

## Recovery of DNA from fired and unfired cartridge casings: comparison of two DNA collection methods

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### ABSTRACT

For over 10 years, various studies have attempted to increase the recovery of DNA from ammunition by modifying the DNA collection, extraction, purification, and amplification procedures, with varying levels of success. This study focused on the “soaking” method of Montpetit & O’Donnell [1] and the “rinse-and-swab” method of Bille et al. [2]. First, testing for the presence of exogenous DNA, 210 boxed cartridges (brass, steel, and nickel-plated) from nine manufacturers were swabbed and DNA was extracted, concentrated, and quantified. Extracts that quantified > 0 ng/μL (44 of 210) were amplified and genotyped with GlobalFiler™. Of those, only one extract yielded two alleles indicating that the manufacturing and packaging of ammunition was virtually DNA free. Next, to obtain a baseline comparison of two DNA collection methods on a non-metallic substrate and identify a suitable number of cells to spot on cartridges, different DNA input amounts of primary human adult epidermal keratinocytes (HEKa) were tested. Thereafter, 300 brass and 300 nickel-plated, cartridges were spotted with HEKa cells containing ~5 ng of DNA, fired or unfired, and processed with either method. Finally, five methods representing hybrids of the soaking and rinse-and-swab methods were tested to determine if variations of those methods could be used to increase DNA yield and recovery. The results show that the soaking method consistently yielded more DNA than the rinse-and-swab method from a non-metallic substrate. However, the comparison study demonstrated that both methods performed comparably for cartridges. On average, the soaking method recovered 0.25 ng of DNA (5.1% recovery) and the rinse-and-swab method recovered 0.28 ng (5.8% recovery). However, average recoveries were significantly different among three analysts and considerable variation in yields were observed, possibly due to storage time. Furthermore, consistent with prior reports, the DNA recovered from brass casings was only 16% of that recovered from nickel-plated casings and the average yield of DNA from fired casings was reduced to 67% of unfired casings. Moreover, DNA extracts from brass or nickel-plated casings did not appear to contain amplification inhibitors and only 30/596 appeared severely degraded. Finally, both the published rinse-and-swab and soaking methods yielded more DNA than all modifications of the two methods. Overall, both methods yielded equivalent DNA quantities. Additionally, recovery of DNA from any given cartridge casing may be dependent on storage time as well as the skill, proficiency, and experience of the analyst and may reflect stochastic effects, particularly for casings containing low copy and/or degraded DNA.

### 1. Introduction

Fired cartridge casings (FCCs) are a type of evidence that has received considerable attention in the past decade as a DNA source for solving violent crimes. The analysis of FCCs is an evaluation of touch DNA on a surface known to inhibit and/or degrade DNA. Touch DNA remains a challenging sample type to produce interpretable STR profiles using the standard methodologies in forensic laboratories [3]. Touch

DNA is primarily thought to be composed of DNA brought to the surface of the skin via sweat from eccrine ducts [4], keratinocyte turnover [5,6], and cell-free DNA [4,7]. Sessa et al. examined the amount of time an object needs to be handled to deposit enough DNA for a complete DNA profile. The authors found that garments needed to be handled for just two seconds before enough DNA to process was shed onto the surface of the garment [8]. Moreover, the last person to handle an object is often found to be the major contributor in any DNA recovered [9]. As the

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amount of DNA deposited on a surface by touch can fluctuate between individuals, and even within the same individual depending on several conditions, two groups have set out to create artificial eccrine solutions containing known concentrations of DNA to simulate touch DNA evidence [10,11]. In this work, a similar approach was taken, but with a known amount of keratinocytes, a cell type found in genuine eccrine prints.

The goal of obtaining an interpretable DNA profile from FCCs is to link an individual to a crime involving a firearm. When processing FCC evidence, decisions must be made about whether it should be expedited for swabbing and DNA analysis or examined for another form of evidence, such as fingerprints. One study [12], used specialized swab storage devices to determine if touch DNA could still be recovered two months after swabbing. The results suggested no statistically significant differences in touch DNA recovery from swabs processed immediately versus two months after swabbing. Nonetheless, whether DNA analysis is immediate or delayed, the process of extraction and purification of DNA can result in losses exceeding 60% of the original amount of DNA on the sample [13]. Thus, direct PCR has been proposed as an alternative for touch DNA samples. Direct PCR skips the purification and quantification steps to maximize the template DNA available for STR amplification [3,14]. However, by skipping purification, PCR inhibitors can remain in the sample, potentially reducing the number of alleles observed in the resulting electropherograms [15,16]. Additionally, quantification is a Quality Assurance Standard (QAS) requirement for samples to be entered into the National DNA Index System (NDIS).

The difficulty in producing DNA profiles from FCCs is largely thought to stem from the inhibitory effects of copper ions [17] and degradation from the high temperatures during firing [1,15]. Studies examining the effect of temperature on DNA degradation stemming from the conflagration of smokeless powders have differed in their conclusions. In some instances, the observed temperatures on the surface of the casing and the expelled gas did not reach temperatures sufficiently high enough to degrade DNA [18]. However, Thanakiatkrai and Rerkamnuaychoke found that fired casings lost approximately 30% of surface DNA, possibly due to DNA deposition, collection, or extraction processes [15]. Although evidence is scant for the specific mechanisms by which copper ions inhibit amplification of and/or degrade DNA, one possibility is that the ions bind to DNA and destabilize the double helix [19]. Efforts to reduce the amount of metal ions co-extracted with the DNA should improve downstream amplification of the DNA analysis workflow [17].

Attempts have been made to ameliorate the recovery of touch DNA through modifications of most aspects of the sampling, extraction, and purification steps. Tonkrongjun et al. used a fluorescent dye to more reliably locate touch DNA for a more targeted sampling [20]. Researchers have made modifications to the sampling method by employing the double swab (wet/dry) technique [21] and the use of tape lifts in lieu of swabbing [22]. Samples have been concentrated using Microcon Centrifugal Filters to increase the odds of developing full profiles. Semi-automatable direct lysis methods using liquid handlers, like the Hamilton MicroLab STAR AutoLys robot have shown significant increases in DNA recovery compared to double swab methods by reducing the number of steps requiring human interaction [23]. The addition of 0.5 M ethylenediaminetetraacetic acid (EDTA) to a cotton swab has been found to reduce the effects of copper ions in the extract as EDTA is known to chelate metal ions in solution, reducing the likelihood of copper ion coextraction [24].

Two published methods for the recovery of DNA from FCCs were tested. The first method, developed by Montpetit and O'Donnell and referred to as the "Soaking" method, involves the use of a digestion buffer and Proteinase K (ProK) added to the cartridge or cartridge casing in a 5 mL tube. Following incubation, it is swabbed with a single cotton swab to recover any remaining buffer and DNA. The swab and lysate are extracted further and purified using the EZ1 DNA Investigator kit (QIAGEN, Germantown, MD) on an EZ1 Advanced XL (QIAGEN, Hilden, Germany) [1]. The second method, developed by Bille et al., and

referred to as the "Rinse-and-swab" method, adds bovine serum albumin (BSA) and a tripeptide to the digestion buffer to serve as a metal ion chelating agent. The buffer is rinsed over the casing several times, swabbed with a nylon swab, rinsed again with the same buffer, then swabbed with a cotton swab. Both swabs are co-extracted followed by purification with the QIAamp DNA Investigator kit (QIAGEN) on the QIAcube (QIAGEN) [2]. The two methods have demonstrated interpretable amounts of DNA recovery from FCCs, which supports the use of FCCs as standard evidence for submission into forensic DNA analysis. In this study, we sought to evaluate the soaking and rinse-and-swab methods for their ability to recover DNA from fired and unfired cartridges.

This study was divided into four phases. The first was conducted to determine if unopened boxes of ammunition contain exogenous DNA from the manufacturing and packaging process. Next, the performance of the DNA collection methods was tested on a non-metal substrate. This also served to determine an appropriate DNA input to use for subsequent experiments. Subsequently, the soaking method and the rinse-and-swab method were evaluated using brass and nickel-plated, fired and unfired cartridges. Finally, various components of both DNA collection methods were combined as hybrid methods in an attempt to produce greater DNA recoveries.

## 2. Materials and methods

### 2.1. Sampling for exogenous DNA from manufactured ammunition

A total of 210 cartridges randomly selected from nine manufacturers and 25 different boxes were tested for the presence of exogenous DNA. The description of manufacturers and cartridges sampled for exogenous DNA can be found in [Supplemental Table 1](#).

Ten cartridges were randomly selected from each lot of ammunition and sampled for exogenous DNA. A single cotton swab containing 50  $\mu$ L nuclease-free water was used to swab each cartridge. Each swab head was then placed in an Investigator Lyse&Spin basket (QIAGEN) with 450  $\mu$ L of Lysis Buffer (Buffer G2, ProK, and Dithiothreitol (DTT)) and incubated at 56 °C for one hour with low agitation. Following incubation, the sample tubes were centrifuged at 11,228 x g for five minutes and the spin basket with swab head was discarded. The sample lysates were processed using the EZ1 DNA Investigator Kit on the EZ1 Advanced XL using the Large Volume Protocol and the DNA was eluted in 50  $\mu$ L of water. If the samples were not processed immediately, they were stored at -20 °C until processing.

### 2.2. Evaluating two DNA collection methods using a non-metallic substrate

A total of 240 1.1 mL polypropylene mini tubes (TaKaRa Bio USA, Inc., San Jose, CA) were spotted with two sets of eight different DNA concentrations of cell suspension, with two concentrations overlapping between the sets, and processed by both DNA collection methods to determine a sufficient deposition amount for cartridge cases and to serve as a baseline for DNA recovery.

#### 2.2.1. Keratinocytes

Human Epidermal Keratinocytes, i.e. adult HEKa cells (Thermo Fisher, Waltham, MA), were used as the source of DNA for this project. The stock cell concentration averaged 1500 cells/ $\mu$ L using a Countess II FL Automated Cell Counter (Invitrogen, Waltham, MA) and triplicate readings.

Ten suspensions of HEKa cells were prepared in nuclease-free water, gently mixed to keep the cells intact, and 2  $\mu$ L were spotted onto the mini tubes and allowed to dry overnight. Once dry, each mini tube was separated from their strip and placed in a 5 mL polypropylene screw-cap tube (Axygen, Union City, CA) to minimize potential contamination. The first set of DNA input amounts included 2, 1, 0.5, 0.25, and 0.125 ng of

DNA ( $n = 120$ ), and the second set of DNA input amounts included 20, 10, 5, 2, and 1 ng of DNA ( $n = 120$ ). In total, 12 replicates of each DNA input amount, except 24 replicates for 2 ng and 1 ng, were processed using the soaking method ( $n = 120$ ) and the rinse-and-swab method ( $n = 120$ ).

One HEKa cell suspension (5 ng) was prepared for a third set of samples ( $n = 48$ ) and three analysts processed the DNA-spotted mini tubes with both collection methods.

### 2.2.2. "Soaking" collection method and extraction

This method follows a protocol described by Montpetit & O'Donnell [1]. DNA-spotted mini tubes were placed inside individual 5 mL tubes with the opening in line with the opening of the 5 mL tube. An aliquot of 800  $\mu$ L of digest buffer (10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 2% SDS) and 32  $\mu$ L of ProK was added to each tube, ensuring that the liquid did not get inside the mini tube. Samples were incubated at 56 °C for 30 min on a Nutator (BD Diagnostics, Franklin Lakes, NJ) at low speed in a digital incubator (Labnet International, Inc., Corning, NY). Following incubation, sterile forceps were used to hold the mini tube while the surface of the tube was swabbed with a sterile cotton swab (Puritan, Guilford, ME). The swab head was placed in a Lyse&Spin basket tube and the digest buffer/ProK solution from the 5 mL tube was added to the basket, which was incubated at 56 °C for 90 min in a thermomixer (Benchmark Scientific, Sayreville, NJ) with low agitation. Once incubation was complete, the samples were centrifuged at 11,228  $\times$  g for five minutes and the spin basket was discarded. Lysates were extracted using the EZ1 DNA Investigator Kit on the EZ1 Advanced XL and eluted in 40  $\mu$ L of TE. Extracts proceeded to DNA concentration and DNA quantification as described in Sections 2.5 and 2.6 below. These samples were not genotyped. This protocol was used as described for unfired cartridges and fired casings.

### 2.2.3. "Rinse-and-swab" collection method and extraction

This method follows a protocol described by Bille et al. [2] with modifications (Bille, personal communication). A rinse solution was prepared using 500  $\mu$ L Buffer ATL (QIAGEN) and 30  $\mu$ L BTmix (2 mg/mL UltraPure™ BSA (Invitrogen) and 62.5 mg/mL Gly-Gly-His (GGH) tripeptide (MilliporeSigma, Burlington, MA)) per sample. Each sample was held at a 45° angle with sterile forceps over a disposable 15 mL beaker (Fisher Scientific, Waltham, MA) while one side of the sample was rinsed with 500  $\mu$ L of rinse solution. The sample was turned a quarter turn then rinsed again with 400  $\mu$ L of the collected rinse solution from the beaker. This process was repeated twice more for the two remaining sides and then a final time on the bottom of the sample. Following the first set of rinses, the sample was swabbed with a sterile foam swab (Puritan) and the swab head was placed in a Lyse&Spin basket tube. The entire 5x rinse process was performed a second time using 400  $\mu$ L of collected rinse solution from the same beaker. After the second set of rinses, the sample was swabbed with a sterile cotton swab (Puritan) and the swab head was placed in the same Lyse&Spin basket tube with the foam swab head. The collected rinse solution from the disposable beaker and 25  $\mu$ L of ProK were added to the Lyse&Spin basket containing both swab heads. The samples were incubated at 56 °C for 3 h in a thermomixer with shaking at 900 rpm. Samples were spun at 11,228  $\times$  g for three minutes and the spin basket discarded. Sample lysates were extracted using the QIAamp DNA Investigator Kit on the QIAcube using a custom protocol for surface and buccal swabs (Bille, personal communication), which uses two elutions of 50  $\mu$ L for a final elution of 100  $\mu$ L in ATE buffer instead of the normal volume of 50  $\mu$ L. Extracts proceeded to DNA concentration and DNA quantification as described in Sections 2.5 and 2.6 below. These samples were not genotyped. This protocol was used as described for unfired cartridges and fired casings.

## 2.3. Evaluating two DNA collection methods using fired and unfired, brass and nickel-plated ammunition

A total of 600 cartridges were processed with the two DNA collection methods described above to compare DNA recovery, variability amongst analysts, effect of storage time (time since DNA deposition), and differences in recovery for cartridge types and fired/unfired status.

### 2.3.1. Sample preparation

HEKa cells (2  $\mu$ L at 2.5 ng/ $\mu$ L, 5 ng total) were deposited, by one analyst, just above the rim of 300 brass Winchester Ranger 9 mm Luger, full metal jacket (Winchester Ammunition Inc., East Alton, IL) and 300 nickel-plated Speer G2 Gold Dot 9 mm Luger, hollow point (Speer Ammunition, Lewiston, ID) cartridge cases. Spotting cells in suspension was a reproducible proxy for touch DNA. The cartridges were allowed to dry for a minimum of three hours before being placed back in their original boxes or loaded into a magazine.

### 2.3.2. Material decontamination and preparation

Prior to loading and firing of cartridges, ten Glock 9 mm (Glock Inc., Smyrna, GA) magazines were sanitized and verified DNA free. Each magazine was disassembled and placed in a large beaker containing 10% bleach with agitation at 130 rpm in a large incubator shaker (New Brunswick Scientific Co. Inc., Edison, NJ) for 30 min. The bleach solution was removed from the beaker, NERL™ high purity water (Fisher Diagnostics, Middletown, VA) was added, and the magazine components were rinsed for 15 min with agitation. The magazine components were placed in a DrySafe™ Forensic Evidence Drying Cabinet (AirClean® Systems, Raleigh, NC) until completely dry, then reassembled. The components were swabbed and tested for the presence of DNA before and after decontamination.

A net assembly (Caldwell Shooting Supplies, Columbia, MO) was used to catch cartridge cases once ejected from the firearm during live fire. The net was similarly decontaminated and swabbed for DNA before and after decontamination.

### 2.3.3. Magazine loading and discharge

The 10 DNA-free magazines were divided into two groups: five magazines for brass cartridges only and five magazines for nickel-plated cartridges only. The cartridges were loaded into the magazines using an autoloader (Glock Inc.) to prevent the deposited DNA from being scraped away by any component of the magazine or pressure from the surrounding cartridges. Each magazine was loaded with ten (10) DNA-spotted cartridges.

Of the 600 cartridges used for this experiment, 300 cartridges (150 brass and 150 nickel-plated) were fired with a Glock 19M9 mm Luger (Glock Inc.) into a Bullet Recovery System (EMARK Industries, West Falls, NY). The cartridge casings were automatically ejected from the firearm and caught in a net attached to the water tank by an adjustable arm. Magazines were fired alternating between brass and nickel-plated loaded magazines. After firing 10 rounds, each magazine was reloaded twice, resulting in 30 fired cartridges from each magazine. The collected casings were stored in 5 mL tubes until processed. Four ejected, brass casings were not recovered in the net and were not included in analysis.

For the remaining 300 unfired cartridges, 10 cartridges were loaded per magazine, left in the magazine for two hours, and then unloaded and stored in 5 mL tubes until they were processed. The workflow used for processing the cartridges can be found in Supplemental Fig. 1.

### 2.3.4. Collection and extraction

Three analysts processed 596 cartridges (four unrecovered during firing) with each analyst processing 10 cartridges per magazine, five by each of the collection methods, for both fired and unfired cartridges, both brass and nickel-plated, resulting in ~200 cartridges per analyst. Thus, for each analyst, half of the cartridges ( $n = 100$ ) were processed with the soaking method (2.2.2) and half ( $n = 100$ ) were processed with

**Table 1**

Descriptions of hybrid methods. Shown are the rinse-and-swab (R&S) method (4.1) and soaking method (4.3) for comparison against five alternative hybrid methods (4.2, 4.4, 4.5, 4.6, and 4.7). A check mark (✓) denotes that the condition was used in the experiment.

DNA Collection Method	Experiment number						
	Exp 4.1	Exp 4.2	Exp 4.3	Exp 4.4	Exp 4.5	Exp 4.6	Exp 4.7
Rinse-and-swab (R&S) collection	✓	✓					
R&S rinse buffer w/BTmix	✓	✓		✓	✓		
R&S incubation (3 h)	✓	✓			✓		✓
R&S purification (QIAcube)	✓						
Soaking collection			✓	✓	✓	✓	✓
Soaking rinse buffer			✓				
Soaking rinse buffer w/BTmix						✓	✓
Soaking incubation (90 min)			✓	✓		✓	
Soaking purification (EZ1)		✓	✓	✓	✓	✓	✓

the rinse-and-swab method (2.2.3). The 596 cartridges were randomly divided between the three analysts. Extracts were concentrated, quantified, and genotyped as described below.

#### 2.4. Evaluating protocol modifications for two DNA collection methods using fired brass casings

A total of 175 fired brass casings (Winchester Ranger 9 mm Luger, full metal jacket) were processed with the two unmodified DNA collection protocols and five modifications of the two protocols (25 cartridge casings each) to determine if any efficiencies could be gained by a hybrid approach.

##### 2.4.1. Sample preparation and firing

Cartridges were spotted with 2  $\mu$ L of a 2.5 ng/ $\mu$ L HEKa cell suspension and fired as described previously in 2.3. Collected casings were stored in 5 mL tubes until they were processed. A single analyst processed the 175 casings in this study. Twenty-five DNA spotted casings were processed for each of the seven protocols (Table 1): (1) the rinse-and-swab protocol as described above, without modification, as a baseline for all the modified protocols, (2) the rinse-and-swab method as described, except the EZ1 DNA Investigator Kit and EZ1 Advanced XL were used for purification in lieu of the QIAamp DNA Investigator Kit and QIAcube, (3) the soaking protocol as described above, without modification, as a baseline for the modified protocols, (4) the soaking protocol wherein the rinse-and-swab rinse solution was used in place of the soaking digest buffer, (5) the soaking protocol wherein the rinse-and-swab rinse solution was used in place of the soaking digest buffer and the incubation time was increased from 90 min to three hours, as in the rinse-and-swab protocol, (6) the soaking protocol wherein the rinse-and-swab BTmix was added to the soaking digest buffer, and (7) the soaking protocol wherein the rinse-and-swab BTmix was added to the soaking digest buffer and the incubation was increased from 90 min to three hours, as in the rinse-and-swab protocol.

#### 2.5. DNA extract concentration

All sample extracts were concentrated using either a ThermoSavant SPD1010 SpeedVac® System (Thermo Fisher) or a ThermoSavant ISS110 SpeedVac® System (Thermo Fisher). Samples were reconstituted

in 15  $\mu$ L nuclease-free water.

#### 2.6. DNA quantification

All extracts were quantified using the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher) on the Applied Biosystems™ 7500 Real-Time PCR System with the HID Real-Time PCR Software (Applied Biosystems, Waltham, MA), following the manufacturer's protocol [25].

#### 2.7. Amplification and STR genotyping

All extracts that provided a quantification value  $> 0$  ng/ $\mu$ L for the small autosomal (SA) target with the Quantifiler™ Trio kit were amplified using the GlobalFiler™ PCR Amplification Kit (Thermo Fisher) on a ProFlex PCR System (Thermo Fisher) following the manufacturer's protocol for 28 cycles [26]. The maximum volume of each extract used for amplification was 10  $\mu$ L for concentrations  $\leq 0.1$  ng/ $\mu$ L and higher concentrations were diluted to 0.1 ng/ $\mu$ L. Fragment analysis was performed on the Applied Biosystems™ 3500xL Genetic Analyzer with an injection voltage of 1.2 kV and an injection time of 24 s (Thermo Fisher). Data were analyzed using GeneMapper™ ID-X v1.6 (Thermo Fisher). The analytical threshold was set to 150 Relative Fluorescent Units (RFU) and the stochastic threshold was set to 725 RFU.

#### 2.8. Statistical analysis

To ascertain statistical significance, a Levene's Test for Equality of Variances was performed to determine if the groups being compared were of equal variances. Next, an Independent Samples, two tailed *t*-Test Assuming Equal (or Unequal) Variances, as appropriate, was performed to determine if means were significantly different with alpha 0.05. Additionally, one-way or two-way ANOVA was performed, when necessary, followed by Tukey Honestly Significant Difference (HSD) Post-Hoc Test or Bonferroni correction to determine significance between variables. All statistical analyses were completed in IBM SPSS Statistics Version: 28.0.1 (IBM Corporation, Armonk, NY).

### 3. Results and discussion

#### 3.1. Sampling for exogenous DNA from manufactured ammunition

In this experiment, 44 of the 210 concentrated DNA extracts, processed for exogenous DNA, resulted in a small autosomal (SA) target quantification value above zero. Quantification values ranged from 0.063 pg/ $\mu$ L to 2.29 pg/ $\mu$ L (Supplemental Table 2). No sample produced a SA target value above the lowest Quantifiler™ Trio DNA standard (5 pg/ $\mu$ L) (Supplemental Table 2). However, one sample (V8) resulted in two GlobalFiler™ alleles when amplified and genotyped. The two alleles that appeared in sample V8, were an 'X' at Amelogenin and a '16' at D2S441, which cannot rule out human exogenous DNA; however, they are not consistent with the analysts' STR profiles. Additionally, eight samples also quantified the large autosomal (LA) target and therefore produced degradation index (DI) values that ranged from 0.85 to 18.91, with 7 samples exhibiting DIs from 1 to 10, defined as slight to moderate degradation with or without PCR inhibition according to the manufacturer's guide [25]. One sample had a DI  $> 10$ , defined as significant degradation, with or without inhibition. However, DI values, or SA/LA ratio, should be interpreted with caution since one or both targets were often below the lowest quantification standard, suggesting stochastic effects.

Although only two alleles were identified in one sample out of 210, we cannot rule out prior degradation of the DNA on cartridges due to storage time of indeterminate length or degradation due to nonuse of a chelating agent while swabbing. However, these possibilities may be less likely for two reasons. First, Wan et al. [27] compared tape-lifting to wet swabbing and concluded that an aqueous collection method is not

required to negatively affect DNA processing. Additionally, Holland et al. [24] tested the recovery of DNA from both brass and copper when using EDTA versus water and found that while recovery was higher for cartridges swabbed with EDTA, the difference was not statistically significant. Furthermore, Bille et al. [2] performed an “effects of additives” study on brass ammunition with water and three chelating agents. Their results concluded that while mean DNA recovery was higher for two of the chelating agents, the chelating agents did not produce significantly higher recoveries than water. Second, Moore et al. processed ten cartridges to establish the level of background DNA that may come from the manufacturing process. Of the ten cartridges, only two samples produced STR profiles with one allele each. They concluded that ammunition obtained from a manufacturing facility was primarily DNA free [23]. In addition, Montpetit & O'Donnell [1] reported that the manufacturing process is largely automated with few human quality assurance checks (less than 1% of manufactured ammunition), for ammunition from Federal Ammunition (Anoka, MN). Given these reports, together with the results presented here, the likelihood of substantial levels of exogenous DNA on unopened boxes of cartridges may be low.

### 3.2. Evaluating two DNA collection methods using a non-metallic substrate

Cultured HEKa cells were used in this and subsequent experiments as a proxy for skin cells and cellular touch DNA material. The primary purpose of this experiment was to identify a sufficient amount of cells to spot to enable a comparison of DNA yields via the two collection methods. Additionally, sterile polypropylene mini tubes were spotted in lieu of metal cartridges to mitigate inhibitory or degradation effects, which may be caused by metal ions.

The two sets of DNA input amounts tested were combined and included 20, 10, 5, 2, 1, 0.5, 0.25, and 0.125 ng. Of the 120 samples processed with the soaking method, 36 samples were “flagged” because the quantity mean was below the lowest standard of 5 pg/μL for the Quantifiler™ Trio kit. Concentrations ranged from 0.0898 pg/μL to 533 pg/μL, while average concentrations ranged from 0.493 pg/μL (0.125 ng) to 384 pg/μL (20 ng). Recovery rates ranged from 0.9% to 104.5%, while average recovery rates ranged from 4.7% (0.125 ng) to 50.5% (0.25 ng) (Supplemental Tables 3 and 4). DI values were below two, suggesting no-to-slight degradation. Generally, DNA mean concentrations, and recovery rates increased from lower DNA inputs to higher DNA inputs. The increase in mean concentration is predominantly due to the increase in DNA input. Approximately one-third of the sample replicates (37/120) tested with the soaking method resulted in > 50 pg/μL of DNA, including all 20 ng, 10 ng, 92% (11/12) of 5 ng, and 8.3% (2/24) of 2 ng samples. All quantification data metrics may be found in Supplemental Table 3.

Of the 120 samples processed with the rinse-and-swab method, 55 samples were “flagged” because the quantity mean was below the lowest Quantifiler™ Trio standard. Additionally, two samples failed to give quantification results altogether. Concentrations ranged from 0.194 pg/μL to 50.5 pg/μL, while average concentrations ranged from 0.621 pg/μL (0.125 ng) to 20.1 pg/μL (10 ng). Recovery rates ranged from 0.9% to 80%, while average recovery rates ranged from 2.3% (20 ng) to 23.3% (0.5 ng) (Supplemental Tables 3 and 4). Similar to the soaking method, DI values were all below two, suggesting virtually no degradation present. Unlike the soaking method, only one sample (20 ng) resulted in a quantity > 50 pg/μL. The remaining 119 samples resulted in quantities below 40 pg/μL. The DNA quantities resulting from 2 ng–0.125 ng were deemed too low to provide valuable comparison data and were significantly lower ( $p < 0.001$ ) than 20 ng–5 ng inputs, except for 0.25 ng input for the soaking method. Overall, low average recovery could have been due to DNA adhering to the mini tubes, analysts performing a new method, poor DNA recovery from swabs, and/or extraction method among other reasons. All quantification data metrics may be found in

### Supplemental Table 3.

Based on these results, it was decided that spotting a large DNA input amount, such as 10 ng or 20 ng, on the substrate was not comparable to a real-world situation. Furthermore, depending on shedder status and the amount of contact an individual has with a substrate, 5 ng, or approximately ~800 cells' worth of DNA, may more closely resemble a real-world situation and should yield sufficient DNA to make a comparison between the two collection methods.

Therefore, 5 ng of DNA was chosen to be used for the remaining experiments in this study. An experiment was performed to confirm that 5 ng of DNA would fulfill the requirements of the remaining experiments. Three analysts processed 48 mini tubes, 24 mini tubes per collection method, which also assessed reproducibility among the three analysts.

For the soaking method, both Analysts One and Three produced similar results. Average concentrations and recovery rates were 69.1 pg/μL and 16.6% and 69.8 pg/μL and 16.8%, respectively (Supplemental Table 5). Likewise, for the rinse-and-swab method, both Analysts One and Three produced similar results; however, Analyst Three's average DNA recovery was slightly higher than Analyst One. Average concentrations and recovery rates were 30 pg/μL and 18.0% and 18.3 pg/μL and 11.0% recovery, for Analysts Three and One, respectively (Supplemental Table 5).

Conversely, Analyst Two produced seemingly disparate results for this experiment than Analysts One and Three. For the replicates processed with the soaking method, the average concentration was 127 pg/μL and the average recovery rate was 30.4%. For the samples processed with the rinse-and-swab method, the average concentration was 9.4 pg/μL and the average recovery rate was 5.6% (Supplemental Table 5). Compared to Analysts One and Three, Analyst Two produced concentration and recovery results that were two times higher for the soaking method. However, for the rinse-and-swab method, Analyst Two produced concentration and recovery results of less than half of what was produced by Analysts One and Three. One possibility for these results could be that a separate HEKa cell suspension was created for Analyst Two's replicates. However, the same HEKa stock was used to create the 2.5 ng/μL (~800 cells) suspension. Another possibility for this difference could simply be variability among analysts' technique and time since DNA deposition.

If the non-metallic substrate data for all three analysts is combined and averaged, the soaking method produced a mean concentration of 98 pg/μL and a recovery rate of 23.5%, which was significantly higher ( $p < 0.001$ ) than the rinse-and-swab method, which resulted in a mean concentration of 16.8 pg/μL and a recovery rate of 10.1%. All quantification data metrics may be found in Supplemental Table 6.

### 3.3. Evaluating two DNA collection methods using fired and unfired, brass and nickel-plated ammunition

For this experiment, 600 cartridges, 300 brass and 300 nickel-plated, were processed using two DNA collection methods, the soaking method and the rinse-and-swab method. Of the 300 brass and 300 nickel-plated cartridges, 150 cartridges of each material were fired and the remaining 150 were processed unfired. Three analysts planned to process 200 cartridges each, 100 with the soaking method and 100 with the rinse-and-swab method. Analysts Two and Three only processed 198 cartridges each because of the loss of four cartridges during the firing process, while Analyst One processed 200 cartridges. A total of 299 cartridges were processed with the soaking method and 297 cartridges with the rinse-and-swab method. Of the 596 cartridges processed, 13 were found to have low level contamination in the STR profile and were excluded from STR analysis. Reagent blanks, defined as cartridges with no DNA added but processed in the same manner as the respective soaking and rinse-and-swab methods, were processed with each automated extraction. Contamination in 11 of the 596 samples (1.8%), not including reagent blanks, was due to analyst contamination and were

**Table 2**

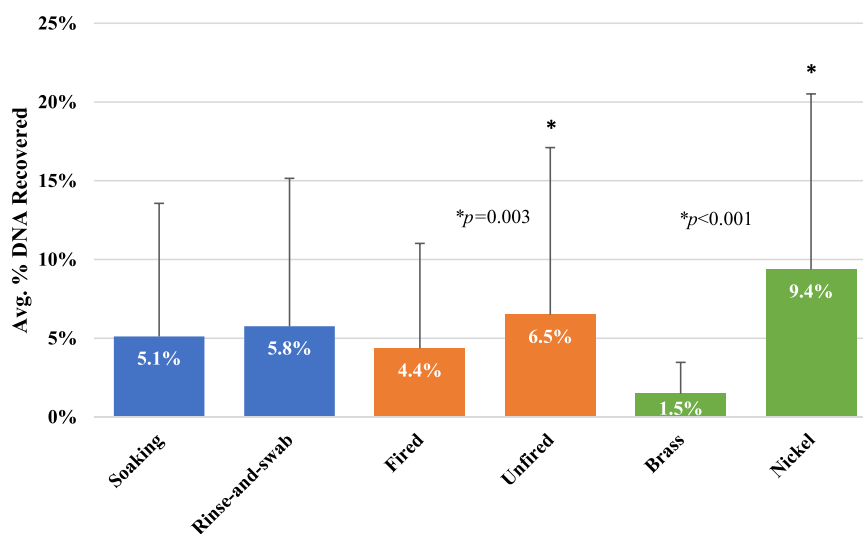
Minimum, maximum, and mean DNA yields from cartridge cases. DNA concentrations (pg/ $\mu$ L) and percent recoveries for both collection methods on brass and nickel-plated cartridges, fired and unfired.

	Soaking	Rinse-and-swab	Fired	Unfired	Brass	Nickel
<b>Min Conc. %</b>	0.13 pg/ $\mu$ L 0.031%	0.045 pg/ $\mu$ L 0.027%	0.045 pg/ $\mu$ L 0.027%	0.13 pg/ $\mu$ L 0.031%	0.045 pg/ $\mu$ L 0.027%	0.13 pg/ $\mu$ L 0.031%
<b>Max Conc. %</b>	170 pg/ $\mu$ L 40.74%	86.6 pg/ $\mu$ L 51.96%	145 pg/ $\mu$ L 34.70%	170 pg/ $\mu$ L 51.96%	48.3 pg/ $\mu$ L 17.26%	170 pg/ $\mu$ L 51.96%
<b>Mean Conc. %</b>	21.3 pg/ $\mu$ L 5.11%	9.57 pg/ $\mu$ L 5.76%	12.3 pg/ $\mu$ L 4.36%	18.6 pg/ $\mu$ L 6.50%	4.16 pg/ $\mu$ L 1.47%	26.7 pg/ $\mu$ L 9.37%

**Table 3**

Recoveries above or below 50 pg/ $\mu$ L by method and type. Shown are the number and percentage of samples that quantified above or below 50 pg/ $\mu$ L of DNA for both collection methods, fired and unfired cartridges, and brass and nickel-plated cartridges.

	Combined	Soaking	Rinse-and-swab	Fired	Unfired	Brass	Nickel
<b>&lt;50 pg/<math>\mu</math>L</b>	542	256	286	280	262	296	246
<b>%</b>	91.25%	85.91%	96.62%	94.92%	87.63%	100%	82.55%
<b>&gt;50 pg/<math>\mu</math>L</b>	52	42	10	15	37	0	52
<b>%</b>	8.75%	14.09%	3.38%	5.08%	12.37%	0%	17.45%
<b>Total</b>	594	298	296	295	299	296	298
	100%	100%	100%	100%	100%	100%	100%



**Fig. 1.** Recovery of DNA from cartridges. Results comparing the soaking (n = 299) and rinse-and-swab (n = 297) methods, fired (n = 296) and unfired (n = 300), and brass (n = 297) and nickel-plated (n = 299) cartridges for average percent of DNA recovered. Error bars denote  $\pm$  SD. Asterisk(\*) denotes significance.

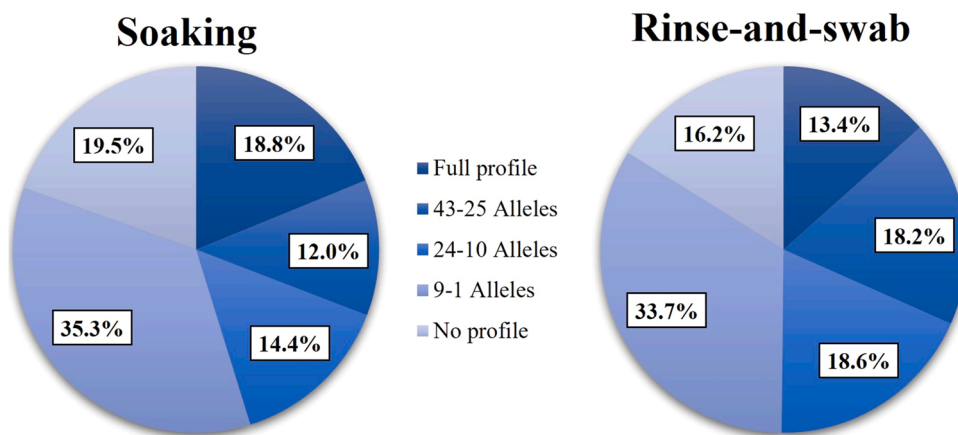
removed prior to analysis. Additionally, five of the 62 reagent blanks (8%) showed one to 14 alleles of either analyst contamination (three alleles in one of the five contaminated reagent blanks) or HEKa cell line contamination (four out of five contaminated reagent blanks). We have determined that six of the 596 samples (1.0%) associated with the HEKa cell-contaminated reagent blanks were potentially affected. Reagents were tested for contamination and resulted in clean extracts.

### 3.3.1. Soaking method vs rinse-and-swab method

Of the 596 cartridges that were processed, the concentrations of the SA target ranged from 0.045 pg/ $\mu$ L to 170 pg/ $\mu$ L and recovery rates ranged from 0.027% to 51.96% (Table 2). For the samples processed with the soaking method, the concentrations ranged from 0.13 pg/ $\mu$ L to 170 pg/ $\mu$ L and recovery rates ranged from 0.031% to 40.74%. For the samples processed with the rinse-and-swab method, concentrations ranged from 0.045 pg/ $\mu$ L to 86.6 pg/ $\mu$ L with recovery rates that ranged from 0.027% to 51.96% (Table 2). Based on these quantification results, the lowest SA concentrations and highest DNA recovery rates resulted from the rinse-and-swab method. Additionally, DNA quality was determined using the internal PCR control (IPC) cycle threshold (CT) and the DI from quantification. IPC CT values were between 25.3 and 28.4 indicating no inhibition was detected (data not shown), which was

similarly observed in Prasad et al. [28] and was not a major factor for Bille et al. [2]. Moreover, 458 samples (77%) were slightly to moderately (DI, 1–10) degraded and 30 samples (5%) were severely (DI, >10) degraded (data not shown).

The majority of samples (542/594, 91.25%) recovered < 50 pg/ $\mu$ L of DNA with either method and the remaining 52 samples (8.75%) recovered > 50 pg/ $\mu$ L of DNA. Both the soaking method and the rinse-and-swab method resulted in a majority of samples with concentrations < 50 pg/ $\mu$ L, 85.91% and 96.62% respectively (Table 3). However, total DNA recovered was > 50 pg total for 53% of fired samples processed with the soaking method and 61% of fired samples processed with the rinse-and-swab method. Our results were similar to both [1] and [2] for the respective methods, suggesting an appropriate amount of cells (~800) were used to simulate casework scenarios. The average total DNA recovered for the soaking method was 0.25 ng with an average recovery of 5.1%, and for the rinse-and-swab method the average total DNA recovered was 0.28 ng with an average recovery of 5.8% (Fig. 1). The average total DNA and average percentage of DNA recovered for the soaking and rinse-and-swab methods were not significantly different ( $p = 0.38$ ) from one another, indicating that both methods yielded comparable results overall. Considering Phase 2 demonstrated a significantly higher DNA recovery for the soaking



**Fig. 2.** GlobalFiler™ results for DNA recovered from cartridges. Shown are the percentages of samples that produced a range of alleles using the soaking and rinse-and-swab collection methods.

**Table 4**

GlobalFiler™ results for DNA recovered from cartridges. Shown is a breakdown of the number of alleles obtained for soaking versus rinse-and-swab, fired versus unfired, and brass versus nickel.

No. Alleles	Soaking	Rinse-and-swab	Fired	Unfired	Brass	Nickel	Average
<b>44</b>	18.8%	13.4%	15.2%	17.1%	1.0%	31.3%	<b>16.1%</b>
<b>43–25</b>	12.0%	18.2%	18.6%	11.6%	6.8%	23.4%	<b>15.1%</b>
<b>24–10</b>	14.4%	18.6%	15.9%	17.1%	19.5%	13.4%	<b>16.5%</b>
<b>9–1</b>	35.3%	33.7%	34.1%	34.8%	47.6%	21.3%	<b>34.5%</b>
<b>0</b>	19.5%	16.2%	16.2%	19.5%	25.0%	10.7%	<b>17.8%</b>

method on an inert substrate and Phase 3 demonstrated that both methods do not recover significantly different amounts of DNA on metal surfaces, there is a possibility that the BTmix in the rinse-and-swab rinse solution plays a role in maximizing DNA recovery from cartridges, as suggested by Bille et al. [2].

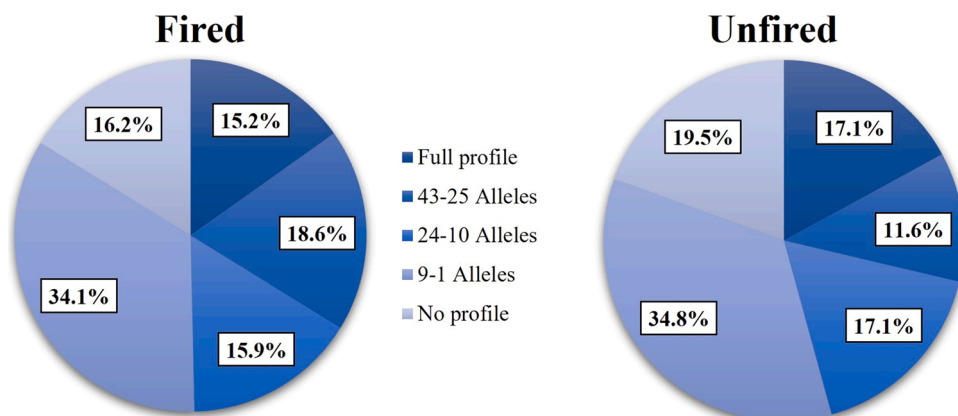
Of the 583 samples suitable for STR analysis (meaning samples not excluded for any reason), 82.2% (479/583) of samples produced at least one allele including 80.5% of samples (235/292) for the soaking method and 83.8% of samples (244/291) for the rinse-and-swab method. Detailed results are summarized in Fig. 2 and Table 4.

Overall, full profiles were observed 16.1% (94/583) of the time, 25–43 alleles were observed 15.1% (88/583) of the time, 10–24 alleles were observed 16.5% (96/583) of the time, 1–9 alleles were observed 34.5% (201/583) of the time, and no profile was observed 17.8% (104/583) of the time (Table 4). In addition, all alleles were consistent with the known HEKa cell profile, with the exception of the 13 profiles mentioned previously that were not included in STR analysis.

**3.3.2. Fired vs unfired cartridges**

The SA target concentrations for fired casings ranged from 0.045 pg/μL to 145 pg/μL and recovery rates ranged from 0.027% to 34.70% and for unfired cartridges, the SA target concentrations ranged from 0.13 pg/μL to 170 pg/μL and recovery rates ranged from 0.031% to 51.96% (Table 2). Unsurprisingly, the lowest concentrations and recovery rates resulted from fired casings.

For fired casings and unfired cartridges, the average total DNA recovered for each was 0.21 ng and 0.32 ng of DNA, respectively, and the average percentage of DNA recovered for both fired and unfired cartridges was 4.4% and 6.5% (Fig. 1). Unfired cartridges demonstrated a significantly higher ( $p = 0.003$ ) average total DNA and average percentage of DNA recovered by approximately 34%. This is an expected observation since it is believed that DNA on fired casings is damaged or physically removed due to some aspect of heat, pressure, expansion of the casing in the firing chamber, and/or ejection from the firearm [1,29,31]. Consistent with our findings, Montpetit & O'Donnell [1]



**Fig. 3.** GlobalFiler™ results for DNA recovered from cartridges. Shown are the percentages of samples that produced a range of alleles from fired casings and unfired cartridges.

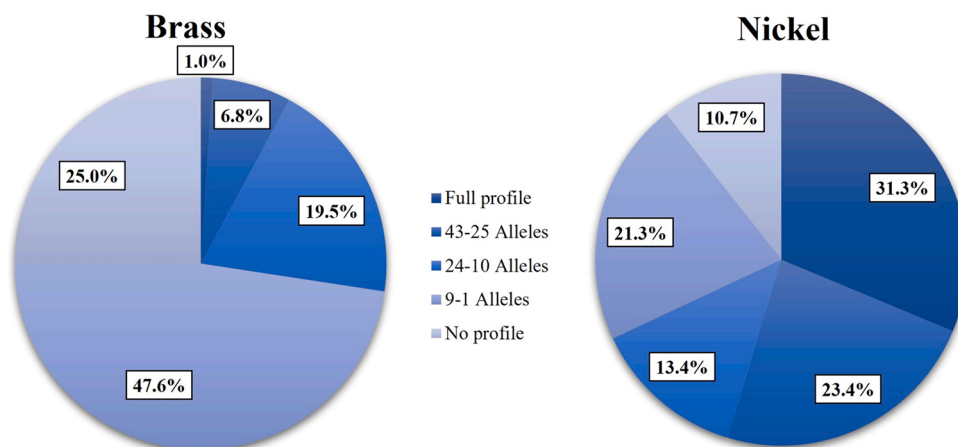


Fig. 4. GlobalFiler™ results for DNA recovered from cartridges. Shown are the percentages of samples that produced a range of alleles from brass and nickel-plated cartridges.

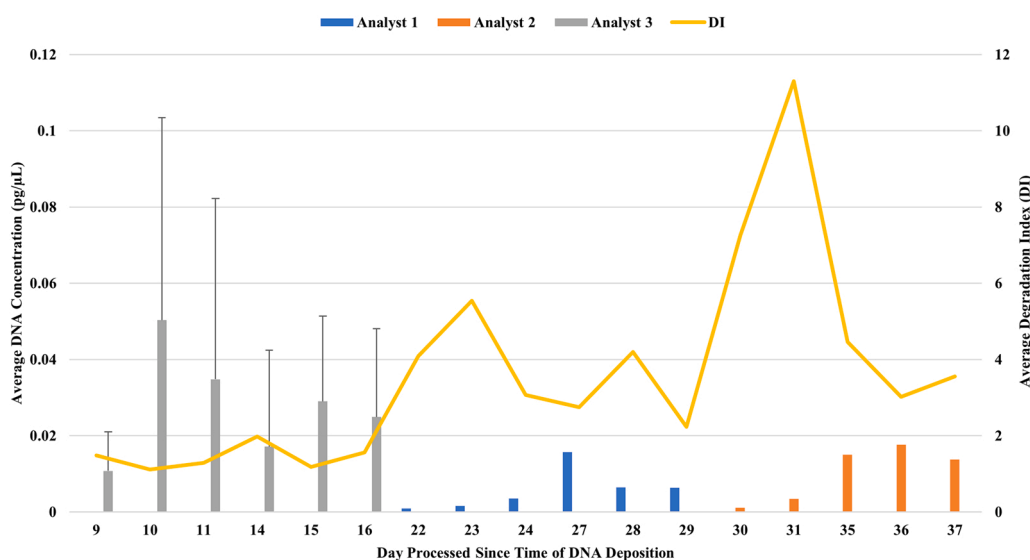


Fig. 5. Average DNA concentration and DI by day processed by analyst. Data shown for the average DNA concentration and average DI collected from cartridge cases processed on different days by each analyst for 596 fired and unfired, brass and nickel-plated cartridges. Error bars denote  $\pm$  SD.

successfully obtained DNA from both fired and unfired cartridges. Additionally, [1] and Moore et al. [23] noted that less DNA quantity was obtained from fired casings than from unfired cartridges.

Of the 583 samples suitable for STR analysis, 290 were fired casings and 293 were unfired cartridges. For 290 fired casings and 293 unfired cartridges, all but 47 fired samples (16.2%) and 57 unfired samples (19.5%) produced at least one STR allele (Table 4, Fig. 3).

Thus, while less DNA on average was recovered from fired casings compared to unfired cartridges, both cartridge types yielded similar STR profiles for the conditions of this study. Consistent with our findings, a previous study reported that STR profiles can be successfully obtained from both fired and unfired cartridges [1].

### 3.3.3. Brass vs nickel cartridges

The average total DNA recovered was 0.07 ng (1.5% recovered) and 0.47 ng (9.4%), for brass and nickel-plated cartridges, respectively (Fig. 1). Nickel-plated cartridges resulted in a significantly higher ( $p < 0.001$ ) average total DNA or 6-fold more than brass, demonstrating the detrimental effect of brass on DNA [17,30]. This finding is consistent with Prasad et al. [28] who found that brass cartridges resulted in less DNA than nickel-plated cartridges when swabbing fired and unfired

ammunition. Two hundred ninety-six brass cartridges resulted in SA target concentrations below 50 pg/μL of DNA, which ranged from 0.045 pg/μL to 48.3 pg/μL and recovery rates ranged from 0.027% to 17.26% (Tables 2 and 3). Of the nickel-plated cartridges processed, 246 recovered DNA concentrations < 50 pg/μL (Table 3). SA target concentrations and recovery rates are summarized in Table 2.

Average DI values for brass and nickel-plated cartridges when processed with the rinse-and-swab method were 3.6 and 2.8, respectively; and 5.7 and 2.2, respectively, for the soaking method (data not shown). The lower values for the rinse-and-swab method may be due to the use of BTmix (BSA and GGH tripeptide) and a reduction of the effects of copper ions on brass cartridges [2].

Of the 583 samples suitable for STR analysis, 292 were brass and 291 were nickel-plated. For brass cartridges, only 3 samples (1.0%) resulted in full profiles, while 75% (219/292) of samples produced at least one allele (Table 4, Fig. 4). In contrast, for nickel-plated cartridges, 31.3% of samples (91/291) produced full profiles, while 89.3% of samples (260/291) produced at least one STR allele (Table 4, Fig. 4).

### 3.3.4. Time since DNA deposition and recovery by analyst

Overall, for both the soaking and rinse-and-swab methods combined,

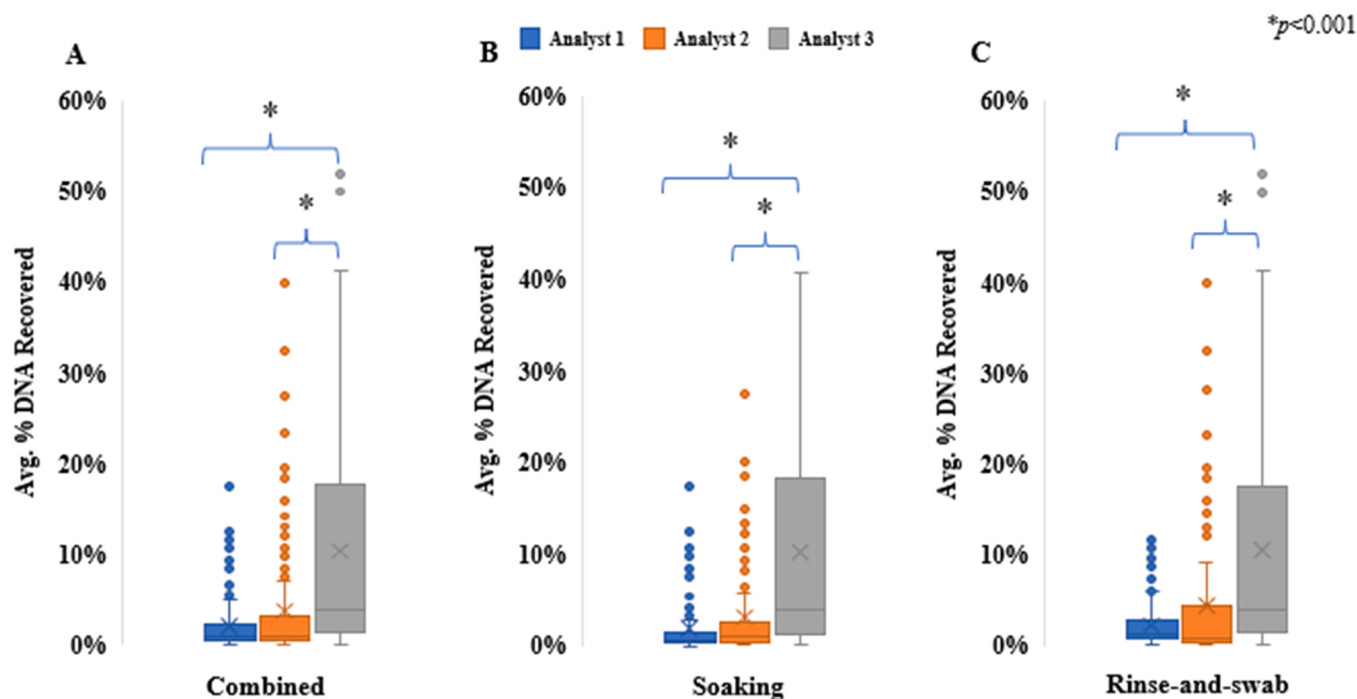


Fig. 6. Average percent of DNA recovered by analyst. Data shown for the collection method in combination (A) versus soaking (B) and rinse-and-swab (C) methods by analyst for 596 fired and unfired, brass and nickel-plated cartridges. Error bars denote  $\pm$  SD. Asterisk(\*) denotes significance.

Analyst One processed 200 samples on days 22 through 29, Analyst Two processed 198 samples on days 30 through 37, and Analyst Three processed 198 samples on days 9 through 16 (Fig. 5, Supplemental Table 7).

Generally, average DNA concentration increased as the time since DNA deposition increased for each analyst, possibly due to the analyst becoming more proficient and consistent with each method. However, there was a large decrease in average DNA concentration from Analyst Three at Day 16 (25 pg/ $\mu$ L) to Analyst One at Day 22 (1 pg/ $\mu$ L), with similar trends in average concentration for Analysts One and Two (Fig. 5). There is a possibility that between Day 16 and Days 22–37, the DNA was more severely degraded (DI 2.2–11.3), therefore resulting in lower DNA concentrations and recoveries for Analysts One and Two (Fig. 5). According to Bille et al. [32], cartridges should be analyzed as soon as possible because there is a loss of DNA over time when working with metal surfaces. Additionally, this trend was also observed when depositing cells on brass cartridge cases [2]. In a traditional casework scenario, an analyst may not typically have 600 cartridges to process, and equipment and space may not be as limited as in a research laboratory. Therefore, the trends observed from storage time (time since DNA deposition) may not pertain to a casework laboratory.

For both collection methods combined, the total DNA quantities for Analyst One ranged from 2 pg to 0.87 ng (0.03–17.47% recovered), 1 pg to 2 ng (0.03–39.9% recovered) for Analyst Two, and 3 pg to 2.6 ng (0.06–51.96% recovered) for Analyst Three (average quantity data not shown). Analyst Three produced average recoveries significantly higher ( $p < 0.001$ ) than both Analysts One and Two (Fig. 6A). However, Analysts One and Two did not produce recoveries significantly different ( $p = 0.231$ ) from one another (Fig. 6A).

Average quantity data and average percent of DNA recovered per analyst for the soaking and rinse-and-swab methods were similar to the data for the methods combined. Like the results for the combined methods, Analyst Three produced significantly higher ( $p < 0.001$ ) average recoveries than Analysts One and Two for both methods. However, there was no significant difference between Analysts One and Two (Fig. 6B & C). Furthermore, for each analyst, the soaking and rinse-and-swab methods were not significantly different from one other ( $p = 0.78$  Analyst One,  $p = 0.28$  Analyst Two,  $p = 0.78$  Analyst Three,

Table 5

GlobalFiler™ summary for the number of alleles (%) by analyst. Results include fired and unfired, brass and nickel-plated ammunition processed with both soaking and rinse-and-swab collection methods.

Analyst	Number of Alleles (%)				
	44	43–25	24–10	9–1	0
Analyst 1	7.9%	14.1%	10.5%	46.1%	21.5%
Analyst 2	4.1%	13.3%	10.3%	44.6%	27.7%
Analyst 3	36.0%	17.8%	28.4%	13.2%	4.6%
Average	16.0%	15.1%	16.4%	34.6%	17.9%

data not shown).

Of the 583 samples suitable for STR analysis, Analyst One processed 191 samples, Analyst Two processed 195 samples, and Analyst Three processed 197 samples. Analyst Three processed samples that resulted in mostly full profiles, 25–43 alleles, and 10–24 alleles, which would be considered higher quality and quantity data (Table 5). In contrast, Analysts One and Two processed samples that resulted in mostly 1–9 alleles or no profile, suggesting again that storage time or time since DNA deposition may have played a role in DNA recovery. Analysts One and Two produced more similar, but lower quality results throughout the study, whereas Analyst Three produced higher quality, more optimal results. These data demonstrate that DNA yields for both methods were highly variable, analyst dependent, and/or potentially affected by sample storage times. However, Bille et al. [2] suggests variability in results for any object containing trace DNA.

#### 3.4. Evaluating protocol modifications for two DNA collection methods using fired, brass casings

For the final phase of the study, 25 fired, brass casings were processed using the soaking method, the rinse-and-swab method, and five other modified versions of the two methods (Table 1).

For experiments 4.1–4.7, concentrations ranged from undetectable (observed in all methods) up to 48 pg/ $\mu$ L (experiment 4.1, the rinse-and-swab method) and recoveries ranged from 0% (experiments 4.4 and 4.5,

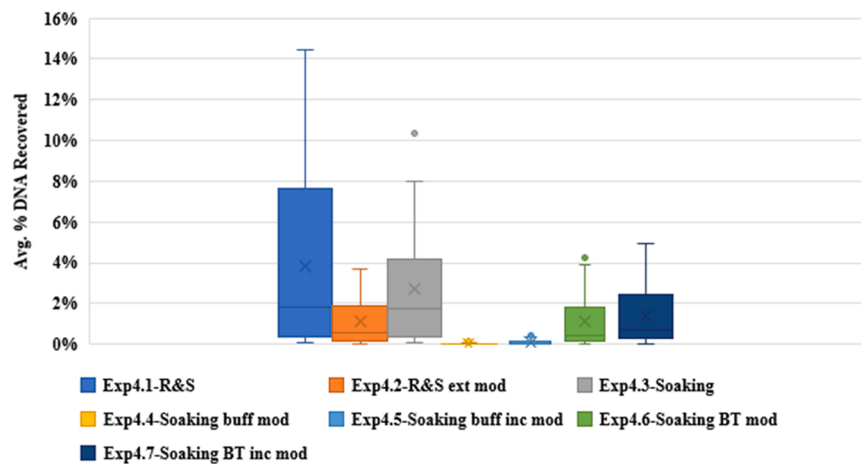


Fig. 7. Percent DNA recovered from hybrid methods. The average percent DNA recovered from various modified, hybrid collection methods compared to the rinse-and-swab (Exp 4.1) and soaking (Exp 4.3) methods, each experiment  $n = 25$  fired, brass casings. Error bars denote  $\pm$  SD. See Table 1 for modified method details.

Table 6

Summary of average DNA concentration (pg/ $\mu$ L) and average percent DNA recovered for hybrid methods. Exp 4.1, rinse-and-swab and Exp 4.3, soaking methods. See Table 1 for modified experiment (Exps 4.2, 4.4, 4.5, 4.6, 4.7) details.

	Average DNA concentration (pg/ $\mu$ L)	Average % DNA recovered
Exp 4.1	12.9	3.87%
Exp 4.2	3.7	1.11%
Exp 4.3	9.2	2.75%
Exp 4.4	0.2	0.03%
Exp 4.5	0.5	0.10%
Exp4.6	3.8	1.13%
Exp 4.7	4.7	1.40%

two modifications of the soaking method) to 14.48% (experiment 4.1) (Fig. 7). In experiment 4.4 (the soaking method using the rinse-and-swab rinse solution), 13 of the 25 samples processed resulted in no quantification value (Supplemental Table 8). Similarly, in experiment 4.5 (the soaking method using the rinse-and-swab rinse solution and three-hour incubation), 8 of 25 samples resulted in no quantification value (Supplemental Table 9). The methods resulting in the largest average DNA concentrations and recoveries were the rinse-and-swab method (13 pg/ $\mu$ L, 3.87%) and the soaking method (9 pg/ $\mu$ L, 2.75%), and the two methods were not significantly different from one another ( $p = 0.59$ ). A summary of results can be found in Table 6. STR analysis was not conducted on these samples. In conclusion, no improvement was observed in this study with any of the modified collection methods, and the two published methods examined in this article remained the most optimal collection methods. However, given the variability, results may not be consistent if repeated on a different day or performed by another analyst.

#### 4. Conclusion

The first phase of this study demonstrated that very little, if any, exogenous DNA was deposited during the manufacturing and packaging process for the cartridges tested here. Next, a comparison of the two collection methods on an inert substrate demonstrated the variability of the two collection methods among analysts as well as temporally for a given analyst. This phase also assisted in determining an appropriate cell concentration to simulate touch DNA for the purposes of this study. We found that the soaking method recovered significantly more DNA than the rinse-and-swab method for five out of the eight DNA concentrations tested on an inert substrate. A comparison of the two methods on cartridge casings showed that (1) there were no significant differences

between either the soaking or rinse-and-swab collection methods, suggesting that the chosen method may be based on user or laboratory preference, (2) both methods yielded highly variable DNA recoveries, although STR profiles were successfully obtained from both unfired cartridges and fired casings for the amount of cells spotted in this study, (3) ~34% less DNA was obtained from fired casings compared to unfired cartridges, (4) ~85% less DNA was obtained from brass than nickel-plated ammunition, (5) DNA recovery may be analyst dependent, (6) DNA recovery may be dependent on storage times or time since DNA deposition, (7) DNA degradation was typically mild or not observed, and (8) overall, amplification inhibitors did not appear to be co-extracted. Finally, the authors combined both the soaking and rinse-and-swab methods in an attempt to improve DNA yield. However, such modification experiments did not improve upon the two original methods; therefore, the soaking and rinse-and-swab methods appear optimal as originally described.

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## Declaration of Interests

The authors have no competing interests to declare.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.fsigen.2022.102726](https://doi.org/10.1016/j.fsigen.2022.102726).

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