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Research paper

High-throughput DNA extraction of forensic adhesive tapes

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ABSTRACT

Tape-lifting has since its introduction in the early 2000's become a well-established sampling method in forensic DNA analysis. Sampling is quick and straightforward while the following DNA extraction is more challenging due to the "stickiness", rigidity and size of the tape. We have developed, validated and implemented a simple and efficient direct lysis DNA extraction protocol for adhesive tapes that requires limited manual labour. The method uses Chelex beads and is applied with SceneSafe FAST tape. This direct lysis protocol provided higher mean DNA yields than PrepFiler Express BTA on Automate Express, although the differences were not significant when using clothes worn in a controlled fashion as reference material ($p = 0.13$ and $p = 0.34$ for T-shirts and button-down shirts, respectively). Through in-house validation we show that the method is fit-for-purpose for application in casework, as it provides high DNA yields and amplifiability, as well as good reproducibility and DNA extract stability. After implementation in casework, the proportion of extracts with DNA concentrations above 0.01 ng/μL increased from 71% to 76%. Apart from providing higher DNA yields compared with the previous method, the introduction of the developed direct lysis protocol also reduced the amount of manual labour by half and doubled the potential throughput for tapes at the laboratory. Generally, simplified manual protocols can serve as a cost-effective alternative to sophisticated automation solutions when the aim is to enable high-throughput DNA extraction of complex crime scene samples.

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1. Introduction

Tape-lifting has since its introduction in the early 2000's [1] become a well-established sampling method in forensic biology for subsequent DNA analysis. Applying adhesive tape, cells/DNA can be efficiently collected from rather large areas of fabrics, solid surfaces or skin [1–3]. Taping for biological traces has been shown to provide higher DNA yields than swabbing for certain fabrics [4–6].

Sampling is quick and straightforward: the tape is simply pressed against the material or surface a number of times. The following DNA extraction process is more challenging due to the "stickiness", rigidity and size of the tape. Cells must be efficiently released while the tape somewhat obstructs manual as well as automated pipetting. Previously, methods based on Chelex beads and filter purification [2,3,7], or commercial kits such as QIAamp DNA Mini and PrepFiler BTA [1,8,9], have mainly been applied, including more or less manual labour. Different strategies for simplifying and improving the DNA extraction process have been suggested, including swabbing the tape with an organic solvent and performing extraction on the swab [10], leaching the tape in a buffer and removing it before automated DNA extraction [4], and applying tape that dissolves in the extraction buffer [2].

At the Swedish National Forensic Centre (NFC) tapes are used for sampling of clothes and other fabrics, in search for DNA from the wearer or touch DNA from a perpetrator. About six thousand tapes are processed (sampling – DNA extraction – analysis) each year, out of a total of about 55,000 analysed crime scene samples. The use of tapes is growing due to their wide applicability and the simplicity in sampling larger areas. This places an increased demand for efficient handling downstream.

Here we present the development and validation of a simplified and efficient direct lysis DNA extraction protocol for adhesive tapes that requires limited manual labour. The method developed is applied with SceneSafe FAST tape, which we previously found to be a suitable alternative to replace an in-house tape used in casework for several years [11]. The DNA yield of the developed method is compared to that of a commercially available automated DNA extraction system developed for tapes. The new protocol is subsequently validated for casework. Finally, we show how the

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method performs in routine casework, in terms of DNA yield, usability of the generated STR DNA profiles and sample throughput. The applied strategy of developing a simplified manual DNA extraction protocol provides a cost-effective high-throughput alternative to commercial automated DNA extraction and purification platforms.

2. Material and methods

2.1. Preparation of samples

Clothes worn under controlled conditions were applied as reference material and used to evaluate DNA yield, whereas a variety of "normally" worn clothes were applied to investigate impact of different fabrics. The reference material, prepared and sampled as previously described [11], consisted of long sleeved T-shirts and button-down shirts initially washed and then worn by one volunteer per type of garment for two office working days, in total about 30 h. Six to ten equal areas were sampled for each type of clothes, tape and DNA extraction method. Ten caps, ten gloves and ten sweaters worn by volunteers were collected and sampled following the internal standard operational procedure used for casework [7]. Equal areas of the clothes were sampled with an in-house tape [7] and SceneSafe FAST Box, K545 (SceneSafe, Burnham on Crouch, UK). The STR DNA profiles of all volunteers were known and comparisons were made to verify that the DNA profiles resulting from the analyses were from the wearers.

2.2. DNA extraction

In the reference method, the in-house tape was extracted with a Chelex based (Bio-Rad Laboratories, Hercules, CA, USA) method as previously described [7,12]. Each tape was cut in pieces and divided into three tubes due to its size and rigidity. The extracts of the three tubes were pooled and the volumes reduced to 200 μL using Amicon Ultra-2 (Merck Millipore, Darmstadt, Germany) [13].

Two simplified DNA extraction protocols based on direct lysis were developed at Applied Microbiology, Lund University, Lund, Sweden. In the developmental process, different lysis buffers were compared with respect to DNA yield: TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0, Medicago AB, Uppsala, Sweden) and 5% Chelex in MilliQ water, both with 0.1 μg/μL Proteinase K (Sigma-Aldrich, Saint Louis, USA), with and without 0.2% Tween 20 (Sigma-Aldrich). The addition of three supplements (0.2% sodium dodecyl sulfate solution (SDS, BioUltra, Sigma-Aldrich), 0.1 μg/μL bovine serum albumin (BSA, Roche, Basil, Switzerland) and 0.2 M D-(+)-Trehalose dehydrate (Sigma-Aldrich)) to 5% Chelex with 0.2% Tween 20 and 0.1 μg/μL Proteinase K, was also evaluated. Subsequently, the initial leaching and pelleting step of the reference method was compared with direct lysis, either adding 250 μL lysis buffer, or 1 mL with a final volume reduction to 200 μL using Amicon Ultra-2 (Fig. 1).

The two DNA extraction protocols developed consist of the following steps: 1 mL lysis buffer (5% Chelex solution or TE buffer, containing 0.2% Tween 20 and 0.1 mg Proteinase K) was added to each tape. The samples were kept at room temperature for 30 min including vortexing, followed by two incubation steps in a heating incubator (56 °C for 45 min and 100 °C for 20 min) with vortexing in between. The sample volumes were then reduced to 200 μL using Amicon Ultra-2.

PrepFiler Express BTA Forensic DNA Extraction kit on Automate Express (Thermo Fisher Scientific, Waltham, MA, USA) was used to extract DNA from SceneSafe FAST tapes. The extraction was performed according to the provided user guidelines [14] with the modifications that a larger input lysis buffer volume was applied to fully cover the tape, 500 μL instead of the recommended 230 μL, and that the lysates were further run on standard protocol 1. The elution volume was adjusted to 200 μL with TE buffer to enable direct comparisons between the methods.

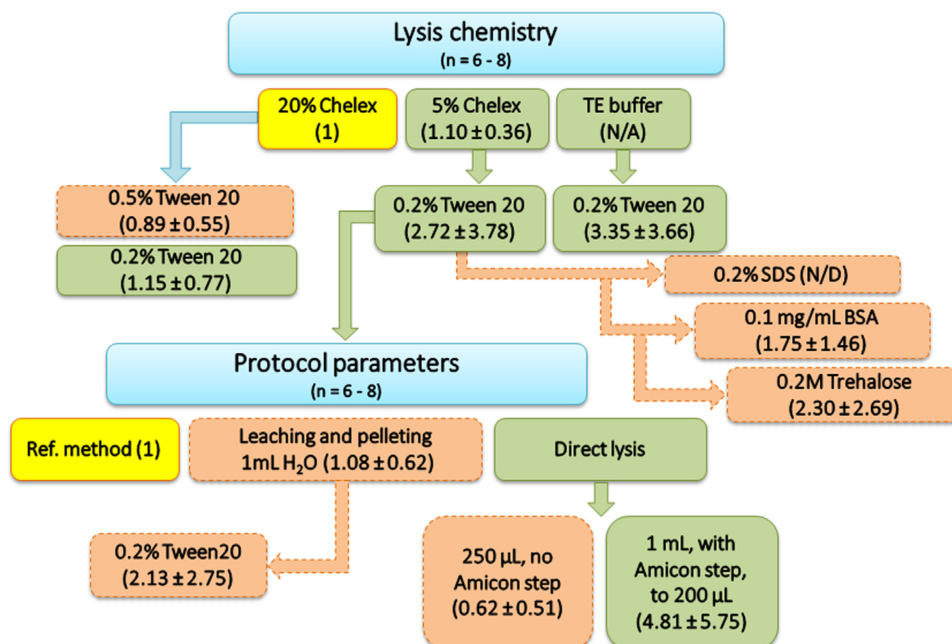

Fig. 1. A schematic overview showing the process of developing a simplified DNA extraction protocol for tapes. Results are presented in brackets as the ratio between the mean DNA concentration of a given protocol and the mean DNA concentration of the reference method (yellow) ± standard deviation. The best performing protocols are given in green, while orange boxes with dashed lines highlight protocols that gave no further improvements. First, lysis buffer composition was evaluated, where proteinase K (0.1 μg/μL) was added in all protocols. The lysis buffer containing 5% Chelex, 0.2% Tween 20 and 0.1 μg/μL proteinase K was then applied in the evaluation of different protocol parameters. In these comparisons, both the in-house tape and SceneSafe FAST were applied. BSA: bovine serum albumin, SDS: sodium dodecyl sulfate, N/A: not applicable, N/D: not detected. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

One extraction negative control including all lysis reagents was processed with each DNA extraction batch. An overview of the tested DNA extraction protocols is seen in Table 1.

2.3. Quantification and STR analysis

In the process of protocol development, the extraction efficiencies of the different protocols were evaluated with quantitative PCR using a LightCycler Nano instrument (Roche Diagnostics, Basel, Switzerland). Reactions included: 1 x ExTaq buffer (TaKaRa Bio Europe/Clontech, Saint-Germain-en-Laye, France), 0.2 mM deoxynucleoside triphosphate (Roche Diagnostics), 4 mM $MgCl_2$ (Roche Diagnostics), 0.3 μM of primers RB1_80 F and RB1_235R, 0.2 μM 6FAM-RB1 212-MGB hydrolysis probe described in Niederstätter et al. [15] (Life Technologies, New York, USA), 1 U Ex*Taq* HS DNA polymerase (TaKaRa Bio Europe/Clontech), 4 μL sample extract and SuperQ water up to a total reaction volume of 20 μL. Reaction conditions were as follows: an initial heat-activating step at 95 °C for 2 min, followed by 45 cycles of 10 s at 95 °C, 20 s at 60 °C and 30 s at 72 °C.

For the comparison between the two developed methods and PrepFiler Express BTA, the in-house validation, as well as in routine casework, quantification was performed using Quantifiler HP DNA Quantification kit on an Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific) with a linear range of quantification from 0.003 to 20 ng/μL. STR analysis was performed applying PowerPlex ESX 16 Fast System (Promega Corporation, Madison, WI, USA), GeneAmp PCR System 9700, ABI 3130xl, and GeneMapper ID-X Software v1.3 (Thermo Fisher Scientific).

Samples were normalized prior to STR amplification according to the manufacturer's recommendations (0.5 ng DNA in PCR). For diluted samples, the total sum of peak heights (TPH) in relative fluorescence units (rfu) was compensated with the dilution factor. The difference in expected value between extraction methods was tested statistically with independent two sample *t*-test with unequal variances. Binomial test and paired samples *t*-test were applied when comparing the DNA yield for "normally" worn clothes between direct lysis with Chelex and the Chelex reference method. The tests were performed both on DNA concentrations (ng/μL) and TPH (rfu). The *p*-value for the binomial test was computed assuming the number of samples generating higher yield with the direct lysis method is binomially distributed with parameter 0.5.

At NFC, taping is the principal sampling method for clothes and fabrics, such as sweaters, gloves and balaclavas. Taping is not yet routinely applied for solid surfaces. Data from 1,000 routine casework tape samples extracted with the Chelex reference method and 1,000 samples extracted with the developed method were retrieved from the laboratory LIMS. In routine (standard) analysis, extracts with DNA concentrations below 0.01 ng/μL (i.e. below 175 pg template DNA for amplification) are generally not processed further. Obtained STR typing results were divided into four categories: single-donor profiles, clear major profiles, mixtures, and negative profiles (no recorded allele peaks).

3. Results and discussion

3.1. Development of high-throughput DNA extraction protocols for adhesive tapes

The objective of the developmental process was to find a simplified and efficient manual DNA extraction protocol, by minimizing the number of pipetting steps and optimizing buffer content. First, lysis buffer composition was evaluated, adding proteinase K in all tested protocols. The lysis buffers containing 5% Chelex or TE buffer, Tween 20 and proteinase K gave the highest DNA yields (Fig. 1). Addition of the detergent SDS gave complete PCR inhibition, whereas the protein BSA and the osmoprotectant trehalose gave no further improvements. The lysis buffer with 5% Chelex, Tween 20 and proteinase K was then applied in the evaluation of different protocol parameters. Adding lysis buffer directly to the tape gave better results than leaching and pelleting followed by lysis. Thus, the steps of centrifugation and supernatant removal in the "classic" Chelex protocol [12] could be eliminated, reducing the amount of manual labour, the time spent and elevating yield (Fig. 1). Adding 1 mL lysis buffer and performing volume reduction applying Amicon Ultra gave higher yields than adding 250 μL buffer without volume reduction (Fig. 1). The experiments resulted in two direct lysis DNA extraction protocols as described in Materials and Methods (Table 1) including 5% Chelex or TE buffer, both substantially simplified and generating higher yields compared with the reference method.

3.2. Comparison between the developed methods and PrepFiler Express BTA

The developed direct lysis protocol with Chelex gave higher DNA yields than PrepFiler Express BTA/Automate Express for both types of controlled reference material (Fig. 2). For T-shirts and button-down shirts, the direct lysis protocol gave mean DNA concentrations of 0.029 and 0.032 ng/μL, compared with 0.017 and 0.026 ng/μL for PrepFiler Express BTA. However, the differences were not statistically significant ($p = 0.13$ for T-shirts and $p = 0.34$ for button-down shirts). Direct lysis with TE buffer performed better than PrepFiler Express BTA for T-shirts (mean: 0.025 ng/μL, $p = 0.04$) but gave low DNA yields for button-down shirts (<0.003 ng/μL).

The total sum of peak heights (TPH) of STR analysis electropherograms showed similar trends as the corresponding DNA concentrations: mean TPH was higher for direct lysis (Chelex) than for PrepFiler Express BTA, although the differences were not significant (mean TPH 56,900 vs. 34,000 rfu (T-shirt, $p = 0.24$), and 31,300 vs. 24,200 rfu (button-down shirt, $p = 0.54$)). Direct lysis (TE buffer) gave mean TPH of 51,700 rfu for T-shirts ($p = 0.13$ for

Table 1
Overview of the evaluated DNA extraction methods.

	Chelex Reference Method	PrepFiler Express BTA/Automate Express	Direct lysis with Chelex	Direct lysis with TE buffer
Tape type	In-house	SceneSafe FAST	SceneSafe FAST	SceneSafe FAST
Number of tubes per tape	3	1	1	1
Number of manual pipettings	20	3	5	5
Manual labour per sample (min)	10	3	5	5
Extraction time per batch of samples (hrs)	3.5	1.5	3	3
Maximum number of samples per technician and robot per working day	50	100	120	120
Price per sample for DNA extraction reagents (list price in Euro)	5.90	7.10	5.90	5.90

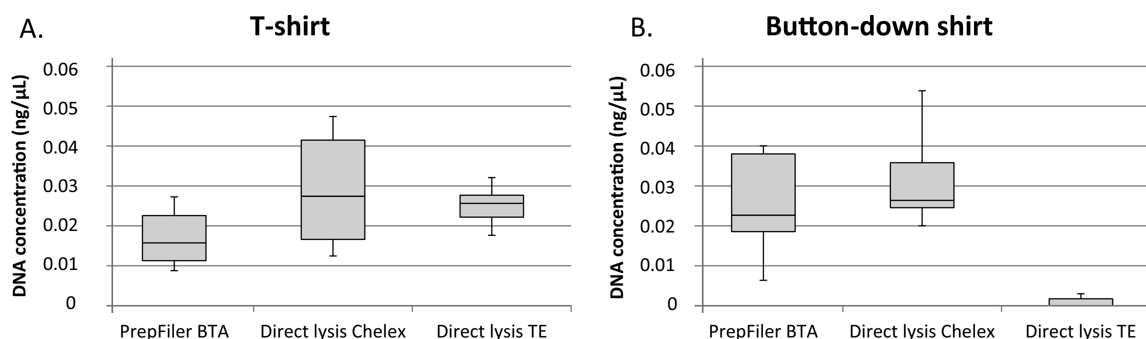

Fig. 2. Comparing DNA yield for PrepFiler Express BTA on AutoMate Express with the developed direct lysis protocols. The box plots show the median, the first and third quartiles and the highest and lowest DNA concentrations (ng/μL) within each group. A) Sleeves of T-shirts (n = 7 for PrepFiler Express BTA and direct lysis with TE buffer, and n = 6 for direct lysis with Chelex). B) Cuffs of button-down shirts (n = 8 for PrepFiler Express BTA and n = 7 for direct lysis with Chelex and TE buffer).

comparison against PrepFiler Express BTA) and 1,660 rfu for button-down shirts ($p = 0.01$). Complete, well-balanced electropherograms were generated for all samples for both direct lysis with Chelex and PrepFiler Express BTA, as well as for T-shirts with TE buffer direct lysis. The uneven performance for direct lysis with TE buffer could be due to lowered stability of DNA. Chelex is well-known to have a high chelating capacity for divalent metal ions, which serve as co-factors for nucleases [12], and the EDTA in the applied TE buffer likely gives a poorer chelating effect. Direct lysis with Chelex was chosen for in-house validation and implementation in routine casework with SceneSafe FAST tape, due to the good DNA yield and amplifiability, as well as the potential for high-throughput analysis and lower cost for reagents and consumables compared with PrepFiler Express BTA (Table 1, Fig. 2).

3.3. In-house validation of the direct lysis protocol

In-house validation of tape DNA extraction entailed investigating DNA yield, reproducibility, DNA extract stability and contamination risk for direct lysis with Chelex. Comparing the DNA yields for the reference material (T-shirts and button-down shirts), direct lysis with Chelex gave higher mean DNA concentrations than the Chelex reference method (Table 2), although the differences were not significant ($p > 0.05$). Both methods produced complete, well-balanced electropherograms for all analysed samples (data not shown).

For various types of “normally” worn clothes (caps, gloves and sweaters), direct lysis generated higher DNA yields than the Chelex reference method (Fig. 3), with the exception of sweater collars. However, binomial test and *t*-tests showed no significant differences in DNA yield between the methods ($p > 0.05$, Supplementary Table S1). Complete DNA profiles were produced by both methods (data not shown).

Reproducibility tests of direct lysis with Chelex comparing DNA yields between operators showed no significant differences ($p = 0.20$, data not shown). The stability of DNA extracts was considered good as two weeks storage in refrigerator (8 °C) had no effect on DNA yield or electropherogram quality (data not shown). All 13 analysed extraction negative controls were blank, suggesting a low risk to introduce contaminating DNA during the process.

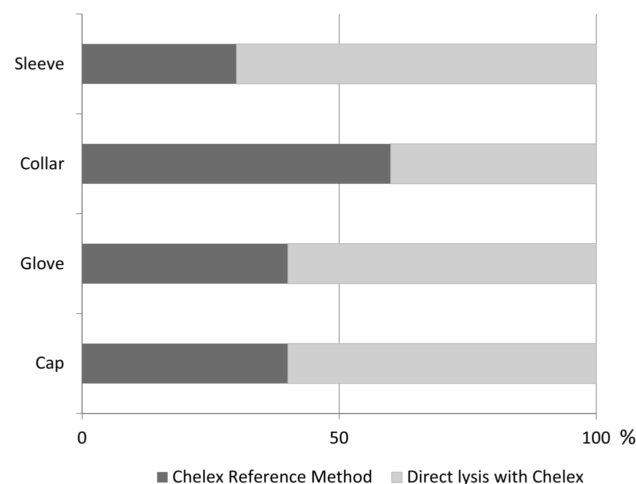

Fig. 3. DNA yield for taping of various types of clothes in in-house validation. The results are presented as percentage of samples giving a higher DNA concentration (ng/μL) for each of the methods (Chelex reference method and direct lysis with Chelex). n = 10 for caps, pairs of gloves and sweaters (collar and sleeve sampled, giving 20 sampled areas).

Direct lysis with Chelex was thus considered suitable for casework and was implemented at the laboratory in September 2015.

3.4. Performance in casework

Following the introduction of SceneSafe FAST and direct lysis with Chelex in casework, the fraction of crime scene tape samples with DNA concentrations above 0.01 ng/μL (in-house limit for performing a standard STR analysis) increased from 71% to 76% (Fig. 4). The increase was statistically verified (See Supplementary Material). These results follow the trend indicated in the in-house validation, i.e. that the new set-up provides at least as good DNA yields and amplifiability as the former (Table 2, Fig. 3).

The fraction of single-donor profiles and clear major profiles increased from 20% to 26% after implementing SceneSafe FAST and

Table 2
DNA yields for direct lysis with Chelex and the Chelex reference method. Mean DNA concentrations (ng/μL) and STR total sum of peak heights, TPH (rfu), standard deviation and *p*-values are given for the two methods. T-shirts: n = 10, button-down shirts: n = 10 for the Chelex reference method, n = 7 for direct lysis with Chelex.

	DNA concentration			Total sum of peak heights, STR analysis		
Material	Chelex Reference Method	Direct lysis with Chelex	*p*-value	Chelex Reference Method	Direct lysis with Chelex	*p*-value
Button-down shirt	0.023 ± 0.010	0.032 ± 0.011	0.14	20,100 ± 17,700	34,200 ± 34,500	0.38
T-shirt	0.035 ± 0.023	0.042 ± 0.020	0.48	62,200 ± 60,700	55,282 ± 43,000	0.77

Table 3
Usability of STR DNA profiles from tape sampling in casework. The results are presented as percentage of samples that generated single-donor profiles, clear major profiles, mixtures, and negative profiles. n = 698 for the Chelex reference method, n = 756 for direct lysis with Chelex. The sum is not 100% as rounded values are presented.

Method/STR analysis result	Single donor profile	Clear major profile	Mixture	Negative profile
Chelex Reference Method	7%	13%	77%	2%
Direct lysis with Chelex	9%	17%	73%	0%

direct lysis with Chelex (Table 3). In a recent study [16], it did not prove possible to selectively lift the outermost cells on clothes by applying tapes with different levels of "stickiness". Applying a less sticky tape simply resulted in lowered DNA amounts, not a higher incidence of single-donor/clear major profiles. Those findings imply that the differences in numbers of single-donor profiles and mixtures seen in the present study are related to differences in the sampled material rather than the tape type or extraction procedure.

When the current generation of STR typing kits (including PowerPlex ESX 16 Fast) was introduced it was speculated in the forensic community that these would generate increased levels of mixtures due to their improved detection limits. Comparing the fraction of mixtures in casework found here with results from applying the previous kit AmpFlSTR SGM Plus there is an apparent increase of mixtures, from 61% [7] to 77%, corroborating this assumption. The high level of mixtures is not surprising due to the well-documented easy transfer of cells/touch DNA [17].

The introduction of the developed direct lysis protocol has reduced the amount of manual labour by half compared with the Chelex reference method, and doubled the possible throughput for tapes at the laboratory (Table 1). The new process enables a quick scale-up within existing resources, e.g. if specific cases require high numbers of analyses within a few days.

4. Conclusions

Achieving high-throughput DNA extraction of adhesive tapes is a challenge for forensic DNA laboratories. We have handled this issue by developing a substantially simplified, manual DNA extraction protocol. The focus of the developmental process was to remove unnecessary steps in the procedure (e.g. pelleting and supernatant removal), and improve yield by adding extraction agents (e.g. Tween 20).

The developed direct lysis protocol gave DNA yields comparable with PrepFiler Express BTA/Automate Express, to a lower cost for reagents and consumables. The throughput time is longer for the direct lysis protocol, but the total throughput capacities are similar for the two methods.

Apart from providing higher DNA yields compared with the previously used method, the introduction of the developed direct lysis protocol also reduced the amount of manual labour by half and doubled the potential throughput for tapes at the laboratory. Moreover, the reduction of pipetting steps and sample transfers lowers the contamination risk. Generally, simplified manual protocols can serve as a cost-effective alternative to sophisticated automation solutions when the aim is to enable high-throughput DNA extraction of complex crime scene samples.

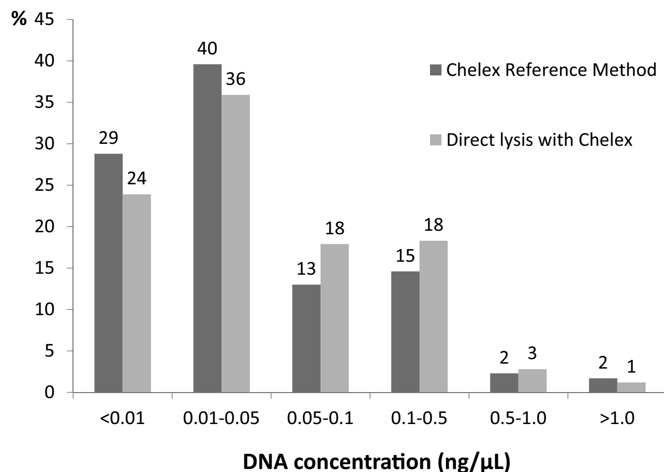

Fig. 4. DNA yield in casework. Percentage of samples with DNA concentrations (ng/µL) in different intervals, where 0.01 ng/µL is the NFC internal threshold for performing standard STR analysis. n = 1,000 for each method.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fsigen.2016.06.004.

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