes a quantification step to adjust DNA concentration for optimal PCR amplification, but rapid DNA instruments skip this step, saving time by transferring samples directly to amplification after lysis. Without quantification, the DNA input cannot be adjusted, potentially affecting results. To compensate, rapid DNA instruments use two types of cartridges: one for high DNA quantities and one for low. Each cartridge is optimized to maximize DNA recovery and produce interpretable results. However, using too little DNA can result in partial or incomplete genetic profiles[26].

7.4 PCR Amplification

Both traditional DNA analysis and the ANDE 6C rapid DNA system utilize similar or identical PCR amplification chemistries, depending on the platform in use. These methods typically target a standardized set of loci, including the 20 core CODIS loci established by the FBI, along with additional loci. As a result, DNA profiles obtained through rapid and traditional DNA processing methods can be directly compared. The amplification chemistries employed in these systems have received FBI NDIS (National DNA Index System) approval in the United States. This approval allows reference DNA profiles produced with these chemistries to be uploaded into the CODIS database, provided that they comply with all FBI standards and guidelines[31, 32].

7.5 Electrophoresis

DNA separation and identification in both rapid and conventional DNA procedures are carried out using a process called electrophoretic separation. However, the ANDE 6C system's approach to electrophoresis differs somewhat from that of other DNA platforms. In the ANDE 6C, single-use electrophoretic separation channels are pre-molded and injected directly into the cassette, ensuring a new capillary is employed for each run. Since there is no mechanism available to confirm the quality of the capillaries before use, operators must rely on the manufacturer's quality control systems to ensure proper functionality[30].

7.6 Data Interpretation

The ANDE 6C system handles data interpretation through predetermined rules similar to those used in traditional DNA analysis. It employs expert system software, specifically the FAIRS software, to automate data analysis. This automation allows non-expert operators to run the rapid DNA system efficiently. The system uses defined interpretation criteria and quality flags to identify DNA profiles requiring further review. For samples that meet these criteria, no manual review is needed[33].

However, when dealing with low-level DNA samples or mixtures from multiple DNA donors, more significant differences can arise in the data interpretation algorithms[34]. For these types of complex samples, the ANDE 6C Expert System is designed to detect and highlight profiles that suggest the presence of DNA from two or more contributors, helping ensure the accuracy and reliability of the results[30].

8. FUTURE PERSPECTIVE OF ANDE 6C RAPID

In the future, empirical research will be crucial for identifying key factors such as the optimal sample size, appropriate protocols for quick preparation, and effective reagent interactions, all of which will contribute to the establishment of standardized procedures for rapid sample collection and DNA analysis. The choice of platform will largely depend on its intended application and operational setting. Although rapid DNA systems have already significantly influenced forensic science and law enforcement, further advancements are needed to close existing performance gaps and overcome barriers to wider adoption[13].

For example, the ANDE 6C system, designed for crime scene use, was the focus of a joint statement by ENFSI, SWGDAM, and the Rapid DNA Crime Scene Technology Advancement Task Group, as discussed in a July 2020 letter to Forensic Science International: Genetics. This letter highlighted five areas where improvements in rapid DNA systems are essential: the integration of human-specific controls, fulfilling quantification requirements, providing exportable raw data, embedding an automated expert system, and enhancing peak height ratio balance for low-level and mixed samples[34, 35].

Moreover, addressing the need for increased sensitivity in rapid DNA systems is critical for better detecting trace DNA samples. Once these advancements are fully developed and

validated, rapid DNA technologies could offer performance comparable to that of conventional laboratory methods[1, 34, 36].

9. CONCLUSION

The ANDE 6C Rapid DNA System has shown remarkable promise in generating DNA profiles across various studies and applications, notably in Disaster Victim Identification (DVI) scenarios. Its ability to produce full DNA profiles comparable to traditional methods has been validated through numerous laboratory tests and real-world deployments, such as during the 2018 California wildfires, which resulted in a high success rate for victim identification. However, challenges persist, particularly regarding its sensitivity to degraded samples, where its performance lags behind that of conventional DNA methods. Moreover, the efficacy of the ANDE 6C is influenced by adherence to proper sample preparation protocols, as deviations can lead to a significant drop in profile generation success. Despite these limitations, the ANDE 6C stands out as an innovative tool for rapid DNA analysis, especially in urgent identification contexts. With its versatility, it can accommodate a wide range of sample types and is designed to be user-friendly, making it accessible even for law enforcement personnel with limited DNA expertise. The system's portability is another strength; it can be set up in various locations, including police stations, crime scenes, vehicles, booking stations, prisons, medical examiner offices, hospitals, sexual assault kit collection centers, mass casualty sites, borders, ports, and embassies. Impressively, the ANDE 6C can generate a DNA profile from five samples within two hours, even under standard office temperature conditions. However, it is essential to note that Rapid DNA systems, including the ANDE 6C, still exhibit lower sensitivity compared to lab-based methods, particularly when handling degraded samples. This underscores the need for ongoing research and development to enhance yield rates and sensitivity. Additionally, developing standardized procedures and expanding the range of compatible sample types will be crucial for the broader adoption of the ANDE 6C in forensic applications and disaster victim identification scenarios.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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