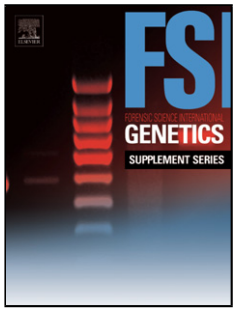

Biological stain collection – absorbing paper is superior to cotton swabs

K. Janssen, M. Aune, M. Olsen, G.-H. Olsen, T. Berg

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1. Title: Biological stain collection – absorbing paper is superior to cotton swabs

2. Names of authors: K. Janssen, M. Aune, M. Olsen, G.-H. Olsen and T. Berg
Authors' affiliation address: Centre of Forensic Genetics, UiT The Arctic University of Norway, Tromsø, Norway

Corresponding author kirstin.janssen@uit.no, + 47 77620905

3. Abstract:

Biological evidence at crime scenes often contains very small amounts of DNA. Therefore, it is important to use the most effective sampling devices and procedures for stain collection. Currently, cotton swabs moistened with water are widely used, also in our laboratory. However, several studies have shown that other methods may be more efficient.

In this study, we compared the DNA sampling efficiency of cotton swabs (Puritan) and pieces of absorbing paper (Kimtech) moistened with two liquids, water and ethanol. An initial experiment with blood stains deposited on glass slides showed that DNA yields were highest for samples collected with absorbing paper and ethanol.

To reflect casework conditions, we tested cotton swabs with water versus absorbing paper with ethanol on a range of used items and clothing from four surface classes: leather, plastic, natural and synthetic fabrics. We found that DNA yields were higher when using absorbing paper and ethanol than with cotton swabs and water. These findings were significant for all surface classes except synthetic fabrics for which there was a trend in the same direction though.

These results suggest that pieces of absorbing paper moistened with ethanol can improve the efficiency of stain collection, especially when stains are expected to contain low amounts of DNA. However, user-friendliness could still be improved and contamination risk reduced if an easy-to-handle collection device based on absorbing paper was developed.

4. Keywords:

Touch DNA; swabs; absorbing paper; ethanol

5. Introduction

Biological stain evidence often contains very small amounts of DNA. Therefore, efficient stain collection methods are needed. A widely used method for biological stain collection is wiping the area of interest with cotton swabs moistened with water. However, a number of studies have shown that the types of swabs or devices and

also the type of fluid used have an effect on stain collection efficiency (e.g. Thomasma & Foran 2013, Verdon *et al.* 2014a, 2014b).

In this study, we have compared the sampling efficiency of cotton swabs (25-806 PC, Puritan) and pieces of absorbing paper ("absorbent towel", code 7506, Kimtech) moistened with either nuclease-free water or 70% ethanol.

6. Material and methods

In an initial experiment, clean microscope slides were prepared with 10 µl of EDTA-blood diluted 1:10 in 1xPBS. Blood was chosen because it is easy to standardize and deposits as a stain visible to the human eye. Samples were dried in a biosafety cabinet overnight before sampling. The absorbing paper was cut into 2 x 2 cm^2 pieces. Cotton swabs and pieces of paper were moistened by pipetting 40 µl or 70 µl of fluid, respectively. The amount of fluid was tested and adjusted beforehand to ensure optimal stain collection with each method, e.g. the amount of fluid should be sufficient to be able to collect all the blood without leaving fluid on the glass slide. Pieces of paper were handled with sterile tweezers. Sampling was carried out with swabs and paper moisten with either nuclease-free water or 70 % ethanol (n = 12 for each device-fluid combination).

To reflect casework conditions, the DNA sampling efficiency of cotton swabs in combination with nuclease-free water (standard) was compared to absorbing paper in combination with 70 % ethanol (the best combination from the initial experiment, see Results and discussion). A range of used items (small plastic containers, covers, glasses, pens, chargers, head sets and watches) and clothing (shoes, tights, bras, socks, tops, sweaters, hats and gloves), for which it was possible to define two equivalent areas for stain collection, were wiped with both methods in parallel (n=82). The items were divided into four surface classes: plastic, leather, natural and synthetic fabrics. Both swabs and pieces of paper were handled as during routine work: they were slightly wetted, and excess fluid was removed by touching a sterile sheet before stain collection.

DNA was first extracted from whole swab heads or pieces of paper using the PrepFiler Express™ DNA extraction kit on the AutoMate Express™ instrument and then quantified with the Quantifiler® Trio-kit on the 7500 Real-Time PCR System (all Thermo Fisher Scientific). Degradation Index (DI) values of the samples were categorized as "non-degraded" (DI 0 – 1.5) or "mildly degraded (DI 1.5 – 4), according to Vernarecci et al. 2015.

To see if the sampling methods affect DNA-profiling, a subset of DNA extracts from samples reflecting casework conditions (n = 17 sample pairs) was additionally amplified with the AmpFLSTR® NGM SElect™ PCR Amplification Kit on the Veriti™ Thermal Cycler. DNA fragments were analyzed using the 3500xL Genetic Analyzer and GeneMapper® ID-X Software v 1.5 with in-house settings (all Thermo Fisher Scientific).

The data was analysed using the Real Statistics Resource Pack software, Release 6.4.1 downloaded from www.real-statistics.com (Zaiontz, Copyright 2013 – 2019).

Donors of the analyzed biological material have all given informed, written consent.

7. Results and discussion

The initial experiment showed that DNA yields from blood stains deposited on microscope slides were not significantly different if using cotton swabs with water (mean 0.681 ± 0.108 ng/µl), cotton swabs with ethanol (mean 0.627 ± 0.105 ng/µl) or absorbing paper with water (mean 0.790 ± 0.213 ng/µl) (Fig. 1A, ANOVA contrasts, $p > 0.05$). DNA yields from samples collected with absorbing paper and ethanol were between 1.5 and 1.9 times higher compared to the other device-fluid combinations (mean 1.192 ± 0.236 ng/µl) (Fig. 1A, ANOVA contrasts, $p < 0.0001$). None of the samples was degraded (all DI < 1). Thus, similar performance independent of sampling method is expected for DNA-profiling.

For the used items reflecting casework samples, the DNA yield was overall 1.9 times higher when collected with absorbing paper and ethanol (median 0.122 ng/µl, min. 0.005 ng/µl, max. 1.143 ng/µl) than with cotton swabs and water (median 0.065 ng/µl, min. 0.000 ng/µl, max. 0.791 ng/µl) (Wilcoxon Signed-Rank Test for Paired Samples, $p < 0.0001$). Equivalent results were obtained for three out of four surface classes, namely plastic, leather and natural fabrics (Wilcoxon Signed-Rank Tests for Paired Samples, $p < 0.01$, $p < 0.0001$ and $p < 0.01$, respectively, Fig. 1B). For synthetic fabrics, the difference between sampling treatments was not significant.

DI values of the samples ranged between 0.422 and 3.674. There was no significant difference in degradation level between the two sampling treatments (Wilcoxon Signed-Rank Test for Paired Samples, $p > 0.5$). Furthermore, peak heights in DNA-profiles were as expected according to the sample's DNA quantity and quality (data not shown).

These results suggest that using pieces of absorbing paper moistened with ethanol can improve the efficiency of stain collection from items with small amounts of DNA compared to the standard method with cotton swabs and water. However, we experienced that handling small pieces of paper with tweezers was not as easy as using a swab. Furthermore, batch controls are recommended since manual preparation of the paper (cutting) may increase the contamination risk.

8. Conclusion

More DNA is recovered when collecting epithelial cells/touch DNA using pieces of absorbing paper moistened with 70 % ethanol instead of cotton swabs moistened with water. Development of easy-to-handle collection devices based on the material of the absorbing paper instead of cotton would help to increase user-friendliness and reduce contamination risk.

Further studies might be conducted for comparison with other sampling methods and in relation to long-term storage of collected samples.

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Conflict of interest statement: None.

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Figures

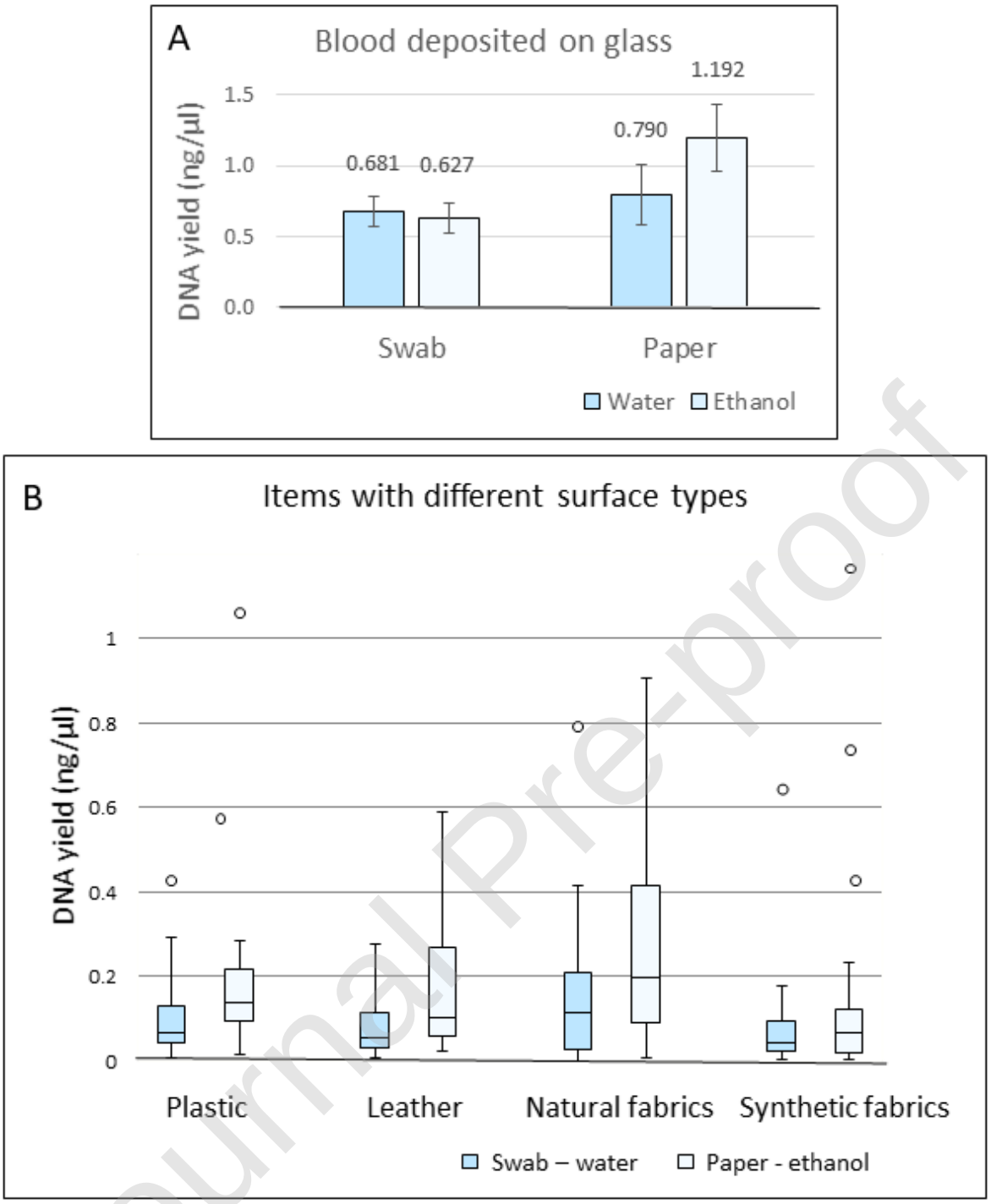

Figure 1: A. Mean ± SD of DNA yields obtained from blood samples deposited on glass slides, collected with cotton swabs or pieces of paper in combination with either water or 70 % ethanol. B. Boxplot of DNA yields obtained for samples collected from items with different surface types, using cotton swabs with water or pieces of paper with 70 % ethanol. - The eluation volume of all DNA extracts was 50 µl.