

Non-differential DNA extraction of post-coital samples submitted as evidence for investigating sexual assault cases in the Philippines

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The number of reported sexual assault cases continues to rise yearly in the Philippines. One major step towards addressing this problem is to use DNA test results in rapidly and accurately identifying assailants.

The common practice among forensic laboratories overseas is to perform differential DNA extraction to isolate sperm fraction. For this study, we employed non-differential methods to maximize DNA recovery. We evaluated two non-differential DNA extraction procedures namely a phenol-chloroform-isoamyl alcohol (PCI) method and a silica-based method for handling post-coital samples. The procedures were assessed based on total human DNA yield, quality of DNA extracted from single-source semen samples, and the proportion of male component from post-coital vaginal swabs and stains. Higher DNA yields but lower DNA quality resulted from the PCI method. The silica-based method using QIAamp DNA Micro kit produced better quality DNA, resulted in higher male proportion for male-female samples, was faster and less tedious, minimized exposure to hazardous chemicals, and generated less waste. In the Philippines, where biological samples may be

compromised due to the tropical climate favoring enzyme-catalyzed degradation of DNA, as well as delays in a victim reporting the incident, we recommend a non-differential silica-based method to maximize recovery of good quality DNA. This should be followed by statistical interpretation using a continuous model to objectively evaluate results that contain the DNA profiles of victim and assailant.

KEYWORDS

sexual assault evidence, rape, post-coital samples, organic extraction, silica-based extraction, non-differential DNA extraction, DNA profiling, forensic genetics

INTRODUCTION

In 2014, Women and Child Protection Units (WCPUs) nationwide received a total of 7,457 reports of sexual abuse (Child Protection Network 2014). Conviction of sexual offenders in those cases that actually proceed to trial mostly relied on eyewitness accounts (Sugue-Castillo 2009). While crime laboratories in the Philippines have the capacity to conduct forensic DNA testing, there is an urgent need to establish an efficient system for routine collection, storage, processing, and interpretation of biological evidence in view of the increasing number of sexual assault cases that are reported each year.

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DNA testing is the most powerful and objective tool for human identification. In sexual assault investigations, the DNA profile from samples that were collected from the crime scene or a victim's body is compared against a suspect's DNA profile. However, DNA testing of sexual assault cases is challenging because samples may be present on various substrates and may have been exposed to harsh environmental conditions and/or contaminants. Exposure to high temperatures and humid conditions accelerate DNA degradation while contaminants may inhibit downstream PCR amplifications that are performed to generate the DNA profile from a biological material. Hence to increase the likelihood of obtaining an interpretable profile, the DNA extraction procedure should effectively remove inhibitors and maximize recovery of DNA contained in biological samples commonly submitted for analysis in a forensic laboratory.

Since the beginning of DNA profiling in forensic investigations in the mid-1980's, organic extraction using phenol, chloroform, and isoamyl alcohol (PCI) has been used routinely for different types of samples. Organic extraction was the earliest procedure used for isolating DNA from biological samples for forensic analysis. The method involves the addition of sodium dodecyl sulfate (SDS) and proteinase K to disrupt membranes and denature proteins, and isoamyl alcohol to enhance interphase formation between the aqueous and organic layers. After centrifugation, two immiscible layers become evident: (1) the organic layer at the bottom which contains proteins, lipids, and other impurities, and (2) the aqueous layer at the top where the DNA is dissolved (Butler 2012). However, the use of the PCI method exposes analysts to toxic organic chemicals, involves several steps where liquid is transferred from one tube to another thus increasing the chance of spillage and contamination, generates toxic organic wastes, and requires a significant amount of time. The entire process may take about 20 hours or more due to an overnight lysis step.

Other methods developed to avoid the use of toxic organic chemicals include solid-phase extraction methods that use solid particles, such as silica beads, to adsorb DNA (Boom et al. 1990). Silica beads which are packed in columns bind DNA in the presence of high concentrations of chaotropic salts such as guanidine hydrochloride, guanidine isothiocyanate, sodium iodide, and sodium perchlorate at low pH. Then buffer is added to wash out proteins and other impurities (Vogelstein and Gillespie 1979; Boom et al. 1990; Duncan et al. 2003). Subsequently, pure DNA is released by increasing pH and decreasing salt concentration.

Because of the physical nature of a sexual assault, most samples submitted to investigations contain the DNA from both the assailant/s and the victim. A modification of the organic extraction method was developed to separate the sperm and epithelial cell fractions by including an additional step known as 'differential lysis' (Gill et al. 1985). However, this differential extraction approach will not be successful in cases where the specimen contains little to no sperm. In fact, in a study by Maiquilla et al. (2011), majority of cytological examinations among sexual abuse patients in Child Protection Units (CPUs) in the country were negative for sperm. The approach also results in considerably less DNA recovered for downstream DNA profiling analysis (Voorhees et al. 2006; Norris et al. 2007; Vuichard et al. 2011). We therefore propose an alternative approach where the DNA from all contributors to the specimen are extracted using a silica-based procedure to maximize recovery of good quality DNA. Autosomal short tandem repeat (aSTR) DNA typing should be conducted followed by mixture interpretation to determine the likelihood of a suspect contributing his DNA to the mixture.

We report here the extraction of DNA from neat semen stains as well as from various post-coital samples, without the differential lysis step using a PCI method and the QIAamp DNA Micro procedure (QIAGEN, Hilden, Germany). The amount and quality of DNA recovered using both methods were compared to evaluate their utility in handling biological samples commonly submitted for analysis in relation to sexual assault investigations in the Philippines.

METHODOLOGY

Samples

Semen samples were submitted by five male volunteers aged 19 to 26 years old. A male-female couple provided a vaginal swab and a male-male couple provided an anal swab from each of five occasions of sexual intercourse. Two pieces of undergarment worn by the female or male receptive partner post-contact and two condoms used by each of the penetrative partners were obtained from separate occasions of coitus (Figure 1). A licensed medical allied professional collected blood samples from volunteers by blotting their blood onto individual FTA[®] Classic cards (GE Healthcare, Little Chalfont, UK). These samples were then used to generate reference DNA profiles. Semen samples were stored at 4°C, while all post-coital samples and FTA[®] Classic cards were air-dried then stored at room temperature (24-27°C) prior to processing. Ethical aspects of the study were approved by the University of the Philippines Manila Research Ethics Board (UPMREB Code: 2012-321-01).



Figure 1. Post-coital samples tested in this study. Vaginal swabs (A) and anal swabs (B) were provided by consenting male-female and male-male couples, respectively. Used condoms (C) and stained pieces of underwear (D and E) were also provided by the volunteers.

Sample preparation and semen detection

Semen samples

Neat semen samples were deposited in 50 μ L aliquots onto two 49 mm² pieces of cotton-polyester and then dried in the dark and at room temperature for approximately 48 hours. The pieces of cloth were cut further into smaller pieces and placed in 1.5 mL microcentrifuge tubes.

Table 1. Types and number of biological specimens processed in this study.

Volunteers	Types of specimens collected	Number of days prior to processing	Number of specimens
Five males	Semen samples	0	5
	Vaginal swabs	63-72	5
One male-female couple	Stained underwear	9-14	2
	Used condoms	53-55	2
	Anal swabs	55-72	5
One male-male couple	Stained underwear	0-1	2
	Used condoms	20-22	2

Post-coital samples

Stained underwear and condoms were photographed through orange barrier filter goggles under blue light emitted by the Mini BLUMAXX™ III LED illuminator (Sirchie, Youngsville, NC, US). Ten small pieces (~100 mm²) were obtained from the underwear where presence of semen was suspected. Two moist cotton swabs were rubbed on the surface of post-coital condoms for a total of four external and four internal swabs per condom. Cotton battings from the vaginal/anal and condom swabs and the underwear cuttings were separately submerged in 400 µL RSID™ Universal Buffer (Independent Forensics, Lombard, IL, US) for two hours at room temperature. RSID™-Semen tests were performed following manufacturer's specifications (Independent Forensics).

Phenol-chloroform-isoamyl extraction

Lysis buffer consisting of 150 µL TEN buffer (10 mM Tris, 1 mM EDTA, 100 mM NaCl), 50 µL 20% Sarkosyl, 7 µL 1M DTT, and 10 µL Protinase K (20 mg/mL) was added to 1.5 mL microcentrifuge tubes containing cuttings with semen. For each post-coital sample, 100 µL of the RSID™ Universal buffer extracted samples were transferred into clean 1.5 mL microcentrifuge tubes. Lysis buffer was also added to each tube followed by incubation at 56°C with shaking at 1,200 rpm for 18–24 hours. Lysates were then transferred into 1.5 mL MaXtract Low Density tubes (QIAGEN). A 300 mL PCI (25:24:1) solution was then added to each tube and centrifuged at 14,000 rpm for 10 minutes. Samples were concentrated using fresh Microcon® 0.5 mL-100K filter units (Merck Millipore, Darmstadt, Germany) and purified following manufacturer's instructions. DNA samples were finally eluted using 40 µL TE⁻ buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). Liquid DNA samples were stored at -20°C until quantitation and amplification.

Silica-based extraction using QIAamp DNA Micro procedure

Lysis buffer consisting of 300 µL Buffer ATL, 20 µL Proteinase K (20 mg/mL), and 20 µL 1M DTT was added to each tube containing cuttings of semen-stained cloth or 100 µL of the DNA eluent consisting of the RSID™ Universal buffer that was incubated with the post-coital samples. Tubes were incubated at 56°C with shaking at 900 rpm for one hour, and centrifuged. Three hundred microliters (300 µL) of Buffer AL and 1 µL dissolved carrier RNA were added, followed by incubation at 70°C with shaking at 900 rpm for 10 minutes. After incubation, 200 µL ethanol (96-100%) was added, and then centrifuged at (20,000 x g) 8,000 rpm for one minute. The supernatants were transferred to QIAamp MinElute Columns

(QIAGEN) and processed according to manufacturer's instructions. DNA samples were eluted in 40 µL Buffer AE and stored at -20°C.

DNA quantitation

Autosomal and Y-chromosomal DNA concentrations were determined via real-time PCR using the Plexor HY System (Promega, Madison, WI, US) in an Applied Biosystems® 7500 Real-time PCR machine (ThermoFisher, Waltham, MA, US) and analyzed with the Plexor Analysis Software (Promega). Total DNA yield was calculated based on total DNA present in the eluate per microliter of starting material, i.e. 50 µL of semen deposited on the cutting for the single-source samples, and 100 µL aliquots from each RSID™-Universal Buffer extract for the post-coital samples. For post-coital male-female samples, the proportion of the male component was calculated by dividing the Y-DNA concentration by the total human DNA concentration.

STR amplification and fragment analysis

DNA extracts from semen and post coital samples as well as reference blood on 1.2 mm FTA® discs were amplified using PowerPlex® 21 System (PP21) and the PowerPlex® Y23 System (PPY23) (Promega) with 0.5 ng DNA template in reduced volume (10 µL) reactions or full reaction volumes (25 µL). PCR amplification was carried out using Applied Biosystems® GeneAmp® 9700 (ThermoFisher) in 30 cycles for liquid DNA samples and 25 cycles for FTA® discs, and detected with the Applied Biosystems® 3500 Genetic Analyzers (ThermoFisher). Resulting electropherograms (epgs) were analyzed using 50 relative fluorescence units (RFUs) as the analytical threshold with GeneMapper® ID-X software version 1.2 (ThermoFisher).

Data analyses

Allele calls on semen and post-coital epgs were compared with the volunteers' reference DNA profiles. The average peak height (PH), average heterozygous peak height ratio (PHR), and average intracolor balance (IB) were used to assess DNA quality. The PHR was calculated by dividing the smaller PH by the larger PH and then expressing the ratio as a percentage. For IB, heterozygous peaks were normalized by calculating the average PH while the homozygous PH were normalized by dividing the value by two. The smallest normalized PH was divided by the largest PH per dye channel and reported as percentage. In Y-STR epgs, peak heights of the heterozygous DYS385 locus were averaged and the balance was calculated by dividing the shortest peak height in a dye channel by the tallest. DYS385 is characterized by a duplication within the Y-chromosome thus resulting in up to two allele types per male contributor (Butler 2012). Paired t-tests and two-way repeated measures analysis of variance (ANOVA) were performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA, US).

RESULTS

Ten semen on cotton-polyester cuttings and 46 post-coital samples (five vaginal swabs, five anal swabs, ten male-female underwear cuttings, ten male-male underwear cuttings, four male-female external condom swabs, four male-female internal condom swabs, four male-male external condom swabs, and four male-male internal condom swabs) were processed in this study. All male-female samples tested positive for semen, while

Table 2. Comparison of DNA quality between organic and silica-based methods for DNA extraction of semen. All parameters measured higher for the silica-based method, albeit significant results are only apparent for intracolor balance.

Parameter	Organic	Silica-based	<i>p</i> -value
Average peak RFU (aSTR)	14,035.01 ± 8777.77	14,884.05 ± 6,664.28	0.8109
Average peak RFU (Y-STR)	18,094.82 ± 10,379.31	21,954.24 ± 8,703.22	0.5186
Average heterozygous peak height ratio	85.72% ± 13%	85.87% ± 9%	0.8777
Average intracolor balance (aSTR)	30.24% ± 19%	52.77% ± 14%	0.0177
Average intracolor balance (Y-STR)	29.22% ± 15%	58.06% ± 19%	0.0779

Boldface indicates significance at 90% confidence level ($p \leq 0.10$)

a few male-male samples were negative for semen (two anal swabs and four external condom swabs) using the RSID™-Semen assay. All samples generated partial to complete DNA profiles.

DNA yield

The PCI procedure produced significantly higher amounts of DNA compared to the use of the silica-based method for single source semen samples ($p=0.0165$). DNA yield per microliter of semen using PCI ranged from 2.79 to 23.94 ng (mean: 13.84 ng; median: 14.27 ng), while the use of the silica-based method produced 0.63 to 9.87 ng (mean: 4.61; median: 2.17 ng). A similar trend was observed among post-coital samples (Figure 2), where DNA yield was affected by both the extraction method ($p < 0.0001$) and sample type ($p < 0.0001$). DNA yields ranged from 0.01 to 159.50 ng/μL (mean: 16.30ng/μL; median: 2.01 ng/μL) using the PCI method. The maximum yield was observed in an internal swab from a condom provided by the male-female couple. On the other hand, the silica-based method produced 0.0007 to 1.70 ng/μL (mean: 0.25 ng/μL; median 0.13 ng/μL), where the highest yield was observed in a vaginal swab.

Contributor DNA recovery from post-coital samples

Despite the higher DNA yield obtained using the PCI procedure, the silica-based method recovered higher proportions of the male component ($p=0.0003$) based on Y-DNA concentration of samples derived from the male-female couple. The male proportion in male-female DNA mixtures extracted using PCI ranged from 0.92% to 89.66% (median: 15.00%); results of the silica-based method ranged from 2.82% to 113.33% (median 39.43%) (Figure 3). The calculated proportion of male DNA in one underwear stain sample which was slightly higher than 100% was attributed to stochastic difference in the amplification levels of autosomal and Y-DNA in the extract.

The resulting eggs (Figure 4) showed peaks generally expected of the sample type and sex-specificity of the multiplex kit used. Autosomal STR DNA profiles generated from male-female and

male-male swab and stain samples indicated the presence of two-person mixtures, i.e. presence of three or four allele peaks in a number of loci. Y-STR typing revealed the single haplotype of the male contributor in male-female samples, whereas male-male swab and stain samples showed alleles of a second male contributor. Where the two male contributors do not share an allele at a locus, two peaks were visible except for the DYS385 locus where four allele peaks were detected. Internal surfaces of condoms only showed male/penetrative partner alleles while the external lining showed alleles of the receptive partner.

DNA quality from single-source semen samples

Multiplex aSTR and Y-STR typing generated full profiles of the semen donor in all samples extracted using PCI and silica-based procedures. All three parameters of DNA quality, namely average PH, average heterozygous PHR, and IB were consistently higher for the QIAamp DNA Micro extracted samples. Significant difference ($p \leq 0.10$) was evident in IB values computed from autosomal and the Y-chromosomal eggs (Table 2).

DISCUSSION

We evaluated two procedures: (1) a phenol-chloroform-isoamyl alcohol method, and (2) a silica-based method for non-differential extraction of DNA from post-coital samples. The PCI procedure continues to be the preferred method for recovering high molecular weight (HMW) DNA (Butler 2012). In our typical casework experience, the standard PCI procedure produces higher amounts of DNA compared to commercial extraction kit protocols. Results of this study consistently generated higher yields for PCI, notwithstanding higher amounts of proteinase K and the use of carrier RNA known to increase extraction efficiency (Kishore et al. 2006) in the QIAamp DNA Micro procedure. We therefore surmise that it is the overnight incubation step in the PCI method which increased DNA yields, however our results indicate that such prolonged lysis with heat and agitation reduced DNA quality.

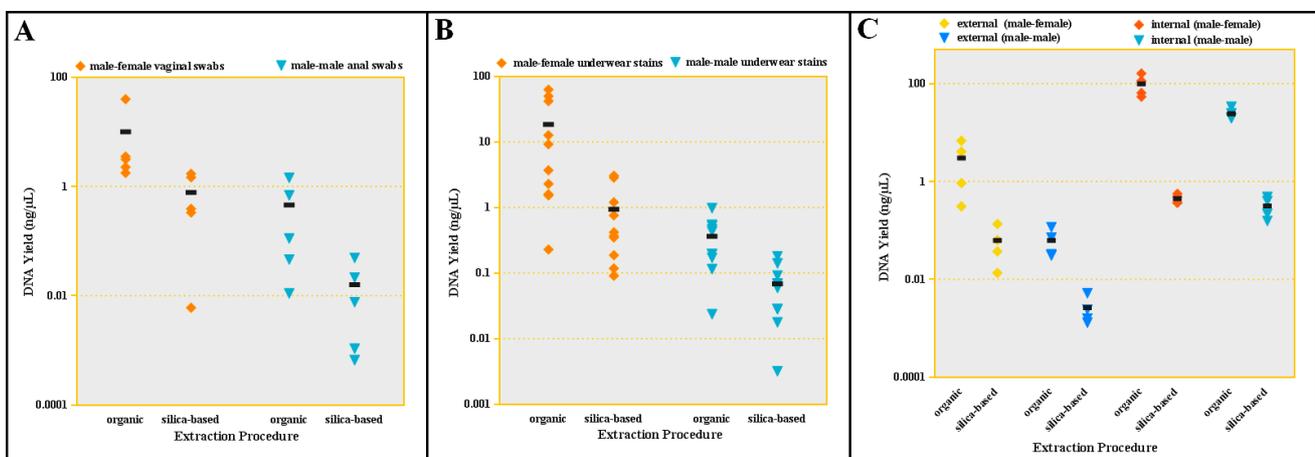


Figure 2. Total human DNA yield from post-coital vaginal and anal swabs (A), underwear stains (B), and condom swabs (C) extracted using a phenol-chloroform-isoamyl method (organic) and a silica-based method. The organic extraction method generally produced higher amounts of DNA compared to the silica-based method. Observed variation in DNA yields is significantly affected by both extraction method ($p < 0.0001$) and sample type ($p < 0.0001$). The y-axes are in a logarithmic scale. Median values are indicated by the black horizontal lines.

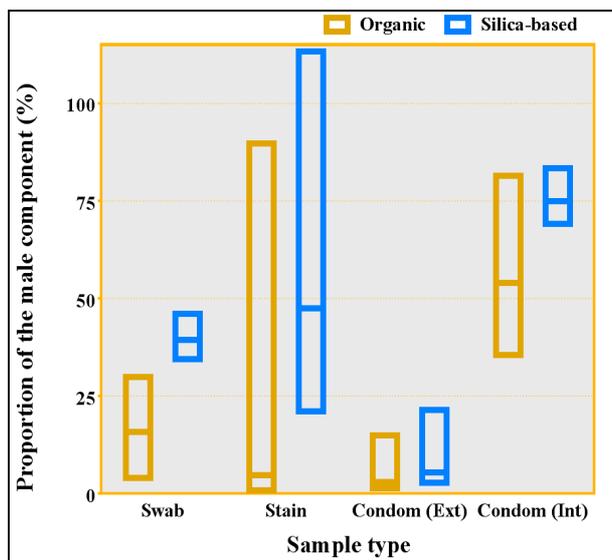


Figure 3. Proportion of the male component among male-female DNA mixtures extracted from post-coital samples using a phenol-chloroform-isoamyl (organic) method and a silica-based method. The silica-based method resulted to higher proportions of the male component ($p=0.0003$). Median values are indicated by the horizontal line within each box plot.

The silica-based method produced less DNA but of better quality. Degradation and inhibition can affect PH (Moretti et al. 2001), heterozygous peak balance (Wallin et al. 1998), and IB (Collins et al. 2004). Of the three measures of DNA quality, statistically significant difference ($p<0.10$) between the DNA extracted using the two methods was observed only in the IB of the resulting eggs. While inhibitors present in semen, such as spermine and spermidine (Ahokas and Erkkilä 1993), can decrease the amount of PCR products resulting in decreased RFU signal, increased stochastic variation and peak imbalance (Gill and Buckleton 2005), the negative effect is much more evident as imbalance within dye channels (Collins et al. 2004). The downward sloping of peak heights indicates lesser efficient amplification of HMW markers and is an indication of degradation or inhibition (Chung et al. 2004; McCord et al. 2011; Nicklas et al. 2012). Hence, a well-balanced amplification within dye channels adds confidence to the accuracy of the DNA profile/s that had been generated. While the study did not quantify levels of inhibitors present in the sample post-extraction, the significant difference in IB between the two methods provided evidence to support the greater efficacy of silica-based procedure in purifying DNA from inhibitors and other contaminants. Similar results were reported for bone (Loreille et al. 2007) and blood samples (Psifidi et al. 2015).

The observed variation in DNA yields reflected differences across sample types. For example, internal condom swabs generated the highest amounts of DNA since semen is retained within the latex. In general, complete DNA profiles may be generated from samples that had been dried and stored at room temperature for an extended period. However, this is not the case for some contaminated samples, e.g. anal swabs with feces.

Microorganisms present in these samples produce nucleases which damage the DNA and compromise the evidence (Butler 2012). In addition, excessive amounts of DNA from microbial sources, e.g. molds, may interfere with downstream procedures (Calacal and De Ungria 2005). Hence, the processing of anal swabs should be prioritized when handling different types of evidence during a sexual assault investigation. Further, all biological samples, especially anal swabs, should be kept at cool temperatures to inhibit microbial growth in these samples.

Excess female to male DNA ratio in a mixture results in preferential amplification of female alleles and non-detection of a few to several male alleles (Krenke et al. 2009). Hence, high efficiency in isolating male DNA is essential in any extraction procedure employed for sexual assault evidence samples. In common casework scenario where there is relative abundance of the victim's epithelial cells compared to the assailant's sperm cells, efficient sperm lysis is needed to maximize the DNA that is recovered from the semen contributor. We further note that this has important implications to mixture interpretation. In a different study, we show that a greater male proportion results to stronger support for the evidence if the prosecution's hypothesis is true (Rodriguez et al., manuscript in preparation). DNA extraction procedures for semen containing samples include dithiothreitol (DTT), a reagent used to disrupt disulfide bridges in sperm nuclear membranes (Butler 2012). However, the longer incubation period in the organic method did not necessarily result in higher proportions of male DNA obtained from male-female samples. Rather, we attribute greater male proportion to higher levels of DTT (20 μmol) used in the silica-based method than in the organic procedure (7 μmol) per reaction.

DNA analysis of sexual assault evidence is made more complex because of the commingling of the victim's and the assailant's DNA. For this reason, many laboratories include the male-specific Y-STR analysis in its repertoire of DNA tests aimed at identifying the male assailant (Ballantyne et al. 2009; Hanson et al. 2009). However, the discrimination capacity of Y-STRs is limited due to haplotype sharing across paternally-related men and the high frequencies of some Y-haplotypes in the population. In addition, haplotypes may not be fully resolved if the victim is male, whose own Y-STR peaks will also be detected in the epg. To generate the autosomal profile of the assailant, many laboratories perform differential extraction to separate a sperm fraction from epithelial cells. However, sperm may not always be isolated from post-coital samples. For example, Maiquilla et al. (2011) found negative sperm result in 86 out of 154 child sexual assault cases which may be due to physiological characteristics of the assailant, e.g. azoospermia, or the degradation of sperm cells before the swabs were collected. Moreover, premature lysis of the sperm into the non-sperm component and the many transfers involved can result in decreased recovery (Ballantyne et al. 2013). Previous studies reported sperm recovery of only up to 10% to 40% from the substrate (Voorhees et al. 2006; Norris et al. 2007). In an inter-laboratory study, Vuichard et al. (2011) reported the loss of up to 64% of female DNA and 98% of male DNA from the separated fractions. To increase efficiency, sperm DNA extraction procedures had been modified (e.g. Uchiyama et al. 2006; Norris et al. 2009; Hudlow and Buoncristiani 2012; Lounsbury et al. 2014), with some procedures eventually packaged as part of different commercial kits (Tsukada et al. 2006; Garvin et al. 2009, 2012). A laboratory workflow may thus involve an initial sperm detection step before differential extraction and autosomal STR typing. If sperm is not detected, the laboratory may resort to non-differential extraction to recover DNA from the semen contributor with the drawback of generating a mixed autosomal epg subject to more complex mixture analysis. While the theory and practice of mixture analysis have been regarded as challenging (Gill et al. 2012) and controversial because of issues such as subjectivity and bias of analysts during interpretation (Dror and Hampikian 2011) and the inaccuracy of the mathematics involved (Buckleton and Triggs 2005), there had been remarkable developments in recent years. Probabilistic and continuous systems with robust algorithms to model possible complicating aspects in epgs, e.g. stutter; drop-in; drop-out; peak height imbalance; and degradation, have been developed and validated (Bright et al. 2013; Taylor et al. 2013).

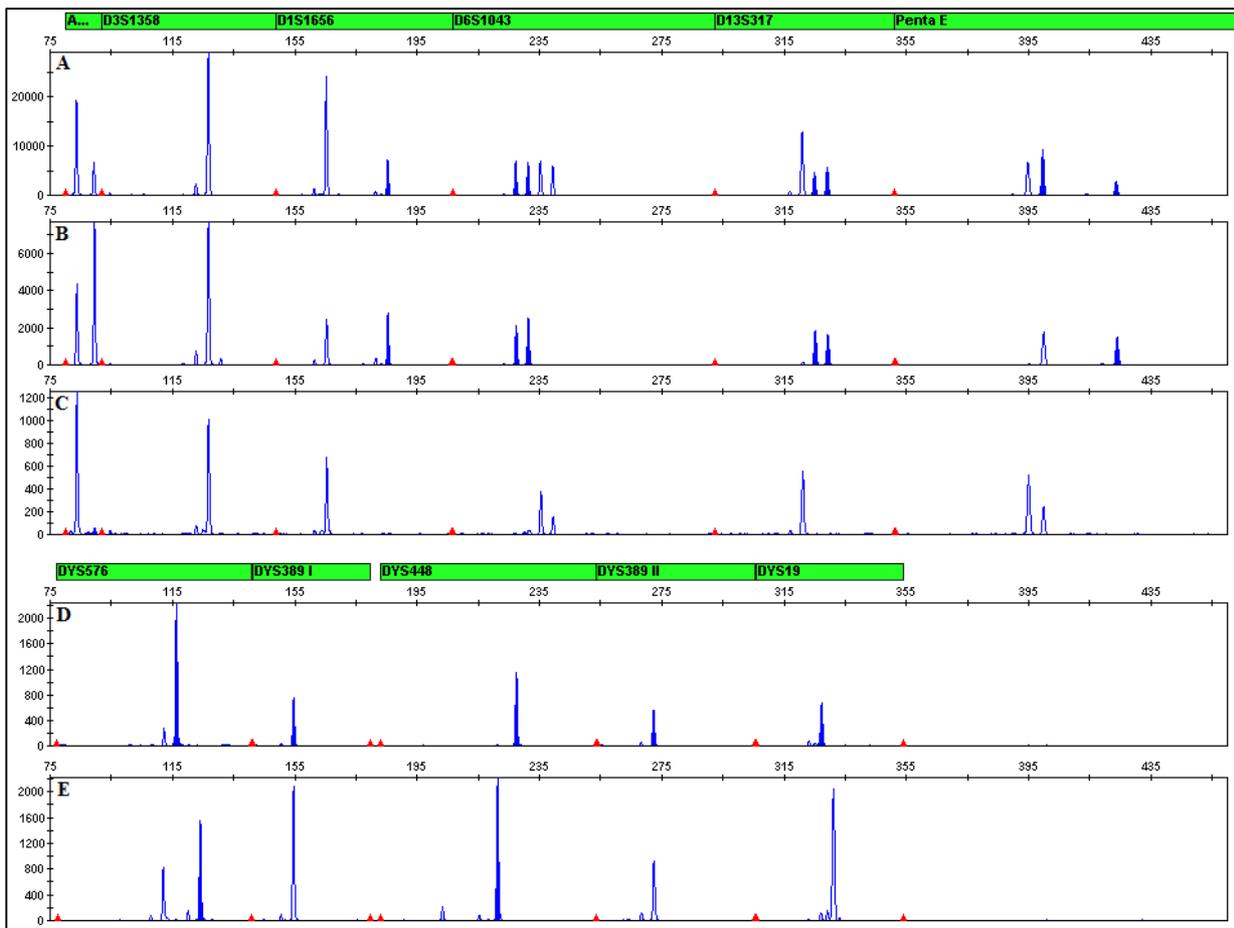


Figure 4. Representative electropherograms (epgs) of post-coital samples showing contributor alleles expected of sample type. (A) An autosomal mixture from vaginal swab; (B) A single male autosomal profile from male-female internal condom surface; (C) A single female autosomal profile from a male-female external condom surface; (D) A single male Y-chromosomal profile from a male-female stain; (E) A Y-chromosomal mixture from an anal swab. Only the blue dye (JOE) channel is shown. Penetrative partner allele peaks are highlighted.

Grant
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JJRB

In the Philippines, collection and proper handling of post-coital samples are not routinely done in sexual assault investigations due to delayed reporting by the victims and the scarcity of medical doctors who are trained to handle such types of evidence. Further, the warm and humid climate threatens the integrity of stored biological samples. Hence, there is a need to educate the community about the value of early reporting and the adoption of efficient procedures for DNA testing of sexual assault cases in the Philippines. We recommend a silica-based non-differential extraction method and the adoption of a statistical approach using a continuous model for autosomal DNA mixture interpretation. Non-differential extraction maximizes DNA recovery and the use of a commercial silica-based kit increases the likelihood of obtaining good quality DNA from biological samples submitted as evidence (anal, vaginal, condom swabs, and undergarment stains). The silica-based method that uses more DTT during the cell lysis stage compared to PCI also results to higher male proportions, is faster and less tedious, generates less waste, and does not expose the analyst to hazardous organic chemicals. The procedure is better suitable for routine processing of sexual assault evidence in order to provide DNA results to investigators in the soonest possible time.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

STATEMENT OF AUTHENTICITY

We herein confirm authenticity of all the data presented and of the procedures described in this article.

INDIVIDUAL CONTRIBUTION OF AUTHORS

JJRB applied for ethics approval, invited some of the participants, designed, and conducted the laboratory experiments and analyses, wrote the manuscript, and prepared

the figures and tables. MCADU conceived the study, provided input in data analysis, and contributed to writing and editing the manuscript. GCC helped in designing the experiments, invited participants, collected some of the samples, and contributed to discussion of the results of the study. RPL provided guidance to JJRBR and contributed to the improvement of the manuscript.

REFERENCES

- Ahokas H, Erkkilä MJ. Interference of PCR amplification by the polyamines, spermine and spermidine. *PCR Methods Appl.* 1993; 3(1):65–68.
- Ballantyne J, Hanson EK. Y-chromosome short tandem repeats. In: Jamieson A and Moenssens A (Eds.). *Wiley Encyclopedia of Forensic Science*. Chichester, UK: Wiley. 2009; 2677–2682.
- Ballantyne J, Hanson E, Green R, Holt A, Mulero J. Enhancing the sexual assault workflow: Testing of next generation DNA assessment and Y-STR systems. *Forensic Sci Int Genet Suppl Ser.* 2013; 4(1):e228–e229.
- Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van der Noordaa J. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol.* 1990; 28: 495–503.
- Bright JA, Taylor D, Curran JM, Buckleton JS. Developing allelic and stutter peak height models for a continuous method of DNA interpretation. *Forensic Sci Int Genet.* 2013; 7:296–304.
- Buckleton J, Triggs C. Is the 2p rule always conservative?. *Forensic Sci. Int.* 2005; 159:206–209.
- Butler JM. DNA Extraction Methods. In: *Advanced Topics in Forensic DNA Typing: Methodology*. San Diego: Elsevier Academic Press. 2012; 29–47.
- Calacal GC, De Ungria MCA. Fungal DNA challenge in human STR typing of bone samples. *J Forensic Sci.* 2005; 50(6):1394–1401.
- Child Protection Network. Annual Report. Manila: Philippine General Hospital Child Protection Unit, 2014.
- Chung DT, Drábek J, Opel KL, Butler JM, McCord BR. A study on the effects of degradation and template concentration on the amplification efficiency of the STR Miniplex primer sets. *J Forensic Sci.* 2004; 49(4):733–740.
- Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. Developmental validation of the 13 CODIS loci, D2S1338, D19S433, and Amelogenin: the AmpFISTR® Identifiler® PCR amplification kit. *J Forensic Sci.* 2004; 49(6): 1265–1275.
- Dror IE, Hampikian G. Subjectivity and bias in forensic DNA mixture interpretation. *Sci Justice.* 2011; 51(4):204–208.
- Duncan E, Setzke E, Lehmann J. Isolation of genomic DNA. In Bowien B, Dürre P. (Eds.), *Nucleic acids isolation methods Stevenson Ranch, California: American Scientific Publishers, 2003; 7–19.*
- Garvin AM, Bottinelli M, Gola M, Conti A, Soldati G. DNA preparation from sexual assault cases by selective degradation of contaminating DNA from the victim. *J Forensic Sci.* 2009; 54:1297–1303.
- Garvin AM, Fischer A, Schnee-Griese J, Jelinski A, Bottinelli M, Soldati G, Tubio M, castella V, Monney N, Malik N, Madrid M. Isolating DNA from sexual assault cases: a comparison of standard methods with a nuclease-based approach. *Investig Genet.* 2012; 3:25.
- Gill P, Buckleton J. Biological basis for DNA evidence. In Buckleton J, Triggs CM, Walsh S.J. (Eds.), *Forensic DNA Evidence Interpretation*. Boca Raton, FL: CRC Press. 2005; 1–25.
- Gill P, Jeffreys AJ, Werrett DJ. Forensic application of DNA fingerprints. *Nature.* 1985; 318: 577–579.
- Gill P, Gusmão L, Haned H, Mayre WR, Morling N, Parson W, Prieto K, Prinz M, Schneider H, Schneider PM, Weir BS. DNA commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods. *Forensic Sci Int Genet.* 2012; 6:679–668.
- Hanson EK, Ballantyne J. The forensic application of Y-chromosome short-tandem repeats, In: Saferstein R (Ed.). *Forensic Science Handbook, vol. III*. Upper Saddle River, New Jersey: Prentice Hall. 2009; 436–466.
- Hudlow WR, Buoncristiani MR. Development of a rapid, 96-well alkaline based differential DNA extraction method for sexual assault evidence. *Forensic Sci Int Genet.* 2012; 6:1–16.
- Kishore R, Hardy WH, Anderson, VJ, Sanchez NA, Buoncristiani MR. Optimization of DNA Extraction from Low-Yield and Degraded Samples Using the BioRobot®EZ1 and BioRobot®. *J Forensic Sci.* 2006; 51(5):1055–1061.
- Krenke BE, Nassif N, Sprecher CJ, Knox C, Schwandt M, Storts DR. Developmental validation of a real-time PCR assay for the simultaneous quantification of total human and male DNA. *Forensic Sci Int Genet.* 2009; 3:14–21.
- Loreille OM, Diegoli TM, Irwin JA, Coble MD, Parsons TJ. 2007. High efficiency DNA extraction from bone by total demineralization. *Forensic Sci Int Genet.* 2007; 1: 191–195.
- Lounsbury JA, Nambiar SM, Karlsson A, Cunniffe H, Norris JV, Ferrance JP, Landers JP. Enhanced recovery of spermatozoa and comprehensive lysis of epithelial cells from sexual assault samples having a low cell counts or aged up to one year. *Forensic Sci Int Genet.* 2014; 8:84–89.
- Maiquilla SB, Salvador JM, Calacal GC, Sagum MS, Dalet MM, Delfin FC, Tabbada KA, Franco SL, Perdigon HB, Madrid BJ, Tan MP, De Ungria MCA. Y-STR DNA analysis of 154 female child sexual assault cases in the Philippines. *Int J Legal Med.* 2011; 125(6):817–824.
- McCord B, Opel K, Funes M, Zoppis S, Jantz LM. An investigation of the effect of DNA degradation and inhibition on PCR amplification of single source and mixed forensic samples. *ncjrs.gov.* 2011.
- Moretti TR., Baumstark AL, Defenbaugh, DA, Keys KM, Smerick JB, Budolwe B. 2001. Validation of short tandem repeats (STRs) for forensic usage: performance testing of fluorescent multiplex STR systems and analysis of authentic

- and simulated forensic samples. *J Forensic Sci.* 2001; 46:647–660.
- Nicklas JA, Noreault-Conti T, Buel E. Development of a real-time method to detect DNA degradation in forensic samples. *J Forensic Sci.* 2012; 57(2):466–471.
- Norris JV, Evander M, Horsman-Hall KM, Nilsson J, Laurell T, Landers JP. Acoustic differential extraction for forensic analysis of sexual assault evidence. *Anal Chem.* 2009; 81:6089–6095.
- Norris JV, Manning K, Linke SJ, Ferrance JP, Landers JP. Expedited chemically enhanced sperm cell recovery from cotton swabs for rape kit analysis. *J Forensic Sci.* 2007; 52: 800–805.
- Psifidi A, Chrysostomos DI, Bramis G, Lazou T, Russel CL, Arsenos G, Banos B. Comparison of Eleven Methods for Genomic DNA Extraction Suitable for Large-Scale Whole-Genome Genotyping and Long-Term DNA Banking Using Blood Samples. *PLOS ONE.* 2015; 10(1):1–18.
- Sugue-Castillo M. Legal outcomes of sexually abused children evaluated at the Philippine General Hospital Child Protection Unit. *Child Abuse Negl.* 2009; 33(3):193–202.
- Taylor D, Bright JA, Buckleton JS. The interpretation of single source and mixed DNA profiles. *Forensic Sci Int Genet.* 2013; 7:516–528.
- Tsukada K, Asamura H, Ota M, Kobayashi K, Fukushima H. Sperm DNA extraction from mixed stains using the Differex™ system. *Int Cong Ser.* 2006; 700–703.
- Uchiyama THR, Maeda I, Nakata M, Ohno RHT, Shimizu K, Akashi R. DNA extraction from sperm found in mixed stains of seminal fluid and vaginal secretion using DNase I. *Forensic Sci Technol.* 2006; 11:105–112.
- Vogelstein B, Gillespie D. Preparative and analytical purification of DNA from agarose. *Proc Natl Acad Sci USA.* 1979; 76:615–619.
- Voorhees JC, Ferrance JP, Landers JP. Enhanced elution of sperm from cottonswabs via enzymatic digestion for rape kit analysis. *J Forensic Sci.* 2006; 51:574–578.
- Vuichard S, Borer U, Bottinelli M, Christian C, Malik N, Meier V, Gehrig C, Sulzer A, Morerod M, Castella V. Differential DNA extraction of challenging simulated sexual-assault samples: a Swiss collaborative study. *Investig Genet.* 2011; 2:11
- Wallin JM, Buoncristiani MR, Lazaruk KD, Fildes N, Holt CL, Walsh PS. TWGDAM validation of the AmpFISTR blue PCR amplification kit for forensic casework analysis. *J Forensic Sci.* 1998; 43(4):854–870.