An integrated system for forensic DNA testing of sexual assault cases in the Philippines

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ABSTRACT

In the Philippines, more than 7000 cases of sexual assault are reported annually. DNA technology is a powerful tool in identifying assailants. However, it is not routinely used in sexual assault investigations due to insufficient government support to cover the high cost of DNA testing and the absence of a national system for sample collection, handling, storage, and DNA testing of biological evidence. In itself, the nature of sexual assault samples containing DNA mixtures presents challenges to laboratory methods and interpretation of results. The sample recovered from the victim may only contain trace amounts of the assailant's DNA, may have degraded due to prolonged storage in ambient conditions which is warm and humid in the tropics, or contaminated with inhibitors, such as in anal swabs. Hence, a closer evaluation of the processes of evidence collection and DNA testing is needed to increase the likelihood of success in generating conclusive results. In this paper, we propose an integrated system for DNA testing of biological samples collected from sexual assault victims considering the limitations of resources and the prevailing warm climate. Recommendations in this work should provide basis for formulating national guidelines for DNA analysis in aid of criminal investigations. The proposed scheme can be adopted by forensic DNA laboratories in the Philippines and in other countries facing similar challenges.

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1. Introduction

More than 7000 cases of sexual assault are reported yearly in the Philippines. Hospital-based Women and Child Protection Units (WCPUs) provide medicolegal and psychosocial services to victims of abuse. To date, there are 113 WPCUs in 57 provinces and 10 independent cities all over the country [1]. Trained examiners in the WCPUs collect specimens from patients with report of sexual contact within the last five days using a prototype sexual assault investigation kit (SAI.Kit) for potential submission for DNA testing.

The Philippine Rule on DNA Evidence outlines the requirements for the issuance of DNA testing orders including post-conviction tests, the protocols for the presentation of DNA evidence at trial, and the subsequent interpretation of results in relation to DNA-based parentage tests [2]. For victims who filed their cases in court and from whom samples were collected, the judge would

order their SAI.Kits in the WCPUs or other such facilities to be transferred to a forensic DNA laboratory. Further, suspect/s will be ordered by the regional trial court judge to submit reference sample/s, following the Supreme Court's ruling that the submission of a reference sample does not violate a suspect's right against self-incrimination (People v. Yatar, G.R. No. 150224). In the Philippines that does not prescribe to a jury system, the judge is the sole trier of fact that decides on the verdict for any given case.

The Philippines currently has three government institutions that house laboratories conducting DNA tests in aid of criminal investigations. These are the Philippine National Police (PNP), with its crime laboratories in its national headquarters and regional offices in Cebu City and Davao City, the National Bureau of Investigation (NBI), and the University of the Philippines through the DNA Analysis Laboratory (UP-DAL). The majority of criminal cases are handled by the PNP and NBI laboratories. The UP-DAL primarily conducts research in forensic genetics while also providing DNA test services when ordered by courts to assist in criminal and civil cases.

DNA technology has been in the country for more than two decades. However, the criminal justice system has not maximized

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its use due to inadequate government support to pay for the high cost of DNA testing and the absence of a national system for routine sample collection, processing, and analysis of evidence. Notably, PNP and NBI require a DNA testing order from the courts prior to accepting SAI.Kits from WCPUs and proceeding with the analysis. Victims and their families are often too burdened to bring their cases to court. Reasons such as the stigma attached to sexual abuse victims, the victim's relationship to the abuser, the stress of repeated appearances in court, and the expensive fees for DNA tests and paperwork for the prosecution, often discourage them from filing their cases. Thus, hundreds of stored kits remain untested [3]. Without an institutional source of funding to manage and store these kits until such time when a case is filed and a DNA testing order is issued, the WCPUs are increasingly becoming selective in the collection of samples from victims. Discussions on the possible disposal of old and untested kits have been on-going because of the need to accommodate new case samples given the limited storage spaces in hospitals that house WCPUs. Even in cases where DNA testing is pursued, the country's geography and climate pose challenges such as the logistics of transport from the WCPUs in remote provinces to forensic DNA laboratories in urban centers and the prevailing warm and humid conditions which accelerate the degradation of samples.

In this work, we put forward a system for DNA testing in sexual assault investigations from sample collection to interpretation of DNA evidence. Procedures were selected to maximize the information generated from diverse types of evidence samples considering local challenges in climate and limited resources. This paper outlines recommendations for laboratory procedures which should form the basis for national guidelines for the inclusion of DNA tests in sexual assault investigations in the Philippines. The proposed scheme can be adopted by forensic DNA laboratories in other countries facing similar challenges.

2. Incidence of sexual assault in the Philippines

The Philippine National Police recorded 7579 cases of sexual assault in 2018 [4] (Fig. 1). The National Capital and CALABARZON regions reported the highest numbers at 1072 and 1036 cases, respectively. However, adjusting for the projected population size [5], the Cagayan Valley and MIMAROPA regions showed the highest figures each at about 95 reported cases per million people. The Bangsamoro Autonomous Region of Muslim Mindanao (BARMM) had the lowest incidence at 33 cases or eight per million people. During the same year, WCPUs reported 8162 cases of sexual abuse where 6858 (84.02%) involved victims who were under 18 years old and were considered minors under Philippine law. Among these minors, 13 to 15-year olds were the most victimized [6] (Fig. 2). The higher proportion of cases involving minors compared to adults are due to preference of families to consult WCPU physicians who are trained to handle child sexual abuse patients. Moreover, these statistics are likely underestimates given the diversity of sociocultural norms in Philippine regions influencing help-seeking behavior of victims as well as the variable access to law enforcement and treatment facilities.

In the 2018 WCPU report, 289 (4%) of the victims were males. Notably, in a 2015 nationwide epidemiological survey [7] of 3866 Filipino children and youth aged 13−24 years old, more males than females have experienced some form of sexual violence (24.7% v. 18.2%) including forced oral or anal/vaginal sex (4.1% v. 2.3%). While it is known that male victims are far less likely to report the abuse than females [8], the high number of male victims in the survey warrants consideration of samples obtained from male victims in the workflow for DNA testing of biological samples in sexual investigations in the Philippines.

3. An integrated system for DNA testing in sexual assault cases

DNA typing is useful in sexual assault investigations due to the likely presence of the assailant's semen on the victim's body or clothing. The entire process is nonetheless challenging. Sufficient high-quality DNA from the assailant is often difficult to recover especially with the delayed collection of samples from the victim. When samples have been collected, the ambient warm and humid conditions may accelerate DNA degradation and compromise specimen quality when not stored properly. In addition, the mixture resulting from the commingling of the victim's DNA and that of the assailant/s is often difficult and subjective to interpret with too much reliance on human judgment. Local forensic DNA laboratories should therefore adopt robust and reliable methods in DNA testing to generate results that are admissible in court.

Briefly, the standard workflow for a DNA test typically proceed through a series of steps [9] while maintaining a chain of custody throughout the process. Specimens submitted to the laboratory undergo presumptive and confirmatory tests for semen. DNA is extracted, and the amount of human DNA is measured. Specific DNA regions, more commonly 20 or more autosomal and Y-chromosomal short tandem repeats (STRs), are targeted and amplified via polymerase chain reaction (PCR). Amplified fragments are then separated and detected through capillary electrophoresis (CE). Typically, the analyst would manually review peaks appearing in the electropherogram (epg) generated by a data analysis software. The weight of the evidence from the resulting profile, which could be from a single source or a DNA mixture, is statistically interpreted using a software system.

In our previous work [10−12], we selected procedures that are already established in forensic practice and are relatively inexpensive considering the limited resources of Philippine laboratories. We evaluated their use in male-female and male-male postcoital samples. Based on our observations, a workflow for DNA testing which maximizes information obtained from sexual assault samples is here proposed (Fig. 3). Philippine laboratories are encouraged to adopt the workflow to suit their particular settings and to conduct in-house validations following internationally recommended guidelines [13].

3.1. Sample collection, storage, and characterization

Reference samples, typically blood or buccal swabs from the victim, consensual partner/s, and suspect/s when available, should be collected to allow comparison of the evidence with known sources. Such samples, when transferred on FTA™ cards may be stored at room temperature and later directly amplified by PCR for rapid reference DNA profiling [14].

Typically, biological sample collection from the consenting victim is performed by the medical examiner. At least two vaginal or anal swabs should be collected as evidence: for vaginal swabs within 120 h [15] and for anal swabs within 72 h post-contact [16]. When available, clothing worn by the victim during and immediately after the assault and condoms recovered from crime scenes should also be submitted. The samples should be air-dried for at least an hour before packaging in the SAI.Kit.

Evidence samples are examined to determine the presence of biological material from which DNA can be extracted. Identifying the tissue source of the DNA support activity level propositions [17]. For example, the presence of semen on a vaginal swab corroborates an allegation of sexual contact with ejaculation. Presumptive tests, such as the use of alternate light sources (ALS) and acid phosphatase (AP) tests, are useful in narrowing down possible locations of semen on a large piece of material. Confirmatory tests, on the other
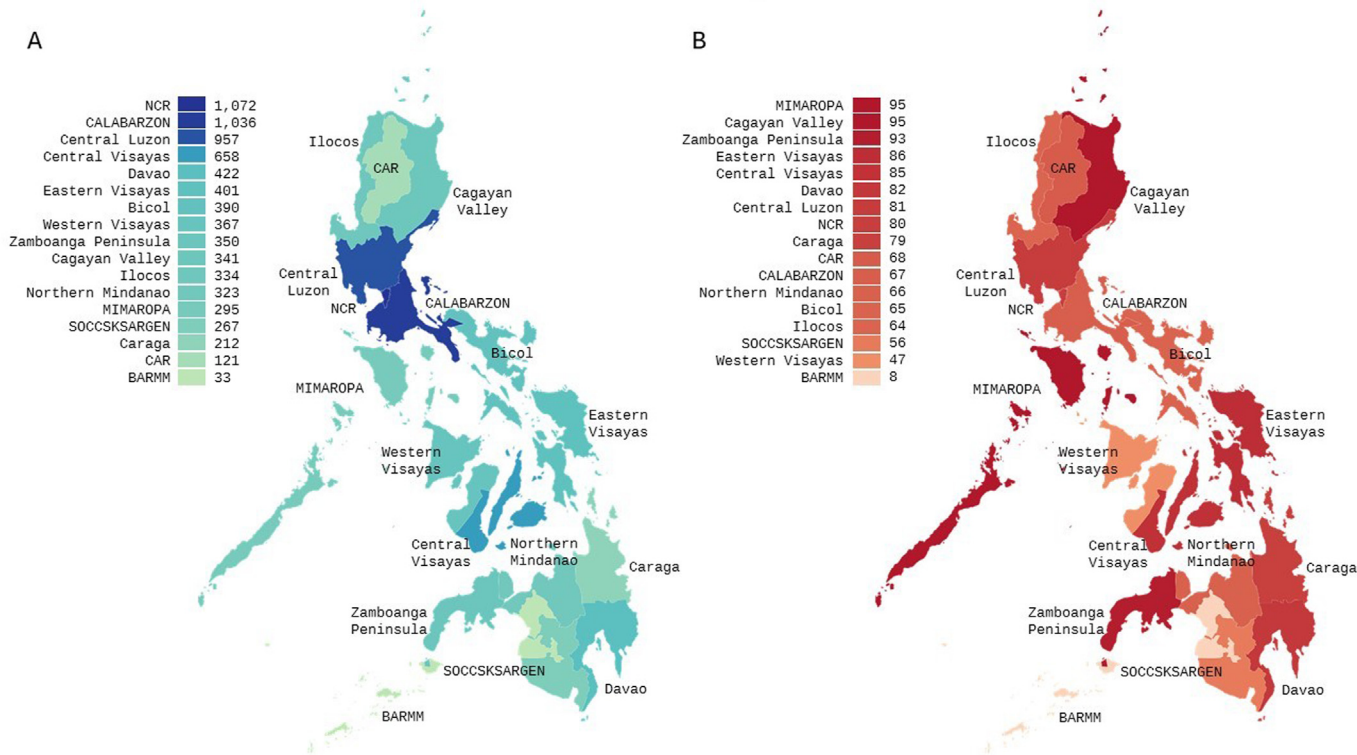
Fig. 1. Maps showing 2018 incidence of sexual assault in 17 regions of the Philippines reported to the Philippine National Police [3] (A) and number of cases per million based on projected population [5] (B).
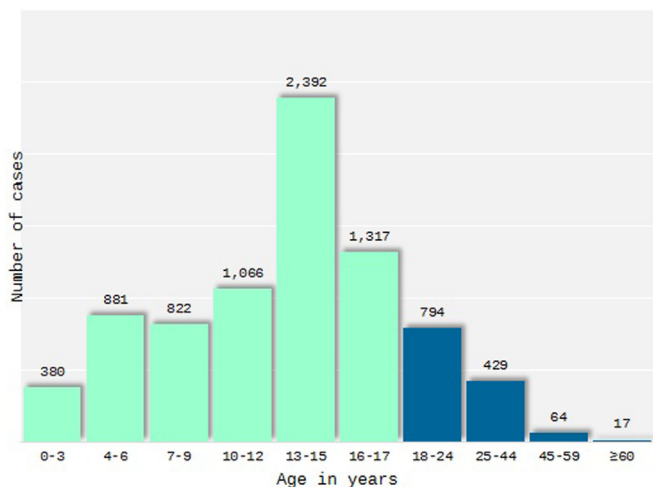

Fig. 2. 2018 incidence of sexual abuse by age in the Philippines seen by Women and Child Protection Units. Light green bars indicate cases involving minors (<18 years old), whereas blue bars show the number of cases for women 18 years old or above (modified from Ref. [6]). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

hand, establish semen presence. Examples of these include assays that detect semen-specific substances such as semenogelin (Sg) and the detection of sperm cells under microscopy.

Visual inspections for stains and condoms first under normal lighting then under blue light (~450 nm) viewed through an orange barrier filter are recommended as semen is most visible at 450 nm [18]. Saliva and urine stains also fluoresce under blue light which could be potential sources of the assailant's DNA. If visual inspection fails, the analyst must weigh the benefit of using an AP test,

which is more sensitive to highly diluted semen, but would require the contact of the material with moistened paper. This may potentially reduce DNA recovery or introduce contaminants to the sample. Blood in stains masks visibility of semen and in such materials, the use of AP tests is recommended.

Presumptive tests should be followed by a confirmatory semen test. Microscopic observation of sperm cells confirms semen presence. However, microscopy is not useful when the assailant is azoospermic or when sperm has disintegrated. Notably, the majority of child sexual assault victims examined by WCPUs over a four-year period were negative for sperm [19]. This suggests that an alternative approach other than microscopy must be adopted for routine screening of swab or stain samples.

RSID™-Semen (Independent Forensics) is an immunochromatographic test specific for human Sg [20]. The kit comes with the RSID™-Universal Buffer used to incubate the sample. This test can detect as little as 0.5 nL of semen and does not cross-react with non-semen containing fluids. The remaining buffer extract may be directly used as starting material for DNA extraction. While sample incubation in the buffer is expected to reduce DNA recovery, a full profile of the semen contributor resulted in the majority of swabs and stains we tested [10].

For DNA extraction, it is recommended that the analyst should prioritize samples which showed the strongest semen positive signals to increase chances of recovering sufficient DNA. Semen negative samples do not rule out sexual penetration and may still generate the non-victim's DNA profile. When there are no other semen positive specimens available, semen negative samples should still proceed to extraction [10].

The storage condition of samples is crucial in preserving DNA integrity. Most air-dried swabs and stains may be kept for long periods at room temperature (RT) without considerable DNA loss or damage. However, molds will likely develop on anal swabs stored
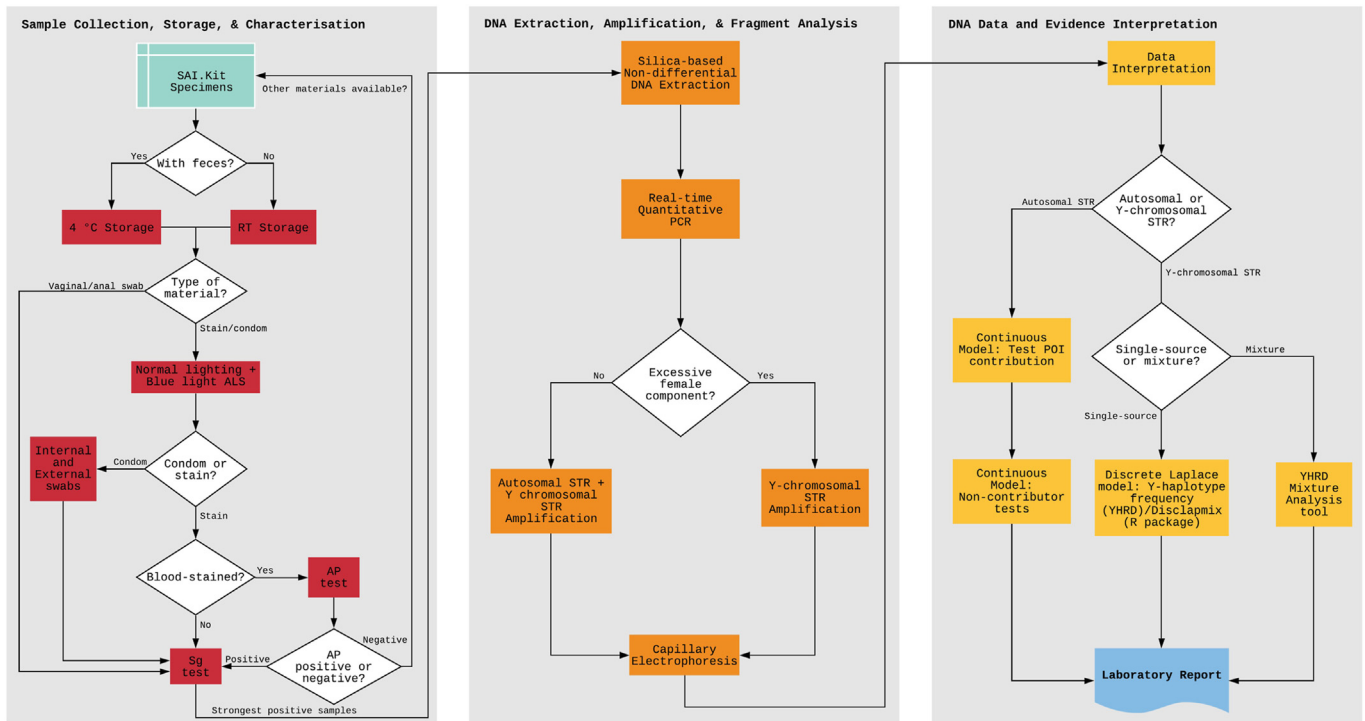
Fig. 3. A proposed integrated system for DNA testing in sexual assault investigations.

in ambient conditions which is warm and humid in the Philippines for most of the year. Mold growth was found to inhibit the detection of semen and resulted in partial DNA profiles [10]. Hence, we recommend the immediate processing of anal swabs when possible. Otherwise, anal swabs should be stored at 4 °C and not at RT in the Philippines.

3.2. DNA extraction, amplification, and fragment analysis

The recovery of non-victim DNA can be challenging particularly with the delayed collection of samples. Other types of specimens on different substrates, for example clothing and latex, may contain inhibitors and contaminants with variable effects on DNA recovery. The laboratory should thus employ an extraction procedure which effectively removes inhibitors and maximizes the recovery of semen DNA.

Many laboratories use a differential extraction technique which separates sperm from the epithelial fraction [21]. However, this can reduce DNA recovery especially with little to no sperm present in the sample [22]. In the proposed scheme, a non-differential approach is adopted to maximize DNA recovery notwithstanding the resulting mixture.

For DNA extraction, the use of a commercially available solid-phase extraction method that uses silica beads resulted in better quality DNA compared with the organic method which uses phenol, chloroform, and isoamyl alcohol [5]. The higher average peak heights and more balanced profiles across dye channels in the silica-based extracted samples indicate less degradation and fewer co-extracted inhibitors. These samples also showed greater semen DNA relative to female DNA which is desirable to successfully detect the assailant's alleles. Further, the protocol involves fewer transfers thus a lower risk of sample-sample contamination.

After extracting the DNA, a quantitation step estimates the amount of total human DNA in the sample. This will enable adjustment of DNA concentrations suitable for PCR amplification.

Most quantitation procedures use real-time quantitative PCR (qPCR) which also measures the amount of male DNA. This step is useful in deciding which marker sets to use, i.e., both Y- and autosomal STRs or Y-STRs only. When there is excessive amount of female relative to male DNA, not all male autosomal alleles may be observed and Y-STR analysis is expected to be more informative [23]. More recent qPCR systems can also determine levels of degradation and inhibition [23] thus predicting the likelihood of successful STR amplification which follows.

In the succeeding step, STR markers are targeted and amplified via polymerase chain reaction. Most commercially available kits use multiplex PCR which simultaneously amplifies 20 or more autosomal or Y-chromosomal STRs in a single reaction. Autosomal STRs are the more suitable markers for identification due to its high variability. On the other hand, Y-STRs are useful in isolating a male profile from a male-female mixture [19,24], in excluding male suspects as possible source of non-victim DNA, in identifying paternal lineages of male perpetrators, and in determining the number of male contributors in a mixture [25]. However, Y-STR haplotypes are shared across paternal relatives and cannot be used to directly identify its source. Without autosomal data, other non-DNA evidence that could provide leads in supporting or negating the contribution of a paternal relative should be considered [26,27]. In all other scenarios when female to male ratio is less than 10:1, using both autosomal and Y-STR profiles will maximize DNA information obtained from a sample.

After PCR, the reaction mix consists of a mixture of DNA fragments representing different alleles of several STR loci. Most forensic DNA laboratories use capillary electrophoresis instruments to separate and to detect these fragments while feeding the raw data into a computer for subsequent analysis.

3.3. Data and evidence interpretation and the laboratory report

Running a sample through CE generates an electropherogram

which graphically represents alleles as peaks measured in relative fluorescence units (RFU). When there is sufficient good quality DNA, alleles can be readily distinguished from artifacts such as stutter. A software (e.g. GeneMapper *ID*-X) automatically calls alleles based on thresholds determined during the laboratory's internal validation [13,28]. The analyst then reviews the epg which requires some knowledge of peak morphologies. In low-level and mixed DNA samples, assigning alleles and genotype combinations can be more ambiguous. Consequently, the forensic community advocates for more probabilistic approaches to account for uncertainties in allele calls and minimize dependence on human interpretation [29,30].

The evidentiary DNA profile is only meaningful when compared to a known or reference profile. Generally, the weight of the evidence depends on the rarity of the profile estimated from a reference database of the relevant population [31,32]. To avoid possible confirmation bias, interpretation of DNA typing results from the evidence should be done before any comparison to a known profile except those assumed to be already present in the mixture (e.g. the profile of the victim) [33].

The likelihood ratio (LR) is the recommended method for evidence interpretation in criminal casework [34], where two contrasting hypotheses (prosecution v. defense) are evaluated. Statistical approaches to calculate LRs vary in the amount of epg information considered ranging from binary to semi- and fully continuous models [35]. Semi-continuous models (e.g. LRmix [36]), which allow for the possibilities of drop-out and drop-in Ref. [37], are a substantial improvement over simplistic binary models which only consider the presence or absence of alleles [38–40]. Continuous approaches such as STRmix™ [30,41] utilize more epg information and generate likely genotype combinations based on models of degradation, stuttering, and peak height variability. The power of LRs to discriminate between true and false propositions was observed to increase with the amount of correct information provided into the calculation. This was maximized in samples whose semen component had no drop-out, had high average peak heights (>5000 RFU), and when the fully continuous model, STRmix™, was used [6].

Allele peak designation in low template samples and mixtures in which the minor component has low proportion can be ambiguous. In LRmix, the analyst may need to thoroughly evaluate the epg of the DNA mixture and decide to remove short peaks presumed to be stutter or to retain these as allele peaks of a minor component. This is not the case with STRmix™ as the fully continuous model takes into account stochastic effects and incorporates probabilities for genotype combinations other than those put forward by the prosecution hypothesis. Non-contributor tests are also performed to rule out false inclusions, where the person of interest (POI) is replaced by numerous randomly generated profiles [36]. Showing that thousands to millions of random profiles are unlikely contributors increases the confidence that the evidence is not ambiguous in supporting the inclusion of the POI. The fully continuous model, which maximizes the use of epg information, allows for non-contributor tests, and is less dependent on human judgment, is a robust and objective tool for interpreting DNA mixtures.

For interpreting Y-chromosomal STR results, the proposed workflow adopts the recommendations of the International Society of Forensic Genetics (ISFG) to use the discrete Laplace model [42]. The discrete Laplace model calculates the haplotype frequency in the population taken as a collection of haplotype subgroups derived from an ancestral haplotype [43]. The Y-Chromosome STR Haplotype Reference Database (YHRD) (http://yhrd.org) and the R package disclapmix [44] (https://CRAN.R-project.org/package=disclapmix) allow for estimating frequencies using this method. The ISFG further recommends considering the relevant metapopulation in the YHRD in calculating these frequencies, particularly the Austronesian metapopulation for Filipino samples.

Y-haplotype mixtures are encountered when there are multiple perpetrators, when the victim has a consensual partner, or when the victim is male. Unfortunately, the developments in Y-STR mixture interpretation generally lag behind autosomal DNA mixtures. The discrete Laplace method had been extended to mixtures [45] and a few studies applied continuous models to Y-STRs [46,47]. These merit further development and should be welcome improvements to casework analysis in the future. Meanwhile, a readily accessible tool for calculating LRs for Y-STR mixtures based on the method described by Wolf et al. [48] is available in the YHRD website.

The final output of the laboratory scheme is a report that contains a description of the analytical methods used, DNA typing results, and the conclusions based on comparisons made between reference and evidentiary samples. Since it is intended to contribute to the resolution of a case, technicians and analysts carefully review the laboratory report for its completeness, accuracy, and clarity. The report may be presented as evidence in court, commonly by a DNA analyst testifying as an expert witness. Given the technicalities in interpreting DNA evidence, the forensic DNA laboratory should formulate its guidelines not only in conducting DNA tests, but also in preparing reports that can be understood by law enforcement, lawyers, and judges [49].

4. Conclusion and recommendations

DNA technology is a powerful tool that can aid sexual assault investigations in the Philippines. Our previous work demonstrated the usefulness of specific protocols in generating DNA profiles from different types of samples encountered during casework. The system for forensic DNA testing of sexual assault samples that we propose here can be adopted by forensic DNA laboratories in the Philippines and in other regions facing similar challenges. Research on emerging technologies is continuously being pursued with the goal of improving the system.

Benefits of forensic DNA technology are not maximized in the country due to the inadequate resources and infrastructure allocated for the conduct of DNA tests. Without routine DNA tests in criminal investigations, court litigations will continue to rely heavily on verbal testimonies resulting in lengthy trials and delayed justice for victims. The proposed system described here should lead to formulation of national guidelines for DNA testing and increase the likelihood of identifying actual perpetrators of sexual assault. Further, a national database of offender and crime scene profiles established through the passing of a DNA law should provide leads for ongoing investigations, link unsolved crimes without suspects, and identify repeat offenders [50]. Consequently, an increased public trust in the judicial process is expected to encourage victims to promptly report cases of sexual violence. These developments are major steps towards improving the criminal justice system in the Philippines.

Declaration of competing interest

The authors declare no conflict of interest.

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