SPECIAL ARTICLE

A Retrospective Look on the Use of DNA Evidence in a Sexual Assault Investigation in the Philippines

Gayvelline C. Calacal, RMT, MSc, Frederick C. Delfin, MSc and Maria Corazon A. De Ungria, PhD

DNA Analysis Laboratory, Natural Sciences Research Institute, University of the Philippines Diliman

ABSTRACT

In 1998, biological samples were collected from the body of a 17-year old female rape-homicide victim within 24 hours post-contact. In the absence of a sexual assault investigation kit, locally available medical supplies were used to collect biological samples. The victim's family filed a case naming the victim's uncle as the assailant. More than a year into the trial, samples from the victim and the accused were tested for DNA. The vaginal smears yielded DNA profiles originating from at least two persons, with one DNA source being male. Upon discovery, the victim's age, the state of her body, and medicolegal examination results supported the allegation of sexual assault rather than consensual sex. This paper described the DNA testing conducted for this rape with homicide case. The prosecution used the DNA test results to support the charges against the accused, who was eventually convicted and sentenced to death in 2001. Upon automatic review in 2004, the Philippine Supreme Court affirmed the conviction and dismissed the defense's claim that DNA testing violated the defendant's right against self-incrimination. The defendant's death conviction was commuted to life imprisonment when the Death Penalty was suspended via Republic Act No. 9346 in 2006. The case described here is considered one of the DNA landmark cases cited in the Philippine Rule on DNA Evidence of 2007.

Keywords: sexual assault, DNA evidence, Short Tandem Repeats, criminal investigation, Philippines

INTRODUCTION

DNA analysis is a reliable scientific procedure used for forensic human identification in sexual assault investigations. Human genomic variations are used to differentiate individuals and identify the source of a biological sample.[1] Since DNA is the same in every cell of the body, identification is possible by comparing the DNA from a biological sample left at a crime scene and the reference DNA profile generated from a known source. Further, DNA is chemically stable under a wide range of conditions, giving time for its recovery, even from aged biological samples.

In sexual assault investigations, biological samples recovered from the victim's body usually contain the assailant's DNA in the transfer of biological material. Any sexual contact, consensual or not, would result in biological material, e.g., skin cells, blood, saliva, or semen, between the persons involved. Thus, the likelihood of recovering biological material in sexual assault cases is greater compared to other crimes. The presence of male DNA increases the value of the medical examination of a female victim's body. Therefore, medical doctors should collect biological samples using a sexual assault investigation kit as soon as possible or up to five days post-contact.[2-3]

In the Philippines, the majority of convictions in sexual assault cases rely on testimonial evidence. The reliance on testimonies that may not be supported by physical evidence

Corresponding author: Maria Corazon A. De Ungria, PhD
DNA Analysis Laboratory
Natural Sciences Research Institute
University of the Philippines Diliman
Quezon City 1101, Philippines
Email: madeungria@up.edu.ph

leads to prolonged litigation in already overburdened courts, further slowing down the judicial process. The subjective nature of testimonies contributes to the high rate of wrongful convictions, including death convictions. In 2004, the Philippine Supreme Court reported 71.77% of the death penalty cases it reviewed from 1993 to 2004 were modified or vacated, translating to 651 out of 907 appellants saved from death.[4]

Before forensic DNA testing started in the Philippines in 1997, microscopic examination of vaginal smears was the usual procedure to confirm sexual contact between a female victim and a male perpetrator. However, negative microscopy findings may result from the assailant's use of a condom, his inability to produce sperm (azoospermia), or ejaculate at the time of contact. Unlike microscopy, forensic DNA technology provides a more robust tool in sexual assault investigations for as long as samples are collected, handled, and stored correctly.[5] Even in the absence of sperm cells, the epithelial cells in semen are an excellent source of male DNA that can identify its human origin. The use of a sexual assault investigation kit (SAI.Kit) that guides the doctor step by step during the victim's medical examination is recommended.[3] After sample collection and the chain of custody of samples documented, all of these, including associated records, should be securely stored. This chain of custody contributes towards sample preservation and fulfills one requirement for the admissibility of DNA evidence in court.

DNA profile/s generated from the victim and crime scene samples must then be compared to an accused's reference profile. If the accused's DNA profile is consistent with the DNA profiles generated from the evidence, then the accused is identified as a potential source. Statistical analysis is conducted to assess the weight of the DNA evidence using relevant population databases.[1] Conversely, the non-matching DNA profiles observed can exclude an accused as a possible source for the DNA profile/s, but this exclusion does not automatically mean that he was not involved in the crime.

This paper reports one of the first cases of sexual assault that used DNA evidence in the Philippines. The protocols used in handling the biological material and the analysis of the genetic profile results are presented to show the critical contribution of DNA evidence towards the resolution of sexual assault cases in Philippine courts.

CASE

Case Background

On the evening of 30 June 1998, the victim's grandmother found her lifeless, cold and naked body sprawled on the 2nd floor of their house. The body was immediately brought to the Rural Health Unit (RHU) for medicolegal examination while investigators processed the crime scene. The victim's undergarments, denim pants, blouse, sandals, and bag were recovered. The investigators also found a dirty, blood-stained white shirt within 50 meters of the house. Eyewitness accounts revealed that the only person near the grandmother's house that day was the victim's uncle by affinity. He lived within a 100-meter radius of the grandmother's house. At about 12:30 pm that day, the suspect came out of the grandmother's house wearing a white shirt with a collar. Sometime later, the suspect was seen wearing a black shirt instead. The victim's cousin also informed the police that the victim had told her of an incident involving the suspect. Apparently, on 25 June 1998, the suspect tried to force the victim to have sex with him, but she managed to reject his advances.

Postmortem Examination and Findings

On 1 July 1998, a medical doctor of the RHU examined the victim's corpse. By this time, the body was already in *rigor mortis*. The victim died from multiple stab wounds. Records showed a total of 11 wounds with five incisions causing the victim's small intestine to spill out of the body, and the estimated time of death to be between nine (9) to twelve (12) hours before the examination. There was no hymenal laceration, contusion, and hematoma in the vaginal cavity. The victim had bruises and swelling on her right forearm. The attending physician noticed fluids in the vaginal cavity and collected this sample by flushing the area with five milliliters (mL) of saline solution (0.9% sodium chloride). The wash solution was aspirated with a sterile syringe, placed in a clean test tube, and centrifuged to concentrate the cells. The cell pellet was used to prepare four vaginal smears- only two of which were Gram-stained. Under microscopy, sperm cells were detected in the two stained slides. The two stained slides were submitted to the Regional Trial Court together with a third unstained slide. Because he was the first doctor to examine the body soon after recovery from the crime scene, the medical doctor opted to keep the fourth unstained slide in the RHU. Before handling this case, the doctor attended a medicolegal training program organized by the Department of Pathology, College of Medicine in UP Manila. He realized the value of the samples he collected and wanted to ensure a backup in case samples were misplaced during the investigation.

On 3 July 1998, a medicolegal officer of a provincial office of the National Bureau of Investigation (NBI) performed a second examination. This time, the examination was done on the embalmed body of the victim. He collected vaginal, anal, and oral samples, which he later sent to NBI Manila for microscopic examination. The victim's blouse and the suspect's fingernail scrapings were also turned over to NBI-Manila to test for the presence of human blood and to identify the blood group classification. NBI-Manila released negative microscopy results for human spermatozoa for all samples collected from the victim's body by the NBI provincial doctor. The suspect's fingernail scrapings yielded negative serological results. The blood on the victim's blouse was type "B," consistent with the victim's known blood type.

DNA Analysis and Findings

In November 1999, the Commission on Human Rights Regional Office requested the DNA Analysis Laboratory, Natural Sciences Research Institute in UP Diliman (NSRI-UPD DNA Analysis Laboratory) to conduct DNA tests to help resolve the case. By January 2000, the NSRI-UPD DNA Analysis Laboratory had coordinated with the provincial prosecutor's office to release three vaginal smears submitted by the medical doctor from the RHU. In addition, the laboratory had directly contacted the medical doctor of the same unit to obtain the remaining unstained vaginal smear under his custody. The provincial NBI office did not have the second set of vaginal smears because these were already turned over to NBI Manila.

Meanwhile, in Manila, the laboratory coordinated with NBI to release the victim's blouse, soaked with her blood. Based on the autopsy report, the victim died of multiple stab wounds.

All samples and material were collected from these agencies following proper chain of custody procedures. During the transfer, individual samples were placed in separate clean and properly labeled paper envelopes. The persons who turned over the samples put their signatures, the time, and the date over the envelopes' seal. The receiver countersigned these seals, then transported the samples to the NSRI-UPD DNA Analysis Laboratory for DNA testing.

An organic extraction method was used to extract DNA from samples collected from the victim, e.g., four vaginal smears, bloodstain from her blouse, and buccal samples from her living parents following specific procedures sourced from the US Federal Bureau of Investigation.[6] For each DNA extraction, a tube containing only the reagents used, the so-called reagent blank or negative control, was simultaneously analyzed along with case samples. A modified two-step differential organic technique separated the male and female fractions for the four vaginal smears.[6] Briefly, the smears were resuspended in lysis buffer for 30 minutes at room temperature with occasional pipetting. The lysates were collected in a tube then incubated at 37˚C and 70˚C for one hour each. The female fraction was then collected and placed in a separate tube. A second lysis step was performed by adding dithiothreitol (DTT) to the buffer to lyse the sperm cells, followed by overnight incubation at 56˚C. Both female and male fractions were purified using organic reagents, and the final volume was reduced using a micro concentrator (Millipore). The parents' DNA was used to confirm the victim's reference DNA profile generated from genotyping her bloodied blouse.

After submitting the DNA test results from analyzing the crime scene samples to the trial court, the defendant was ordered to provide blood samples for DNA testing and comparisons. The defendant's blood was collected directly onto an FTA™ card, dried, and purified following the manufacturer's instructions (Flinders Technology Associates, Fitzco Inc).

DNA amplification and fragment analyses were done using single reactions at seven autosomal STR (aSTR) markers and the human amelogenin gene or HUMAMEL (human Amelogenin) for sex-determination following published procedures.[7-8] Undiluted, 1:2, and 1:10 dilutions of DNA extracts were PCR-amplified to generate DNA profiles. The positive DNA control consisting of commercial DNA with known genotype was analyzed simultaneously with the case samples to indicate that all reagents and equipment worked. In contrast, a separate tube that did not have any DNA or the so-called negative DNA control was included. This control will alert the analysts to the presence of contaminants during the amplification process. DNA test results were analyzed, and a Random Match Probability (RMP) was calculated using a local population database.

A schematic diagram of the DNA analysis conducted is shown in Figure 1.

Only partial DNA profiles were generated because of the extended period and storage conditions from sample collection to DNA testing. A summary of the results is presented in Table 1.

Table 1. DNA test results of samples submitted as part of the investigation

Sample Source	Sample type (# of sample/s or sites extracted)	RTC code	Results of DNA Analysis
Victim	Vaginal smear, unstained	* Item #1	Presence of Y-DNA; mixed profile at 5 STR DNA markers in the male fraction
	Vaginal smear, stained	"H"	Presence of Y-DNA; mixed 2-person profile at 5 STR-DNA markers
	Vaginal smear, stained	"I"	Presence of Y-DNA; mixed 2-person profile at 5 STR-DNA markers
	Vaginal smear, unstained	"J"	Presence of Y- DNA; mixed 2-person profile at 5 STR-DNA markers; male fraction yielded genotypes consistent with the accused
	Blouse, (2 cutting sites)	"B"	DNA profile at eight DNA markers; no Y-DNA was detected
Victim's parents	Buccal swab, (2 swabs)	"XX"	Single-source profile at eight DNA markers for each buccal swab; specific code provided per sample was specific for her mother or her father
Accused	Blood on FTA, 2 x (2.0 mm) blood discs	"Z"	Single-source profile at eight DNA markers

*FTA™: Flinders Technology Associates; *Item #1: not submitted to the Regional Trial Court during the testimony of the medical doctor*

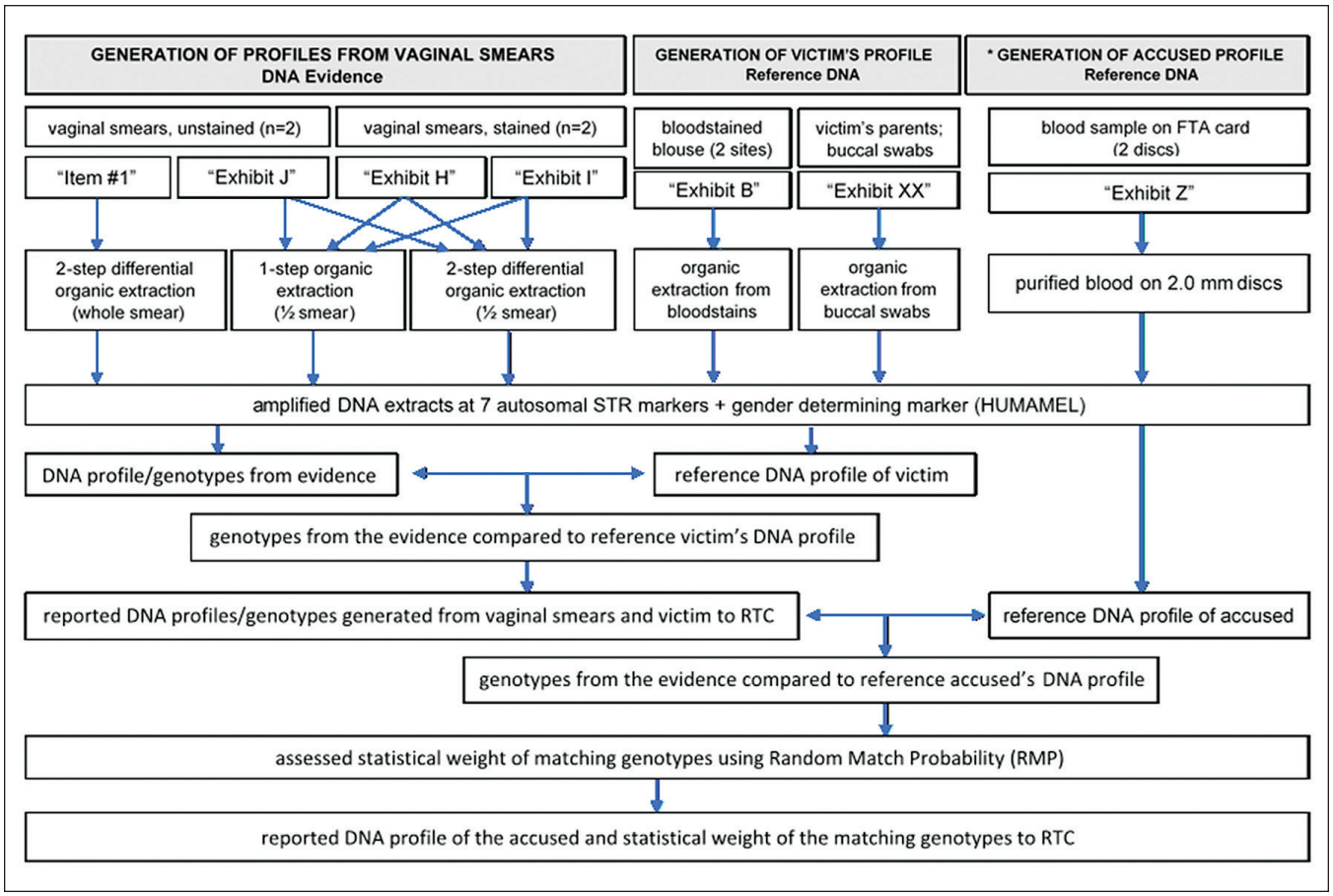

Figure 1. Schematic diagram of the DNA analysis conducted on evidence and reference samples.

**The DNA profiles from the vaginal smears and victim were generated and reported to the RTC before generating the accused's DNA profile.*

Due to the extended storage of the four vaginal smears that increased the extent of degradation, the individual results were collated and used to generate a composite genotype. The four DNA markers, HUMvWA, HUMDHFRP2, HUMTH01, and HUMCSF1PO, together with the human sex-determining marker human amelogenin, were successfully amplified to generate profiles of at least a two-person DNA mixture. The Random Match Probability (RMP) calculated using a local database at the time was 3 in 1,000 persons.

Court Decision

On 27 August 2001, the Regional Trial Court found the accused guilty of rape with homicide and sentenced him to death. Upon automatic review, the Philippine Supreme Court affirmed the death conviction on 19 May 2004. The Supreme Court recognized the value of all the testimonial and physical evidence, including DNA presented, 'in forming an unbroken chain that leads to a fair and reasonable conclusion that the accused, to the exclusion of others, is the perpetrator of the crime."[9] The defendant's death conviction was commuted to life imprisonment when the Death Penalty was suspended via Republic Act No. 9346 in 2006.[10]

DISCUSSION

The case reported here is one of the DNA landmark cases in the Philippines. The case took six years, from the investigation to litigation at the Regional Trial Court, and review by the Supreme Court. This period is relatively short compared to other rape with homicide cases, which can be on trial for more than ten years before a resolution or final conviction is reached. Critical factors that contributed to case resolution include 1) the immediate recovery of samples from the crime scene and victim's body; 2) quick collection of testimonial evidence within 48 hours post-crime; 3) the conduct of medicolegal examination on the victim's body soon after recovery and before embalming, and 4) the availability of DNA evidence that was consistent with all the other pieces of evidence presented in court.

Firstly, witness testimonies provided critical information that helped the investigators reconstruct the events leading to the crime. Only the suspect was seen close to the crime scene, had access to the house where the victim was found and expressed any motive to harm the victim. Also, unlike many reported rapes with homicide cases, the victim's body

was brought to the RHU and not a mortuary for medicolegal examination before it was embalmed. Autopsies could provide critical leads to determine the cause of death and any other violations on the victim's person, which in this case, was caused by stabbing. The bruising and swelling of the right forearm indicated the victim's resistance to someone before her death. These observations were consistent with the allegation that the victim was raped then killed to prevent her from identifying her assailant.

The collection of biological fluids in the victim's vaginal cavity during the first medicolegal examination also provided critical evidence in this case. Sperm cells were detected on the vaginal smears under microscopy. This observation was confirmed using DNA tests that showed that Y-chromosomal DNA was in the internal genitalia of the victim immediately before her death (Table 1). The positive detection of sperm cells in the first set of vaginal smears that the RHU doctor prepared, but not in the second set from the NBI provincial branch medicolegal officer, highlights the absolute requirement for medical examination before embalming. The embalming process includes washing the body externally, draining internal body fluid, and its replacement using chemical preservatives. A lot of biological samples that could be tested chemically and genotyped are lost in the process.

Also, all four slides contained DNA profiles from at least two persons- the perpetrator's DNA recovered from sperm and epithelial cells and the victim's DNA from the epithelial cells lining the vaginal cavity.

Firstly, the victim's reference DNA profile was generated using the blood from her blouse immediately recovered at the crime scene. Reverse parentage testing using samples collected from the victim's parents then confirmed the victim's DNA profile.

Meanwhile, the accused's reference DNA profile was generated from his blood collected following the court's order. The accused's and the victim's DNA profiles at five Short Tandem Repeat (STR) DNA markers were consistent with those from the vaginal smears. Moreover, the male DNA profile in the male fraction of the unstained slide submitted to the trial court (Exhibit "J") was consistent with the accused.

Statistical analysis of matching DNA profiles resulted in a Random Match Probability (RMP) equal to 3 in 1,000 individuals. RMP is a statistical estimate of how likely the DNA in the evidence sample matches the DNA of a random person in the population. Thus, RMP is used to assess how rare or common the matching DNA profile is, based on the genotype frequency estimates of a particular population group.[1] Another approach is to report the Likelihood Ratio (LR), which measures the possibility that the accused is the source versus the likelihood that another random individual in a relevant population is the source of the DNA in the evidence.[1] The use of these statistical approaches in assigning the value of genetic evidence varies among forensic laboratories.

At that time, the RMP value was relatively low because of the limited number of autosomal DNA markers that were successfully tested and the adjustments that were done to reduce statistical bias. Also, forensic laboratories used single DNA marker reactions that required more DNA, took longer to run, and were less robust than the current genotyping methods using multiplex DNA reactions and capillary gel electrophoresis.

Finally, to discredit the DNA evidence, the defense argued that the DNA test conducted violated the accused's right to remain silent and his right against self-incrimination. However, the Philippine Supreme Court ruled that collecting biological samples for DNA testing does not violate a person's right against self-incrimination. The Philippine Supreme Court upheld the lower court's decision because 'the essence of this right is against testimonial compulsion and not all compulsion while the right against self-incrimination is simply against the legal process of obtaining from the lips of the accused an admission of guilt."[9] The defense's argument was categorically dismissed, thereby preventing other counsels from using this reason to question the admissibility of DNA testing in other cases. The Supreme Court decision on this case became part of the Rule on DNA Evidence that was eventually promulgated in 2007 to govern its use in Philippine courts.[11]

Limitations of the study

The study was limited by the existing DNA technology that was available in the Philippines at that time.[7] The RMP equal to 3 in 1,000 individuals was not excessively high. The defense could have argued that other persons in the community may have the same five aSTR-DNA profiles. However, the other prosecution evidence corroborated the DNA test results. The court's decision to consider all evidence highlights the value of a multidisciplinary approach to criminal investigations and litigation.

CONCLUSION AND RECOMMENDATIONS

More than twenty years had passed since that day in June when a young victim's lifeless body was found in her grandmother's home in one of the Philippines' rural communities. Since then, many rape with homicide cases have been prosecuted in Philippine courts. Still, the lessons learned from this case have not been completely integrated into a specific protocol for routine sexual assault investigations. Law enforcement must recognize the value of reasonable crime scene investigation, including the recovery of crime scene evidence and timely collection of detailed testimonies, proper evidence handling following a documented chain of custody procedure, the conduct of autopsy and medicolegal examination before embalming, the availability of sexual assault investigation kits to aid in the systematic collection of biological samples from victims and the practical use of DNA evidence at trial. Currently, forensic DNA technology has

significantly advanced with the help of evidence collection kits, more sensitive and robust DNA typing procedures, and improved evidence interpretation guidelines. This case also highlights the need to identify more forensically relevant DNA markers to select the most informative and cost-effective sets that would maximize the use of our limited forensic resources. Moreover, continuous research and expansion of the Philippine forensic DNA database are needed to optimize forensic DNA technology for the fair and swift administration of justice in the Philippines.

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Statement of Authorship

All authors contributed in the conceptualization; analysis, design; data curation and analysis; writing and editing the manuscript, and approved the final version submitted.

Author Disclosure

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REFERENCES

1. Butler JM. Fundamentals of Forensic DNA Typing. Academic Press; 2009.
2. National Institute of Justice. National Best Practices for Sexual Assault Kits: A Multidisciplinary Approach. Washington DC: National Institute of Justice; 2017.
3. De Ungria MCA, Dalet MRM, Sagum MS, Tan M, Madrid BJ. The Development of a Local Sexual Assault Kit: the Philippine Experience. (this issue).
4. Philippine Supreme Court. People of the Philippines v. Mateo, G.R. No. 147678-87. 7 July 2004.
5. Rodriguez JJRB, Laude RP, De Ungria MC. An Integrated System for Forensic DNA Testing of Sexual Assault Cases in the Philippines. Forensic Science International: Synergy. 2021. 1 January; 3:100133.
6. Federal Bureau of Investigation Crime Laboratory. PCR-based Typing Protocols: Laboratory Manual. 1996.
7. Halos SC, Chu JY, Ferreon AC, Magno MM. Philippine Population Database at Nine Microsatellite Loci for Forensic and Paternity Applications. Forensic Science International. 1999 Apr 12;101(1): 27-32.
8. De Ungria MCA, Frani AM, Tabbada KA, Magno MM, Delfin F, Calacal GC, Halos SC. The Philippine Genetic Database of Short Tandem Repeats (STR) in DNA-based Paternity Testing. Philippine Journal of Science. 2002 Jun;131(1):1-8.
9. Philippine Supreme Court. People of the Philippines v. Yatar, G.R. No 150224. 19 May 2004.
10. Republic of the Philippines. An Act Prohibiting the Imposition of Death Penalty in the Philippines. RA 9346. 24 June 2006.
11. Philippine Supreme Court. Rule on DNA Evidence. Manila: 2007.