

TECHNICAL NOTE**GENERAL**

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Comparing Standard and Selective Degradation DNA Extraction Methods: Results from a Field Experiment with Sexual Assault Kits*†

ABSTRACT: A growing number of U.S. cities have large numbers of untested sexual assault kits (SAKs) in police property facilities. Testing older kits and maintaining current case work will be challenging for forensic laboratories, creating a need for more efficient testing methods. **Methods:** We evaluated selective degradation methods for DNA extraction using actual case work from a sample of previously unsubmitted SAKs in Detroit, Michigan. We randomly assigned 350 kits to either standard or selective degradation testing methods and then compared DNA testing rates and CODIS entry rates between the two groups. **Results and conclusions:** Continuation-ratio modeling showed no significant differences, indicating that the selective degradation method had no decrement in performance relative to customary methods. Follow-up equivalence tests indicated that CODIS entry rates for the two methods could differ by more than $\pm 5\%$. Selective degradation methods required less personnel time for testing and scientific review than standard testing.

KEYWORDS: forensic science, selective degradation, DNA, forensic testing, sexual assault kits, rape kits, CODIS

Large numbers of untested sexual assault kits (SAKs) have been found in police property facilities throughout the United States (1–6). This growing national problem has raised concerns among criminal justice system practitioners and victim advocates alike. When SAKs (also termed ‘rape kits’) are not submitted for forensic DNA testing, there is no opportunity for the evidence within these kits to help prosecute perpetrators, protect public safety, and/or exonerate those who have been wrongly accused of crimes they did not commit (1,6). Even if tested years after their original collection, previously untested SAKs still have the potential to provide actionable information for police and prosecutors, so many jurisdictions are deciding to submit their kits for forensic testing, often thousands of SAKs at a time (5), which presents substantial challenges for forensic science laboratories. To meet these increasing demands for testing, it is essential that laboratories have DNA testing methods that are time, staff, and

resource efficient, without sacrificing quality. To that end, in this study, we examined an emerging method that has shown promise in the forensic science literature for its efficiency and quality—selective degradation (7,8). Using actual casework from one U.S. city that had thousands of untested SAKs in police property, Detroit, Michigan, we compared the forensic testing outcomes of SAKs analyzed with selective degradation or standard methods.

Over the past thirty years, there have been revolutionary changes in how biological evidence can be tested and used by the criminal justice system (9–12). The general process of testing crime scene evidence for DNA includes body fluid identification (serology) screening of the samples to determine whether they contain biological evidence (e.g., semen, saliva, blood), extracting the DNA from the samples, quantifying the amount of DNA extracted, characterizing the DNA, and finally analyzing and interpreting the results (9). Over the years, different methods have been developed for the extraction, amplification, separation, and analysis steps, and in the past decade, newer methods have been developed that allows forensic scientists to skip traditional serology screening in favor of faster screening methods that determine whether there is any male DNA in the samples (Y-marker screening methods). The development of short tandem repeat (STR) typing techniques and polymerase chain reaction (PCR) methods have been particularly impactful advancements for offering faster analysis with smaller samples (10). There have also been significant successes in decreasing overall testing time through the use of automation (13) and microchip technology (14,15).

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*Supported by a grant from the National Institute of Justice (2011-DN-BX-0001).

†The opinions or points of view expressed in this document are those of the authors and do not reflect the official position of the U.S. Department of Justice.

Received 10 Dec. 2015; and in revised form 18 April 2016; accepted 24 April 2016.

More recently, other strategies for reducing testing time have emerged, ones that focus on improving efficiency within specific steps of the DNA testing process. One of the more time-consuming processes in testing samples from SAKs is separating the sperm cells from all other cells (e.g., male epithelial cells, female epithelial cells). National epidemiological data indicate that most sexual assault victims are female and most perpetrators are male (16), so the task of separating male and female DNA is necessary for the vast majority of SAKs submitted for forensic testing. Traditionally, the differential lysis method has been used, which “relies on separation of intact sperm from the DNA of digested epithelial cells by centrifugation and careful removal of supernatant, a process that remains unchanged since it was first described in 1985” (7). For decades, standard practice has been to physically separate the offender’s sperm from all other cells by use of a centrifuge and then to wash the sample repeatedly to remove any remaining female DNA. This process for DNA extraction has been criticized for its tediousness, difficulty to automate, and riskiness for losing sperm from the sample (8).

To address these problems, selective degradation methods were created, which utilize chemical techniques for the separation of male and female DNA (see Fig. 1). Nonsperm cells (e.g., male offender epithelial, female victim epithelial cells) are chemically lysed; the sperm heads are resistant to this process and are not lysed. This first step produces a pellet of sperm heads with residual DNA from the lysed nonsperm cells. A nuclease is used to remove remnants of nonsperm DNA from the sperm head pellet. The nuclease is deactivated prior to chemical rupture of the sperm heads, producing a relatively pure male DNA sample for further analysis (7,8). As Garvin and colleagues (2009) explained, “the addition of a degradative agent is inherently easier than a physical separation process and can require only a single pipetting step” (7, pp. 1297).

To evaluate the efficacy of the selective degradation method, Garvin et al. (2009) analyzed postcoital vaginal swabs from consensual vaginal–penile sexual intercourse; swabs were divided lengthwise and then randomly assigned to DNA testing method (standard vs. selective degradation). As expected, the samples treated with the nuclease had far less contamination by female DNA, making identification of the male profile substantially easier. Garvin et al. (2009) also obtained archived swabs from five criminal sexual assault cases and randomly assigned samples to testing condition. In three samples, there was similar performance between the two methods, and for two samples, selective degradation produced superior STR profiles than standard methods. In a follow-up efficacy study, Garvin et al. (2012) compared STR profiles generated by selective degradation and standard methods from three types of samples: semen-spiked female buccal swabs, postcoital vaginal swabs from consensual sex, and four samples from actual criminal sexual assault casework. Across all three types of samples, the STR profiles of male DNA fractions extracted via selective degradation were of equal or better quality than those obtained using standard methods.

The results of these efficacy studies are promising, so a key next step in this line of research is to evaluate selective degradation methods with larger samples of actual sexual assault casework. It is also important to examine whether there are any “downstream” implications of this testing method with respect to whether and how the results may be utilized by criminal justice system practitioners; specifically, in regard to whether a profile will qualify for entry into CODIS. CODIS (Combined DNA Index System) is the U.S. national forensic DNA database,

which consists of reference DNA profiles from arrestees/convicted offenders and from samples obtained at crime scenes (9,17–19). A DNA profile may be eligible for entry into CODIS if it meets specified standards regarding biological quality of the sample, and reasonable assurances that a crime was in fact committed and that the forensic sample is most likely from the alleged perpetrator (9,11,12,18–20). If a DNA profile meets these standards, then it can be compared to the reference samples in CODIS, and if there is a match (termed a “hit”), then law enforcement personnel have a promising investigative lead.

To date, no prior studies have compared rates of CODIS entry stemming from different DNA testing methods to determine whether resulting profiles are more or less likely to qualify for CODIS. Because a CODIS entry is a necessary condition for a CODIS hit, it is useful to examine this outcome as a first step in understanding how testing methods can affect law enforcement and prosecutorial utilization of forensic testing findings. If there are “downstream” differences such that CODIS entry rates for a particular DNA testing method are significantly higher or lower than another method, such findings could be concerning for criminal justice system practitioners. However, if the rates of CODIS entry are functionally equivalent across testing methods, but one method is notably more resource efficient, then such information could be helpful to laboratory administrators and their colleagues in law enforcement and prosecution for establishing methods for SAK testing, particularly if this effect was established with actual criminal sexual assault casework.

Therefore, the purpose of this study was to examine forensic testing outcomes in a sample of 350 SAKs from Detroit, Michigan. This sample was selected from the larger population of approximately 8,500 untested SAKs that were found in a Detroit police department storage facility in August, 2009 (21). When these untested SAKs were discovered, a multidisciplinary action research project was formed with funding from the National Institute of Justice to bring together researchers and law enforcement personnel, prosecutors, forensic scientists, nurses/medical personnel, and victim advocates to develop data-driven response strategies (22). A primary goal of this action research project was to develop empirically informed approaches for resolving large numbers of untested SAKs, which provided an opportunity to evaluate promising DNA testing methods with actual casework. To that end, we randomly selected a sample of SAKs ($N = 350$) and randomly assigned kits to either standard DNA testing methods or selective degradation methods. In contrast to prior formative studies on selective degradation by Garvin and colleagues (7,8), our focus was not the forensic adequacy of the method (i.e., STR profile quality), but rather its potential utility to criminal justice system practitioners. As such, we quantified and compared how many SAKs in each testing group proceeded from serology screening to DNA testing to CODIS-eligible profiles, as well as how the methods compared with respect to the cost of consumable supplies and the amount of personnel effort required to test, interpret, and review the testing results.

Methods

Sample

The sample contained a total of 350 SAKs, which were associated with a total of 344 unique assailants. Descriptive characteristics of the victims, offenders, and sexual assaults associated with these SAKs are documented in Table 1 (for each testing method and for the overall sample). Nearly all of these victims

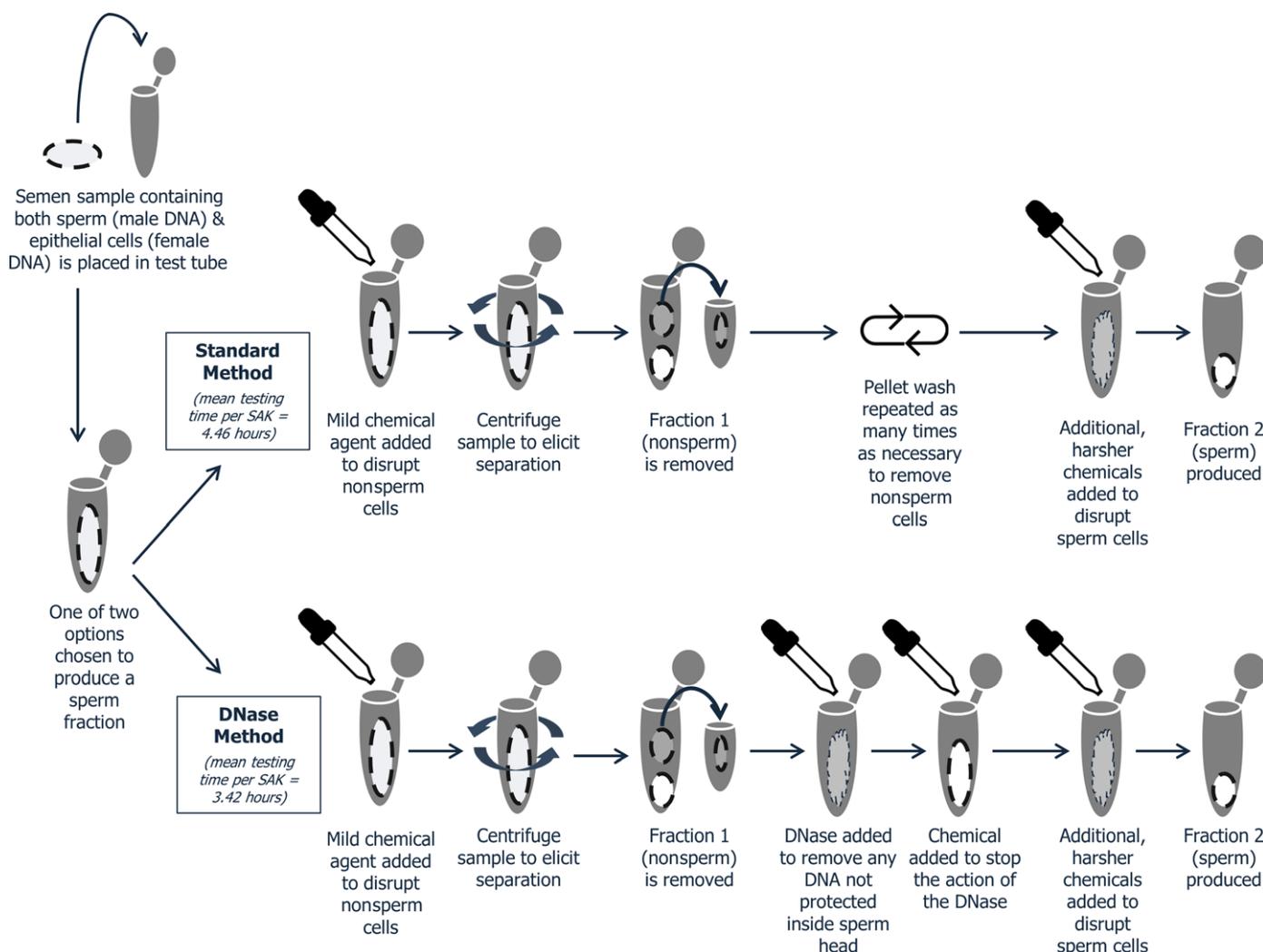


FIG. 1—Comparing standard and selective degradation DNA extraction methods. [Color figure can be viewed at wileyonlinelibrary.com]

were female (96.6%) and the assailants were male (94.9%). It is important to note that in female-perpetrated sexual assaults, selective degradation methods would not be appropriate (regardless of which testing condition to which the kit was assigned), and standard methods would be utilized. In this project, only one SAK associated with a female assailant was assigned to the selective degradation testing group, but it did not pass the body fluid identification (serology) screening, so this was not a concern in this study. Four of the SAKs randomly assigned to the selective degradation group had missing data regarding perpetrator gender; there was no additional information in the medical forensic report to suggest that the offender was female, and given that the overwhelming majority of SAKs received by the state police forensic science division have male perpetrators, testing proceeded as though these four offenders were male. Among the SAKs associated with sexual assaults in which there were multiple perpetrators, the assailants were exclusively male for all but one SAK, which had missing data regarding assailant gender; that kit had been randomly assigned to the standard testing group, so the missing data regarding offender gender were not problematic. With respect to race/ethnicity, most of the victims and assailants were African American (80.0% and 88.9%, respectively), consistent with the demographic composition of Detroit. The victims were, on average, 23.04 years old at the

time of the assault, and the assailants were somewhat older ($M = 28.49$ years old). The SAKs had been collected, on average, 6.55 years prior to the time they were selected for testing, with a range of 4–11 years ago. Accounting for missing data, 21.1% of the assaults were committed by a stranger and 60.9% were committed by someone known to the victim.

Procedures and Measures

We randomly assigned half of the SAKs to each DNA testing method (0 = standard testing; 1 = selective degradation testing). The state police outsourced forensic testing of the SAKs to a private laboratory capable of performing selective degradation testing methods. A site visit was conducted at the vendor laboratory before shipment of the kits to ensure that testing procedures and quality control processes met standards established by the state police forensic science division.

Forensic testing is a multistage process whereby SAKs pass on from one stage to the next only if they meet stage-specific transition criteria (10,12); hence, the last stage of testing reached by each SAK is an ordinal variable. We reorganized the data to record a set of stage-specific binary outcome variables showing whether each SAK continued to the next stage after it reached a given stage (0 = no, 1 = yes). For this study, we focused on

TABLE 1—Victim, offender, and assault characteristics.

	Standard DNA Testing (n = 175)		Selective Degradation DNA Testing (n = 175)		Combined (N = 350)	
	n	%	n	%	n	%
Victim gender						
Female	169	96.6%	169	96.6%	338	96.6%
Male	4	2.3%	6	3.4%	10	2.9%
Missing	2	1.1%	0	0.0%	2	0.6%
Victim race						
African American	131	74.9%	149	85.1%	280	80.0%
Caucasian	37	21.1%	26	14.9%	63	18.0%
Hispanic/Latina	2	1.1%	0	0.0%	2	0.6%
Missing	5	2.9%	0	0.0%	5	1.4%
Victim age						
< 16 years old	43	24.6%	35	20.0%	78	22.3%
≥ 16 years old	128	73.1%	139	79.4%	267	76.3%
Missing	4	2.3%	1	0.6%	5	1.4%
Assailant gender						
Female	2	1.1%	1	0.6%	3	0.9%
Male	162	92.6%	170	97.1%	332	94.9%
Missing	11	6.3%	4	2.3%	15	4.3%
Assailant race						
African American	151	86.3%	160	91.4%	311	88.9%
Asian American/ Pacific Islander	0	0.0%	1	0.6%	1	0.3%
Caucasian	12	6.9%	7	4.0%	19	5.4%
Hispanic/Latina	2	0.0%	1	0.6%	3	0.3%
Multiracial	0	0.0%	1	0.6%	1	0.3%
Missing	10	5.7%	5	2.9%	15	4.3%
Assailant age						
< 22 years old	40	22.9%	30	17.1%	70	20.0%
≥ 22 years old	93	53.1%	94	53.7%	187	53.4%
Missing	42	24.0%	51	29.1%	93	26.6%
Victim-offender relationship						
Stranger	40	22.9%	34	19.4%	74	21.1%
By Sight/nickname	15	8.6%	16	9.1%	31	8.9%
Friend/associate/ family member	79	45.1%	78	44.6%	157	44.9%
Current/past intimate Partner	12	6.9%	13	7.4%	25	7.1%
Missing	29	16.6	34	19.4	63	18.0
Victim age						
Mean (SD)		23.56 (11.18)		22.54 (9.21)		23.04 (10.23)
Range		3–55		11–52		3–55
Missing		4		1		5
Assailant age						
Mean (SD)		28.29 (10.04)		28.71 (9.99)		28.49 (10.01)
Range		8–59		11–61		8–61
Missing		42		51		93
How long ago assault occurred (years as of December 31, 2013)						
Mean (SD)		6.51 (2.35)		6.60 (2.31)		6.55 (2.32)
Range		4–11		4–11		4–11
Missing		29		41		70

two specific transition points in this process. First, we computed how many SAKs passed serology screening (i.e., the samples in the kit contained bodily fluids that could be analyzed), which we termed Stage 0, and proceeded to DNA testing, which we termed Stage 1. The probability that a kit will proceed from Stage 0 to Stage 1 can be quantified as the *DNA Testing Rate*. We did not expect a significant difference in the DNA testing rate between the two testing conditions because what distinguishes the two groups occurs during Stage 1, but we quantified and compared this rate to verify that randomization to testing condition was successful.

For the SAKs randomly assigned to the standard DNA testing condition, if semen was present via Christmas tree staining and microscopic observation, the forensic scientist used a standard

differential extraction method to separate the sperm from the nonsperm cells. For the SAKs in the selective degradation condition, if semen was present, the sample was chemically lysed, separated by centrifugation, and the resulting sperm pellet treated with a nuclease to purify the sperm. Once the sperm cells were isolated, testing in both conditions proceeded per usual PCR-STR methods. The DNA quantitation method used quantitative PCR using an Applied Biosystems 7500 Real Time PCR instrument and Promega's Plexor HY quantitation chemistry. The short tandem repeat (STR) kit was Promega's PowerPlex 16HS chemistry. If no semen was present, the kit testing was halted and no further testing occurred regardless of group.

The second key transition point in the testing process that we examined was whether the resulting DNA profile (whether obtained by standard methods or selective degradation methods) met minimum state requirements for completeness and eligibility for entry into CODIS (Stage 2). The probability that a kit will pass from Stage 1 to Stage 2 was quantified as the *CODIS Entry Rate*. Comparing the CODIS entry rates between the two testing conditions was the key focus of this study. We did not examine whether those CODIS entries resulted in a CODIS hit (i.e., *CODIS Hit Rate*, Stage 3) because a hit depends on the match between the contents of new CODIS entries and other records already stored in CODIS (not on the method used to extract the DNA). Any effect of testing method on the CODIS hit rate *must* be mediated by either what is being entered into CODIS or what was already present in CODIS.

The vendor laboratory completed stages 0 and 1 in the testing process. Forensic scientists at that laboratory recorded whether each SAK passed the serology screening, the presence of sperm (0 = absent, 1 = present), the cost of consumable supplies (in US dollars, including the costs of waste, controls, and reprocessing), and laboratory personnel effort spent on testing and reviewing test results (in hours). Then, state police forensic scientists reviewed the results, entered eligible profiles into CODIS (Stage 2), and recorded CODIS entry outcomes and personnel effort spent reviewing test results (in hours). The testing outcomes for each SAK (whether it advanced from Stage 0 to Stage 1, then from Stage 1 to Stage 2), the presence of sperm, and the staff time and cost of consumable supplies required to test/review the results were recorded and sent to the research team.

The cost and personnel effort data were sometimes recorded as aggregate values for batches of multiple SAKs and other times recorded separately for each SAK. This inconsistency in the level of detail recorded requires that we aggregate these data and present only descriptive summaries, as we are not aware of any statistical method that can adequately quantify the sampling variation expected around the estimates given the inconsistent way the data were recorded.

Data Analytic Plan

We used continuation-ratio models (23,24) to quantify and compare the forensic outcomes (i.e., DNA testing and CODIS entry rates) as a function of DNA testing method. Continuation-ratio models are an appropriate analytic choice for evaluating sequential selection processes (23), which aligned well with our focus on examining SAK progression through these two transition points (or stages) in the forensic testing process.

Our base model regressed stage-specific binary outcomes showing whether a SAK continued passed each stage to reach the next one (0 = no, 1 = yes) on main effects for stage and DNA testing method, plus a stage times method interaction. The

DNA Testing Rate is an unconditional rate that reflects the proportion of SAKs submitted for testing that passed serology screening, indicating that there was sufficient biological evidence present in the SAK to warrant DNA testing. By contrast, the *CODIS entry rate* is a conditional rate that corresponds to the proportion of SAKs that were actually tested (i.e., that passed the screening at Stage 0) that yielded DNA profiles suitable for upload into CODIS. Focusing on the conditional estimate ensures that we have a clean comparison between the two DNA testing methods under conditions where laboratory personnel believe there is enough biological evidence present in the SAKs that extracting an offender’s DNA profile is actually possible.

In addition to reporting these rates, we also report odds ratios (ORs), relative risks (RRs), and number needed to submit (NNS, which is based on the number needed to treat [NNT]; (25,26) to facilitate interpretation of the findings. Relative risk reflects how much more often an event happens (e.g., a CODIS entry) for one group (e.g., SAKs tested with selective degradation methods) relative to another group (e.g., SAKs tested with standard methods). *RR* is computed by dividing the CODIS entry rate for the selective degradation testing group by the CODIS entry rate for standard group, which tells us how much more likely that outcome (CODIS entry) is for the former group versus the latter. The *NNS* is another useful index for understanding differential rates between two groups. Larger absolute values of *NNS* indicate that more SAKs would need to be submitted and tested to obtain a one-unit difference between the groups; if it takes a substantial number of SAKs to yield just a one-unit difference in the focal outcome (e.g., CODIS-eligible profiles), then that suggests the two groups are fairly similar and it may not make practical sense to treat them differentially.

Results from two separate analyses are presented below: (1) a base model that omits covariates; and (2) a model that accounts for a binary covariate, namely the presence of sperm in the SAK, acting as a moderator of the testing method effect. In this context, a moderator is a covariate that modifies how strongly a focal predictor affects the outcome of interest. Here, we consider the possibility that the testing method effect on DNA testing rates and CODIS entry rate depends on whether sperm is present in the SAK. The selective degradation testing method is designed to work by selectively degrading DNA not contributed by a sperm cell. Thus, it has the greatest potential to outperform standard testing when sperm is present. It also has some potential to perform worse when sperm is not present because other assailant DNA that is present may be degraded. Accounting for this potential moderator may be important in understanding when standard versus selective degradation testing may yield different results. To examine whether the testing method influences forensic outcomes after we account for the presence or absence of sperm, we extended the model to include a stage by testing method by presence of sperm interaction effect, then looked at the simple main effect of testing method on each rate separately when sperm was absent and when it was present.

We also directly examined whether the two groups yield conditional and unconditional CODIS entry rates that are *functionally equivalent*. Conventional statistical tests, such as the continuation-ratio model, adopt a null hypothesis that *there is no difference between groups* (i.e., their outcomes are exactly equal). A nonsignificant finding from a conventional test yields only an “absence of evidence” with respect to the hypothesis that two groups have equivalent outcomes. Establishing that groups have equivalent outcomes requires generating credible “evidence of absence” with respect to group differences, which

is the purpose of equivalence tests. These statistical methods adopt the null hypothesis that *the outcomes for the groups are not equivalent* (i.e., the difference is large enough to be important) (27–31). Only when the analysis provides strong evidence refuting that assumption can we conclude that groups are equivalent. Explicitly defining equivalence in advance is crucial for these tests. When rates are expressed as proportions, the margin of equivalence can be expressed as either an odds ratio or an absolute risk reduction ($ARR = p_{SD} - p_S$) (28). We set the margin of equivalence for the ARR at 5% because feedback from forensic science stakeholders (two at the state level and two at the national level) suggested that CODIS entry rates for the two groups that are within 5% of each other ($-0.05 < ARR < 0.05$) would warrant considering the two DNA testing methods functionally equivalent.

We analyzed the data with R 3.2.2 (32) and several R packages (33–40). All data, raw statistical output, and R code used to obtain our results have been submitted for archiving in the National Archive of Criminal Justice Data (NACJD) to promote reproducible research.

Results and Discussion

Conditional Rates for DNA Testing and CODIS Entry: Moderator Omitted

Figure 2 shows the effect of DNA testing method on the testing outcomes when we omit the potential moderator (presence of sperm) from the model. The first panel shows that, as expected, random assignment eliminated systematic differences with respect to the presence of biological evidence (see supplemental file for further interpretation of the difference in DNA testing rates).

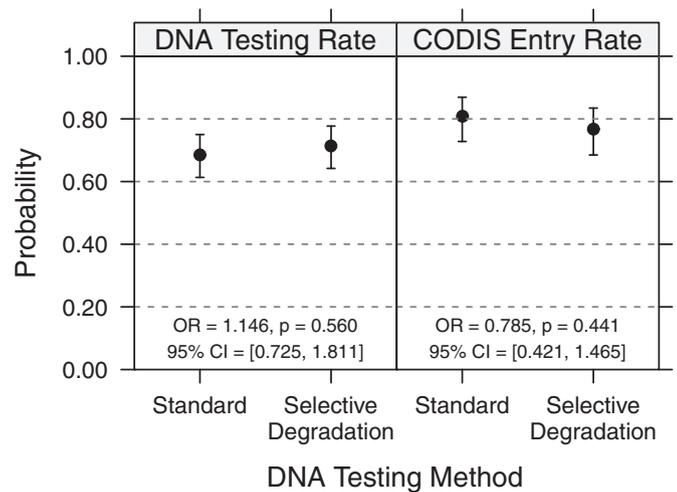


FIG. 2—Testing method effect on DNA testing and CODIS entry rates among SOL-unexpired detroit SAKs. The DNA testing rate is an unconditional estimate (the proportion of SAKs that pass the Stage 0 serology screening to reach the actual DNA test at Stage 1). The CODIS entry rate is a conditional estimate (the proportion of SAKs tested that yielded a DNA profile suitable for upload into CODIS). These estimates generalize to the subpopulation of untested, SOL-unexpired Detroit SAKs (regardless of adjudication status or victim–offender relationship). After selecting N = 350 SAKs from that subpopulation, n = 175 SAKs were randomly allocated to each of the two testing methods. These estimates were obtained from a continuation-ratio model of SAK progression across stages 0–2. The dots mark the estimated rates; whiskers show corresponding 95% CIs. The odds ratios (OR) and associated 95% CIs quantify the simple effect of testing method on the rate named in each panel.

The conditional *CODIS Entry Rate* for SAKs in the standard group was 80.8% (95% CI = [72.8, 86.9]), as compared to 76.8% (95% CI = [68.5, 83.4]) in the selective degradation group (see Table 2). The effect size is very small and nonsignificant (OR = 0.78, 95% CI = [0.42, 1.46], $p = 0.441$). The $RR = 0.95$ (95% CI = [0.83, 1.07]) indicates that SAKs allocated to selective degradation testing are about 0.95 times less likely to yield CODIS entries than standard testing. Furthermore, the $NNS = -24.79$ (95% CI = [-87.64, 38.05]) means that testing about 25 SAKs containing biological evidence via selective degradation testing would likely yield one *less* CODIS entry than we would expect from standard testing of a similar number SAKs containing biological evidence (i.e., 19.04 vs. 20.04 CODIS entries).

The continuation-ratio model without the moderator term does not offer strong evidence for a difference between testing methods, so we also computed an equivalence test to evaluate whether the conditional CODIS entry rates are functionally equivalent. The $ARR = -4\%$ was imprecisely estimated (90% CI = [-12.6, 4.5]), with the lower bound of that CI falling far outside the $\pm 5\%$ margin of equivalence. So, CODIS entry rates for the two groups are not equivalent because the rate yielded by selective degradation testing may be more than 5% lower than the rate yielded by standard testing.

We also examined results of the experiment in terms of unconditional rates when we omit the potential moderator from the model. Because our focus is on conditional CODIS entry rates, we put Figure S1 (which contains the unconditional rates) and interpretive comments in the supplemental file.

Conditional Rates for DNA Testing and CODIS Entry: Moderator Included

Figure 3 shows the effect of testing method on the testing outcomes when we include the presence of sperm as a moderator in

the model. The top panels show the results when sperm was absent; the bottom panels show the results when sperm was present. Comparing Figs 2 and 3 highlights the fact that each panel in the former is essentially a weighted average of the top and bottom panels from the latter (with weights based on the numbers of SAKs with and without sperm). The first panel on each row shows that accounting for the moderator does not alter the finding that random assignment eliminated systematic differences with respect to the presence of biological evidence (see supplemental file for further interpretation of the difference in DNA testing rates).

When sperm was absent, the conditional *CODIS Entry Rate* was 20.0% (95% CI = [05.0, 54.1]) for SAKs in the standard test group and 33.3% (95% CI = [12.9, 62.8]) in the selective degradation group (see Table 3). The wide confidence intervals for these estimates (top right panel, Fig. 3) are likely due to the low DNA testing rates, which effectively reduced the sample size and increased the uncertainty surrounding the estimated proportions and the corresponding effect size. Thus, although the difference in the odds of a CODIS entry was nominally moderate and nonsignificant (OR = 2.00, 95% CI = [0.28, 14.57], $p = 0.489$), it could plausibly be substantially larger and favor *either* group. There is far too much sampling variability to be certain either way because the CI spans a range consistent with a large negative effect to a very large positive effect. The $RR = 1.67$ (95% CI = [0.0, 4.13]) indicates that when SAKs containing biological evidence but no sperm are allocated to selective degradation testing, they are about 1.67 times more likely to yield CODIS entries than similar SAKs allocated to the standard test. Furthermore, the $NNS = 7.50$ (95% CI = [-13.00, 28.00]) means that testing 7.50 SAKs containing biological evidence but no sperm via selective degradation testing would likely yield one *more* CODIS entry than we would expect from standard testing of a similar number SAKs containing biological evidence but no sperm (i.e., 2.50 vs. 1.50 CODIS entries).

TABLE 2—Continuation-ratio model for DNA testing method effect (moderator omitted).

Model Effects							
Parameter	Estimate	SE	Rate	Rate 95% CI	Odds Ratio	OR 95% CI	p -value
Stage							
Stage 0→1: DNA testing rate Standard	0.780	0.163	0.686	[0.613, 0.750]	2.182	[1.585, 3.003]	<0.001
Stage 1→2: CODIS entry rate Standard	1.439	0.232	0.808	[0.728, 0.869]	4.217	[2.676, 6.647]	<0.001
Testing method							
Stage 0→1: Selective degradation effect on DNA testing rate	0.136	0.234	0.534	[0.420, 0.644]	1.146	[0.725, 1.811]	0.560
Stage by Testing Method Interaction							
Stage 1→2: Selective degradation effect adj. for CODIS entry rate	-0.378	0.392	0.407	[0.241, 0.596]	0.685	[0.318, 1.476]	0.335
Derived Contrasts							
Specific Rates	Estimate	SE	Rate	Rate 95% CI	Odds Ratio*	OR 95% CI*	p -value
Stage 0→1: DNA testing rate Selective degradation	0.916	0.167	0.714	[0.642, 0.777]			<0.001
Stage 1→2: CODIS entry rate Selective degradation	1.197	0.212	0.768	[0.685, 0.834]			<0.001
Simple Main Effects	Estimate	SE	Rate [†]	Rate 95% CI [†]	Odds Ratio	OR 95% CI	p -value
Stage 1→2: Selective degradation effect on CODIS entry rate	-0.242	0.314			0.785	[0.421, 1.465]	0.441

These results generalize to the subpopulation of untested, SOL-unexpired Detroit SAKs (regardless of adjudication status or victim-offender relationship). After selecting $N = 350$ SAKs from that subpopulation, $n = 175$ SAKs were randomly allocated to each of the two testing methods. These estimates were obtained from a continuation-ratio model of SAK progression across stages 0–2 that omitted the presence of sperm moderator. Model fit statistics: total $df = 595$, residual $df = 591$, null deviance = 824.8, residual deviance = 680, $AIC = 688$.

*Odds ratios and corresponding CIs are not reported because these contrasts combine coefficients into values that are more meaningful when transformed back into stage-specific transition rates for particular subsets of SAKs.

[†]Rates and corresponding CIs are not reported because these contrasts combine coefficients to directly quantify the simple main effect of DNA testing method on the rate for a particular stage transition; odds ratios are a more meaningful metric for examining a difference between the rates observed in two subsets of SAKs.

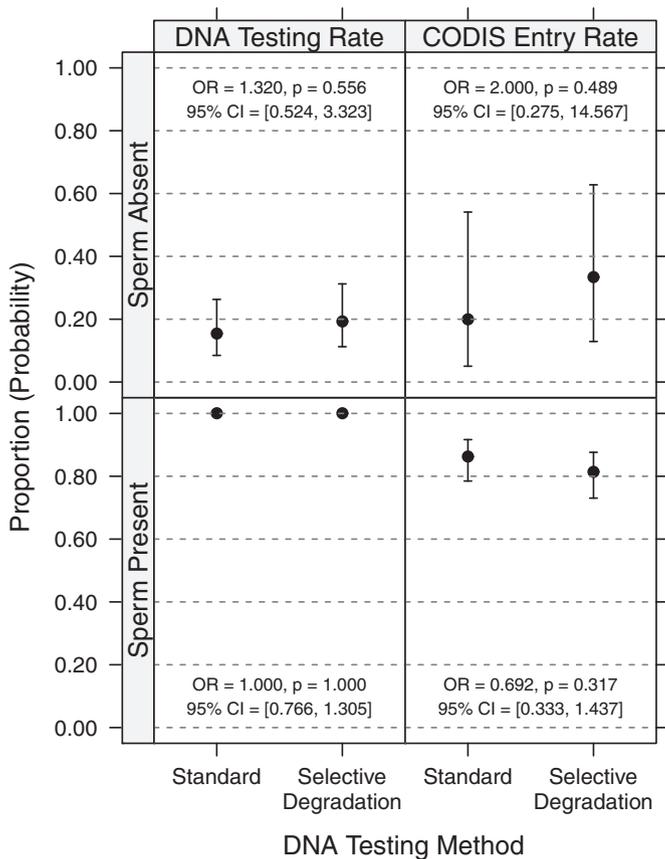


FIG. 3—Simple main effects of testing method on DNA testing and CODIS entry rates among SOL-unexpired detroit SAKs. Depending on Whether Sperm Was Absent From or Present in the SAK. The DNA testing rate is an unconditional estimate (the proportion of SAKs that pass the Stage 0 serology screening to reach Stage 1 (the actual DNA test)). The CODIS entry rate is a conditional estimate (the proportion of SAKs tested that yielded a DNA profile suitable for upload into CODIS). These results generalize to the subpopulation of untested, SOL-unexpired Detroit SAKs (regardless of adjudication status or victim-offender relationship). After selecting N = 350 SAKs from that subpopulation, n = 175 SAKs were randomly allocated to each of the two testing methods. The SAKs were sorted by the presence/absence of sperm in the samples. These estimates were obtained from a continuation-ratio model of SAK progression across stages 0–2 that contained a three-way stage by testing method by presence of sperm interaction effect. The dots mark the estimated rates; whiskers show corresponding 95% CIs. The odds ratios (OR) and associated 95% CIs quantify the simple effect of testing method on the rate named in each panel.

Examining the difference in the conditional CODIS entry rates (ARR) when sperm was absent reinforced the finding from the difference test. The ARR = 13.3% lies above the upper end of the ±5% margin of equivalence and the 90% CI (−17.3, 43.9) is so wide (due to estimating the rates from just 10-12 SAKs per group) that it extends beyond the lower end of the equivalence margin. This indicates that the rates are not equivalent because the difference between them could be larger than 5% in either direction when sperm are absent.

When sperm was present, the conditional CODIS Entry Rate was 86.4% (95% CI = [78.5, 91.7]) for SAKs in the standard test group and 81.4% (95% CI = [73.0, 87.6]) in the selective degradation test group. This would be considered a small, statistically nonsignificant, negative effect (OR = 0.69, 95% CI = [0.33, 1.44], p = 0.317) on the odds of a CODIS entry that could reflect nothing more than random sampling variation. However, the wide CI for the OR is consistent with a range of

plausible effect sizes falling anywhere between a large negative effect and a small positive one. The RR = 0.94 (95% CI = [0.83, 1.05]) indicates that SAKs allocated to selective degradation testing are about 0.94 times less likely to yield CODIS entries than SAKs allocated to the standard test. Furthermore, the NMS = −20.21 (95% CI = [−59.55, 19.12]) means that testing 20.21 SAKs containing sperm via selective degradation testing would likely yield one less CODIS entry than we would expect from standard testing of a similar number SAKs containing sperm (i.e., 16.46 vs. 17.46 CODIS entries).

The equivalence test directly examining the difference in conditional CODIS entry rates also turned up an unusual result when sperm was present. The observed ARR = −4.9% in favor of standard testing lies right above the lower bound for the margin of equivalence. The 90% CI (−13.0, 3.1) therefore spans that boundary with almost half of the interval on each side. This means the rates are not equivalent. The two rates might differ by less than 5% (indicating equivalence), but it is almost equally plausible that standard testing yields a rate more than 5% higher than that yielded by selective degradation testing.

The unconditional rates based on the model including the potential moderator are shown in Figure S2. Because our focus is on conditional CODIS entry rates, our interpretation of those unconditional rates accompanies Figure S2 in the supplemental file.

Comparisons of Testing Costs and Personnel Effort Between Testing Methods

In this experiment, we also examined whether the two methods differ with respect to cost and personnel effort required. Assuming equal performance with respect to forensic outcomes, a testing method that costs less or enables personnel to work more efficiently might be preferable. Table 4 summarizes cost of consumable supplies used during testing, the amount of laboratory personnel time spent on testing and reviewing the results, and the amount of state police forensic science division personnel time spent reviewing the DNA test results. There was little difference in total cost of supplies (\$16.20 total, \$0.09 per SAK) between the two methods, which could be entirely attributable to the difference in costs at the Stage 0 screening.

The aggregate time spent by laboratory personnel on standard testing was 848.50 h, which was 192.50 more hours than laboratory personnel spent on selective degradation testing (656.00 h) for the same number of SAKs (N = 175 each). Most of the difference was time spent on the actual testing (181.75 h) rather than reviewing results (10.75 h). Laboratory personnel spent an average of 4.85 h/SAK on standard DNA testing; they spent an average of 3.75 h/SAK on selective degradation testing. Therefore, selective degradation testing method saved an average of 1.10 h/SAK in laboratory personnel time relative to standard testing. Cumulated across a large collection of SAKs, this may yield substantial savings on personnel costs, but we cannot assess how much sampling variation one might expect in these estimates from the current data. The true average time savings for laboratory personnel could be higher or lower, but we cannot provide a valid CI because the data were already partially aggregated when we received them.

The aggregate difference in state police forensic science personnel time spent on reviewing test results was substantially smaller (a total of 10.85 h). Reviewing results from standard testing took state police forensic science division personnel a

TABLE 3—Continuation-ratio model for DNA testing method effect (moderator included).

Model Effects							
Parameter	Estimate	SE	Rate	Rate 95% CI	Odds Ratio	OR 95% CI	p-value
Stage							
Stage 0→1: DNA testing rate 0 sperm, Standard	-1.705	0.344	0.154	[0.085, 0.263]	0.182	[0.093, 0.357]	<0.001
Stage 1→2: CODIS entry rate 0 sperm, Standard	-1.386	0.791	0.200	[0.050, 0.541]	0.250	[0.053, 1.179]	0.080
Testing method							
Stage 0→1: Selective degradation effect on DNA testing rate 0 sperm	0.278	0.471	0.569	[0.344, 0.769]	1.320	[0.524, 3.323]	0.556
Sperm							
Stage 0→1: 1 sperm effect on DNA testing rate Standard	21.271	0.355	1.000	[1.000, 1.000]	1.73E+09	[8.63E+08, 3.46E+09]	<0.001
Stage by testing method interaction							
Stage 1→2: Selective degradation effect on CODIS entry rate	0.416	1.106	0.602	[0.148, 0.930]	1.515	[0.173, 13.244]	0.707
Stage by Sperm Interaction							
Stage 1→2: 1 sperm effect on CODIS entry rate	-18.039	0.911	0.000	[0.000, 0.000]	0.000	[0.000, 0.000]	<0.001
Testing Method by Sperm Interaction							
Stage 1→2: Selective degradation effect times 1 sperm effect adj. for CODIS entry rate	-0.278	0.490	0.431	[0.225, 0.664]	0.758	[0.290, 1.978]	0.571
Stage by Testing Method by Sperm Interaction							
Stage 1→2: 1 sperm times Selective degradation times CODIS entry rate effect	-0.784	1.174	0.313	[0.044, 0.820]	0.457	[0.046, 4.555]	0.504
Derived Contrasts							
Specific Rates	Estimate	SE	Rate	Rate 95% CI	Odds Ratio*	OR 95% CI*	p-value
Stage 0→1: DNA testing rate 0 sperm, selective degradation	-1.427	0.322	0.194	[0.113, 0.312]			<0.001
Stage 1→2: CODIS entry rate 0 sperm, selective degradation	-0.693	0.613	0.333	[0.129, 0.628]			0.258
Stage 0→1: DNA testing rate 1 sperm, standard	19.566	0.086	1.000	[1.000, 1.000]			<0.001
Stage 1→2: CODIS entry rate 1 sperm, standard	1.846	0.278	0.864	[0.785, 0.917]			<0.001
Stage 0→1: DNA testing rate 1 sperm, selective degradation	19.566	0.085	1.000	[1.000, 1.000]			<0.001
Stage 1→2: CODIS entry rate 1 sperm, selective degradation e	1.477	0.242	0.814	[0.730, 0.876]			<0.001
Simple Main Effects							
Stage 0→1: Selective degradation effect on DNA testing rate 1 sperm	0.000	0.134			1.000	[0.766, 1.305]	1.000
Stage 1→2: Selective degradation effect on CODIS entry rate 0 sperm	0.693	1.001			2.000	[0.275, 14.567]	0.489
Stage 1→2: Selective degradation effect on CODIS entry rate 1 sperm	-0.369	0.369			0.692	[0.333, 1.437]	0.317

These results generalize to the subpopulation of untested, SOL-unexpired Detroit SAKs (regardless of adjudication status or victim-offender relationship). After selecting $N = 350$ SAKs from that subpopulation, $n = 175$ SAKs were randomly allocated to each of the two testing methods. The SAKs were sorted by the presence/absence of sperm in the samples. These estimates were obtained from a continuation-ratio model of SAK progression across stages 0–2 that contained a three-way stage by testing method by presence of sperm interaction effect (the moderator). Model fit statistics: total $df = 595$, residual $df = 587$, null deviance = 824.8, residual deviance = 338.2, $AIC = 354.2$.

*Odds ratios and corresponding CIs are not reported because these contrasts combine coefficients into values that are more meaningful when transformed back into stage-specific transition rates for particular subsets of SAKs.

†Rates and corresponding CIs are not reported because these contrasts combine coefficients to directly quantify the simple main effect of DNA testing method on the rate for a particular stage transition; odds ratios are a more meaningful metric for examining a difference between the rates observed in two subsets of SAKs.

TABLE 4—Cost and personnel effect comparisons: standard testing versus selective degradation.

Variable	Standard (S) (n = 175)	Selective Degradation (SD) (n = 175)	Difference (S – SD)
Total consumable supplies cost (\$)	52,986.76	52,970.56	16.20
SAKs negative at screening (no DNA test)	6,406.12	6389.92	16.20
SAKs positive at screening (DNA test)	46,580.64	46,580.64	0.00
Mean consumable supplies cost per SAK (\$)	302.78	302.69	0.09
Vendor laboratory personnel effort (h)			
Total testing time	780.75	599.00	181.75
Total reviewing time	67.75	57.00	10.75
Total testing + reviewing time	848.50	656.00	192.50
Mean testing time per SAK	4.46	3.42	1.04
Mean reviewing time per SAK	0.39	0.33	0.06
Mean testing + reviewing time per SAK	4.85	3.75	1.10
State police forensic science personnel effort (h)			
Total review time	119.90	109.05	10.85
Mean review time per SAK	0.69	0.62	0.06

Sample included $N = 350$ SAKs (175/group). Cost estimates include waste, controls, and reprocessing. SAK = sexual assault kit.

total of 119.90 h, while reviewing results from selective degradation testing took a total of 109.05 h. On average, that suggests adopting selective degradation testing could save about 0.07 h of

personnel time per SAK. Again, we cannot assess how much sampling variation one might expect for that figure due to partially aggregated data.

Discussion of Findings

A growing body of research indicates that there are likely hundreds of thousands of untested SAKs in police property facilities throughout the United States (1,6). These kits could contain useful, actionable information for law enforcement personnel and prosecutors, so many communities with large numbers of untested rape kits have elected to have all kits tested for DNA (41). In addition, many U.S. states are passing laws requiring that all SAKs be submitted for forensic testing (41–44). It will be challenging for forensic laboratories to process older, previously untested SAKs, and maintain timely turnaround on current case work. Decreasing testing time, without sacrificing testing quality, is one of many strategies that could help meet this challenge. Emerging literature suggests that selective degradation methods may offer a more efficient approach for DNA extraction, and initial efficacy studies indicate that the technique produces good quality STR profiles (7,8).

In this project, we looked further “downstream” in the process of forensic testing to explore whether selective degradation methods yielded comparable rates of CODIS-eligible DNA profiles compared to standard testing methods. Using actual case work from a sample of previously unsubmitted SAKs in Detroit, Michigan, we randomly assigned 350 kits to either standard or selective degradation testing methods. We then quantified and compared the forensic testing results with respect to DNA testing rates and CODIS entry rates. The continuation-ratio model showed no significant differences between the two groups, indicating that the selective degradation method had no decrement in performance relative to customary methods. Follow-up equivalence tests, however, indicated that the CODIS entry rates for the two methods could differ by more than $\pm 5\%$, due to wide confidence intervals around the estimates.

Taken together, these results are somewhat conflicting: The significance test model showed no difference between the rates of CODIS-eligible profiles for standard and selective degradation methods, but the more rigorous test of equivalence could not establish that the rates were statistically equivalent. Future studies should use larger samples because narrower confidence intervals around the estimated difference between the CODIS entry rates associated with standard and selective degradation DNA testing methods will clarify whether these rates differ meaningfully, allowing researchers to discern more definitively whether selective degradation performs at least as well as standard testing with respect to CODIS entry rates. The data from this project can help facilitate sample size planning required to obtain high precision estimates (45,46). Our estimates were not precise enough to determine unambiguously whether any differences in CODIS entry rates between testing methods were small enough to be substantively unimportant.

The findings of this study are clearer regarding the potential *practical* utility of selective degradation methods. We computed number-needed-to-submit (*NNS*) indices, which estimate how many more SAKs would have to be tested to obtain a one-unit difference between the groups. In this study, when sperm were present, the *NNS* = -20.21 , which was quite high and means that we would need to test 20.21 SAKs via selective degradation testing to observe a one-unit change in the number of CODIS entries (one fewer CODIS entry). The practical advantages of selective degradation were also evident in our cost analyses. Materials costs were also similar across the two groups, but the selective degradation method offered a substantial savings in

staffing time for interpretation/review: 1.10 h of laboratory personnel time per SAK relative to standard testing. Cumulated across a large collection of SAKs, selective degradation testing may yield substantial savings on personnel costs, but our results merit replication in other laboratories/settings prior to broad-based implementation.

Study Limitations

It is important to note several limitations of this study that temper the strength of the conclusions that can be drawn from this work. First, it is worth reiterating that selective degradation methods are not appropriate for female-perpetrated assaults, but given that national epidemiological data indicate that the vast majority of sexual assaults are committed by males (1,6), this testing method could be viable for most—but certainly not all—SAK casework. Second, selective degradation has the potential for automation, which was *not* used in this experiment; thus, the potential time savings of this method are *underestimated* in this study. Replication of these findings with automation would provide more sensitive data regarding potential time savings offered by selective degradation. Finally, the focus of this study was not the quality of the STR profiles generated by selective degradation (7,8), but rather, whether there are potential “downstream” differences in the number of CODIS-eligible profiles. We did not explore how these testing results were utilized by criminal justice practitioners in the investigation and prosecution of the cases associated with these SAKs. As such, we do not know whether there were (or will be) any legal challenges to this testing method, which was beyond the scope of this study, but clearly merits examination in future research.

Conclusions

With these caveats in mind, this study was the first large-scale evaluation of selective degradation testing methods with actual SAK casework. Our findings indicate that this method could offer forensic laboratories substantial time savings as a more efficient method for DNA extraction.

Compared to standard testing methods, selective degradation did not produce a significantly higher or lower number of CODIS-eligible DNA profiles, but additional studies are needed because our results were equivocal as to whether the rates were in fact statistically equivalent between the two groups. Additional studies that evaluate both the quality of the STR profiles and the downstream utility and utilization of DNA profiles obtained through selective degradation are needed.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Unconditional DNA testing and CODIS entry rates, by DNA testing method.

Figure S2. Unconditional DNA testing and CODIS entry rates by DNA testing method and sperm absent/present.

Data S1. Comparing Standard and Selective Degradation DNA Extraction Methods: Results from a Field Experiment with Sexual Assault Kits (SAKs).