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Post-Coital DNA Recovery Study in Minority Proxy Couples

Final Summary Report

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Final Summary Overview

Background. Timing limits of 48-72 hours for sample collection following rape is predicated on pre-DNA science. With advancing DNA science, the NIJ OJP funded the Post Coital DNA Recovery (PCDR) study (2009-DN-BX-0023), which focused on timing of DNA detection in a national sample of volunteer proxy couples. The study used a prospective, blinded design of abstinence and timed collections across four 10-day periods, collecting at baseline (10^{th} day of abstinence protocol) with collections on the 4^{th}, 7^{th}, and 9^{th} day following by semen deposits. The blinded samples were tested using *Y-filer* STR (included both female X and male Y) and enhanced *Y-filer* Y-STR (female X eliminated, leaving Y chromosome only) methods. Findings revealed DNA detection after 9 days in over 65% of reproductive aged females. However, subjects were Caucasian (93%) and expanded collection times were not generalizable to minority populations. Evidence reveals that larger percentages of minorities experience rape; and are less likely to report or experience delayed reporting. Published research reports medical differences between races, supporting duplication of the PCDR validated research protocol in minority populations, specifically to determine post-coital DNA recovery timing. Based on literature, the hypothesis is: Minority couples experience different DNA detection levels and influences.

Aims. The Post Coital DNA Recovery in Minority Proxy Couples (PCDR-M) study aims were recruit adult minority couples to repeat the validated PCDR protocol answering the following: What is the level of DNA recovery at timed intervals post-unprotected coitus in minority couples using standard STR and enhanced Y-STR methods? and When compared to a small sample of new and former study non-minority, what are factors influencing post-coital DNA recovery?

Design and Methods. The PCDR-M study is a prospective, mixed methods research design with qualitative and blinded quantitative analytical methods. The analysis includes duplication of

mixed methods perfected in previous PCDR study embracing descriptive (means and standard deviations from continuous data and categorical data frequencies and proportions) and inferential techniques (Chi-square, Fisher's exact, t-test, among others) using interviews, blinded self-reports, and laboratory data. Semi-structured qualitative interviews and adverse childhood events queries occur throughout the study and notes about questions and statements were recorded. Semi-structured interview analysis from the minority population uses NVivo™ for the qualitative research to detect themes related to the participant's research experience. The steps to meet the first aim in the study included use of minority data only in the first analysis. The steps to meet the second aim included determination of differences between PCDR and PCDR-M populations. If not difference, researchers propose to compare data from PCDR and PCDR-M combined data from both studies in the final analysis. DNA recovery identifies a binary outcome analysis using Generalized Estimating Equation (GEE) methods, accounting for repeated methods in population regression analysis. The study received full IRB approval: UAB IRB# 160111005.

Sample, sample activities and data documentation. The sample was volunteer minority monogamous couples screened according to study protocol and inclusion criteria who maintained fidelity to the validated study protocol. The eligible minority couples consented individually, provided demographic and pertinent personal health data, and received detailed protocol instructions, including use of a daily diary card for recording the single sexual activity of unprotected coitus at the beginning of each of four 10-day periods, providing abstinence rules for protected coitus throughout the study protocol and instructions for recording daily health conditions over the length of the study protocol. At the baseline visit, both members of the minority couple brought the completed diary card demonstrating the abstinence protocol and daily health data, providing buccal swabs for DNA identity analysis. The female also provided cervical

and vaginal fornix samples at baseline. Following a 10-day baseline abstinence period, couples had one unprotected coitus and a variable number of protected coitus under the abstinence protocol. At the designated time (at 4, 7 or 9 days respectively), the female submitted to a cervical-vaginal sampling. The sample was put in a paper drying container, sealed, labeled with unique deidentified number, and stored securely until all sample collections were complete. The protocol period ranged from 34 to 39 days. If sample re-collection was necessary, the couple followed the 10-day abstinence protocol and completed new diary cards, appeared for collection of baseline, followed by one unprotected coitus, and returned for re-collection on the designated date, with transfer of samples to the forensic laboratory for DNA analysis. After all collections, the forensic laboratory received the uniquely identified blinded samples and sexual activity data.

Laboratory activities. Once the laboratory received the couple's deidentified samples, the lab developed the samples using the currently available *Yfiler Plus*™ DNA kit. The laboratory data reported in alleles according to the testing method. The laboratory testing method compared STR standard kit methods to the Y-STR enhanced methods perfected in the first study protocol (Post Coital DNA Recovery, 2014). However, during the current minority study (2014-18), it was decided to utilize the *Yfiler Plus*™ kit (27 alleles), rather than *Yfiler*™ kit (17 alleles) because there were scientific advances in methodology and most operational crime laboratories advanced to the use of the *Yfiler Plus*™. All samples in the minority study were subjected to the *Yfiler Plus*™. The *Yfiler Plus*™ kit contains all *Y-filer* loci, plus an additional 10 loci for additional discrimination. Therefore, in the second study with minorities, the lab was still able to compare the *Yfiler*™ loci, obtained from the *Yfiler Plus*™ kit amplification, with the *Yfiler*™ results from the first study. While comparison of the same loci occurred, it should be noted that it is not an direct comparison entirely since *Yfiler*™ and *Yfiler Plus*™ are different kits with likely different

amp efficiencies and possibly different primer sets for some loci. However, these differences are far less than the added benefit of the use of the *Yfiler Plus*™ kits, including the additional discrimination and the potential demonstration of the ability to obtain some male profiles in extended interval post coital samples using only standard methods (i.e. those methods currently used by operational crime laboratories without any additional validations needed as is the case with the enhanced Y-STR approach).

Statistician activities. The statistical evaluators received blinded uniquely identified data that included personal health information, demographic, and study data recorded by the Primary Investigator (PI) and Project Manager (PM) throughout the study, transferred to Excel files. Before transfer of deidentified data to the statisticians and following transfer to *Excel*™, the PI and PM randomly selected 14 charts for quality review (eliminating 4 incomplete or study participant dropouts), reaching 100% accuracy in both data element coding, and data transfer from *Microsoft Word*™ to *Excel*™.

Results. The reporting of results is according to the aims of the study. *Aim 1* is What is the level of DNA recovery at timed intervals post-unprotected coitus in minority couples using standard STR and enhanced Y-STR methods? To answer the query, researchers cast a wide net to the Minority population in a large Southern minority-majority urban and rural community. The inquiring minority population was large (N=778) where persons who self-eliminated remains unknown. First, persons self-screened, then called, and were screened again by the PM. To eliminate coercion between couple members, the persons meeting the inclusion criteria were asked to consent individually. Once individually consented, the couple was asked to begin the protocol, and each was instructed accordingly. The minority sample included 53 males and 53 females (N=106) where at least one person in the couple identified as a minority. Over a 2-year

period there was one duplicate (eliminated). All beginning couples (N=52) completed and submitted accompanying data from collection forms, collectors submitted evaluation data, and the forensic laboratory submitted DNA recovery information, performed with *Yfiler Plus*™ Y-STR and *Yfiler*™ enhanced Y-STR on the submitted samples, reported in alleles. The PCDR-M participant proxy couple females (N=52) self-identified as African American (70%), menstruating more than 2 periods each year (95%), between 18-35 (82.7%), and college educated (78.9%). One in five minority females reported traumas in their lives and couples had between 5 and 20 lifetime sexual partners (49%). The majority reported normal menstrual cycles that varied in length, were equal in reports about pregnancy or not, and the women used a variety of birth control methods, including hormones. There were multiple recollection requests (N=36) resulting in 22 (61.1 %) dropouts, with recollections (n=14 [38.8%]). The minority sample of 52 couples reduced to 39 completed kits and provided a variety of reasons for not completing the study protocol or did not return outreach communication. Of the 39 kits accepted for analysis, 16 (41%) were rejected leaving 23 (44.2%). The reasoning for kit rejection included obvious high allele levels on samples, reflecting non-adherence to the study protocol (such as multiple unprotected or condom failure intercourses, no detectable intercourses, and/or multiple male identities. Of the 23 (59%) kits accepted for study analysis, 19 had full and expected allele levels for baseline and post coitus allele levels (4, 7 or 9 days post coitus) and 4 had 1 timepoint removed, resulting in 130 samples possible for analysis (46 4-days, 44 7-days, and 40 9-days). Of the 130 samples, the *Yfiler Plus*™ standard method revealed 64 (48%) had at least one allele, and 66 (52%) had no detectable alleles. The *Yfiler*™ enhanced method revealed 98 (75%) had at least one allele. *Yfiler Plus*™ standard testing revealed 48% allele detection, and *Yfiler*™ enhanced testing revealed 75% allele detection, duplicating the results of the first study. Of note, standard

detection of few alleles is not necessarily probative, but detection of increasing numbers of alleles with enhanced testing may report a partial profile, allowing discrimination of individuals. The previous PCDR study was a national representation of proxy couples, where the sample was primarily Caucasian (93%). Minorities (7%) participated, but results were not generalizable to minority populations. To prepare to answer the PCDR-M Aim 2, both studies followed the validated PCDR protocol, allowing for comparison of data from the current minority and previous non-minority sample. Researchers determined homogeneity between the two populations where there was no difference between minority and non-minority populations in DNA detection with standard (p=0.7927) or enhanced methods (p=0.4465), allowing for combining the data and comparison. The PCDR-M *Aim 2* is When compared to a small sample of new and former study non-minority, what are factors influencing post-coital DNA recovery? To answer the Aim 2 query, the data from the PCDR-M study data was combined with the previous study data. The combined kits (N=89) revealed 84 had full and expected allele levels for baseline and post coitus allele levels (4, 7 or 9 days post coitus) and 5 had 1 timepoint removed, resulting in 351 samples possible for analysis (89 4-days, 88 7-days, and 86 9-days). Of the 343 samples, Yfiler Plus™ standard method revealed 118 (34.4%) had at least one allele, and 225 (65.6%) had no detectable alleles. Yfiler™ enhanced method revealed 277 (78.9%) had at least one allele. Yfiler Plus™ standard testing revealed 34.4% (31.0% for 2014; 44.3 for 2018) allele detection, and Yfiler™ enhanced testing revealed 78.9% (79.5% for 2014; 77.3 for 2018) allele detection, approximating the results of the first PCDR study, with implications for laboratory protocols and algorithms with extended post coital interval samples.

Table 1. *Comparing standard Y-STR to enhanced Y-STR from combined cervix and posterior fornix samples reflecting recovery in percentages across days*

Laboratory Method Used	Day 4 Collection	Day 7 Collection	Day 9 Collection	Day 10 Collection
STR*	46.90%	26.60%	26.60%	25.00%
Enhanced Y-STR	92.40%	78.80%	78.80%	67.70%

*Y-filer Plus used 17 of 27 loci in PCDR-M to match PCDR study methods

Table 2. *Adjusted Odds Ratio of DNA recovery (cervix or posterior fornix) using repeated measures adjusting for occurrence of hormonal birth control and menstrual period using STR and enhanced Y-STR methods*

HORMONE BIRTH CONTROL				MENSTRUATION			
STR		Enhanced Y-STR		STR		Enhanced Y-STR	
OR (CI)	*p* value	OR (CI)	*p* value	OR (CI)	*p* value	OR (CI)	*p* value
0.2000 (0.0959 to 0.5047)	0.0004	0.6254 (0.2977 to 0.3138)	0.2152	0.5412 (0.2974 to 0.9849)	0.0445	0.5251 (0.2664 to 1.0350)	0.0628

Factors Influencing DNA Recovery. The first PCDR study incidental finding was that combining swabs increases the percent of DNA recovery. In the current study, when swabs are combined and recovery of alleles is evaluated, *Yfiler Plus*™ standard Y-STR vs *Yfiler*™ enhanced Y-STR from the posterior fornix and the cervix, the percentage change in DNA recovery is evident when combining the two swabs (baseline – 22.09 v 63.64%; 4 days – 52.87 v 91.01%; 7 days – 29.07 v 78.41%; and 9 days – 33.33 v 82.56%), providing the evidence for a practice change related to the

collection method, swabbing the cervical and posterior fornix region, starting with the cervical os first, then the cervical body, and last the posterior fornix.

Fidelity. In both PCDR and PCDR-M studies, there was significant dropout and absence of fidelity to the protocol for similar reasons, such as negotiation of sex between partners, condom use, multiple male DNA, and separation. However, the data used to compare the two groups had specific homogeneity lacking statistical significance across all times and with both standard ($p=0.7927$) and enhanced ($p=0.4465$), including the same inclusion and elimination criteria for volunteer proxy couples in both the PCDR and PCDR-M studies; fidelity to the validated PCDR protocol over time for included couples; laboratory methods analysis with *Yfiler Plus*™, followed by identification of identical 17 loci in the first study, standardized laboratory inclusion and exclusion criteria for samples submitted, as well as inclusion and exclusion following testing results; rigorous statistical analysis and interpretation of data results. Any variation from inclusion criteria resulted in elimination from the study, enhancing study protocol fidelity.

Assumptions and Limitations. Assumptions in both the PCDR and PCDR-M included (1) motivation to participate is altruistic for some; (2) motivation to participate is the incentives and coercion for some; (3) negotiating coitus in monogamous couples is a difficult process, fraught with stressful interaction; and (4) fidelity and dropout rates would duplicate first study findings. The limitations in both the PCDR and PCDR-M included (1) a lack of representation for the diverse experiences of rape victims; (2) small PCDR-M sample size; (3) self-selection bias; (4) adherence to the complex protocol; and (4) advances in laboratory science and DNA kits required allele deconstruction to match as closely as possible previous testing methods.

Implications for Criminal Justice. The PCDR study developed an in vivo study protocol establishing a valid scientific foundation for data collected to study expanded post-coital interval

in DNA detection and influencing variables. Since the first PCDR study, expanded post-coital interval detection benefited victims seeking justice by recommending expansion of timing protocols to 9 days. The purpose of the current PCDR-M study was to determine if the first study results were generalizable to the minority populations, if there is a similar influence of menses and hormone birth control use, re-evaluate incidental findings, and discuss implications for the criminal justice system. The barriers to the current PCDR-M study included historical recruitment and retention of minority populations in clinical research, previously noted in scientific research; and advancing science, especially when capacity and workflow improvements are a constant challenge for forensic laboratories with limited resources. In the PCDR-M study period and since the first PCDR study, many laboratories adopted the *Yfiler Plus*™ kit in DNA detection. To compare previous study outcomes, the forensic research laboratory deconstructed the *Yfiler Plus*™, identifying the 17 alleles specifically used in the former study, for use in the current comparison analysis. As with the first PCDR study, the current PCDR-M study compared standard *Yfiler Plus*™ methods to the enhanced *Yfiler*™ Y-STR methods, using 17 identical alleles, and the results are that the standard *Yfiler Plus*™ method is insufficient in DNA detection from both the cervix and posterior fornix at all timed collections (4, 7, 9, and baseline or 10 days). The enhanced *Yfiler*™ Y-STR method was unchanged between the first and second studies. The enhanced Y-STR method is based only on the *Yfiler*™ kit and therefore this portion of the analysis could not be advanced to the use of the *Yfiler Plus*™ as well.

In the interest of criminal justice improvements, influenced by the rape-kit backlog and application of newer DNA detection methods not heretofore available for older kits, this research provides laboratories the evidence to review protocols related to expanded timing of collections to 9 days for ***all*** reproductive-aged women, regardless of race, triaging to the appropriate scientific

method to identify probative samples from the medical forensic clinical setting. Future research uses the information from this study to develop studies that utilize the next generation of advanced *Yfiler*™ Y-STR kits in the enhanced methods as well. The existing laboratory capacities and standards inform improvements in workforce efficiency when making decisions about new evidence supporting extended post-coital interval collections. The incorporation of the new evidence informs the triage process, directing extended post-coital interval samples to the appropriate testing method in laboratories, thereby improving workflow and cost savings.

The PCDR-M study data also demonstrated a similar association of two variables with diminished DNA recovery – menses and hormonal birth control. There is no statistical significance in DNA recovery using combined Y-STR enhanced methods when reporting menstruation (Baseline p=0.0954, Day 4 p=.1372, Day 7 p=0.6824, Day 9 p=0.3626), supporting the notion that the association is with hormone birth control. The combined data revealed no significance in DNA recovery with Y-STR enhanced methods when reporting hormonal birth control, but with the standard STR method, there is statistical significance in DNA recovery declining at Baseline (Day 10, Day 7 and Day 9 (p=0.0457, 0.007, 0.0503) respectfully, but not at Day 4 (p=0.8317), causing pause when choosing the standard STR method for reproductive aged female samples in the extended post coital interval, when hormone birth control is reported.

Another finding is that PCDR-M data, like the data from the previous PCDR study, supports a practice change to a single swabbing in the cervical os structures first, followed by the cervical neck, and last into the posterior fornix fluid pool (to avoid increasing risk of depositing contaminants into the exposed cervical os and canal), improving allele detection from swabbing of one area, not one structure at a time, which has noteworthy implications for research about combining swabbings into regions or areas following rape, possibly increasing detection of DNA

when there is low volume with extended post coital intervals. Future studies quantifying timing and swabbing of areas following rape events promises to benefit criminal justice processes by informing laboratories about triage for probative samples from delayed post-coital sampling, improving efficiency, regardless of laboratory capacity.

After the first PCDR study, communities increased the timing for collections to 5 days, when the combined data reveal detection of DNA to 9 days, implying that advancement in science is ongoing with the potential for probative sampling for reproductive aged women up to 9 days using enhanced *Yfiler*™ Y-STR methods. The minority study results are similar and provide opportunities for justice for reproductive-aged minority victims of sexual assault when there are expanded post-coital intervals upon report. The evidence from the combined data analysis to expand the timing for **all** reproductive aged survivor intervention up to 9 days and develop nimble laboratory triage algorithms for emerging evidence-based DNA detection reflects an understanding that laboratory science is evolving rapidly. For all reproductive aged victims experiencing delayed reporting, justice may be an outcome for these victims with evidence collection during an expanded post-coital interval, where medical forensic personnel have opportunity to implement trauma-informed and patient-centered care, laboratories have the opportunity to implement triage systems based on capacity and emerging science, and all have the potential to improve the survivor's engagement with the criminal justice system. While the majority of reporting rape victims are reproductive aged, lacking is evidence to (1) expand post coital interval collections in a growing population of menopausal females when 1 in 5 will be over the age of 65 in 2030, all without estrogen effect (with implications for child rape), or to (2) understand DNA recovery from the growing population of infertile or vasectomized males.

In future research, using the validated PCDR protocol, the special circumstances of the post coital environment promises to reveal (1) the influence of surgical or medical menopause in elderly victims on DNA recovery, with implications for children who have little or no estrogen; and (2) the influence of low/no sperm on DNA recovery in medically declared infertile males. Finally, the PCDR-M found that when previous PCDR data from validated post-coital recovery protocols were combined, the expanded post-coital interval is generalizable to all populations of reproductive-aged women, regardless of minority status, promising to improve criminal justice benefits for **all** reproductive aged women.