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THE INFLUENCE OF HAND DOMINANCE, HAND WASHING AND SAMPLING TECHNIQUE ON QUANTITY OF DNA RECOVERED FROM HANDLED PLASTIC TUBES

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Summary. There are several previously published studies suggesting that different people deposit different quantities of their own DNA on items they handled, so can be considered good or bad shedders. This study aimed to investigate the amount of DNA deposited on sterile plastic tubes handled by the employees in our lab for 15 seconds. In particular, we wanted to test if there are differences in the amount of DNA deposited by dominant versus non-dominant hand. Moreover, we investigated the amount of DNA deposited before, immediately after and 30 minutes after hand washing. In the end, this study compared two sampling techniques, namely wet and dry swabbing, aiming to define the technique that guarantees better recovery of touch DNA. A samples were collected from 6 individuals and analyzed for differences in DNA quantity between the two hands of the same individual, but also between different individuals and different sampling techniques. In general, our preliminary results have shown that there are no significant differences between dominant vs nondominant hand. Consistent differences were observed between individuals regarding their ability to deposit biological material on handled objects. Sampling technique was factor that significantly influenced the amount of recovered DNA, suggesting that wet swabbing recovered higher DNA amounts compared to dry swabbing. Hand washing can be considered efficient anti contaminant measure as it significantly reduces the amount of biological material deposited on handled object. Further studies are needed to confirm our findings, especially those considering quantification of DNA deposited by individuals for prolonged period of time.

Key words: shedder status, hand washing, DNA amount, forensics.

Introduction

The transfer of DNA to the surroundings can happen in several ways, namely via primary, secondary and subsequent depositions of biological material. Primary transfer refers to DNA deposition via shedding of skin cells during direct contact with an object or a person (so called "touch DNA") or deposition via aerosol such as saliva spray during talking or coughing (Burrill et al., 2019). Touch DNA is one of the most common types of samples processed in forensic laboratories and is considered an important tool for crime investigation especially in cases where the body fluids are absent. If we remember Locard's Exchange Principle which points out that "every contact leaves a trace" (Locard, 1930), it's obviously that perfect crime doesn't exist and its upon investigators' knowledge and dedication to discover and collect often a minute quantities of biological material present in touch DNA samples. Although low in most cases, the DNA concentration in such samples is often enough to generate full DNA profile of the perpetrator. Such low-quantity touch DNA samples supported essen-

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tial evidence which linked suspects with a crime scene many times during our experience in resolving a wide range of criminal cases (thefts, sexual violence, murders and so on).

According to published data, the amount of DNA transferred to a substrate during direct contact (e.g. handling) seems to be independent of duration of the contact, however dependent on the individual characteristics of a handler and features of a handled substrate (Wickenheiser, 2002). Results of Sessa et al. suggest that it is possible to obtain a complete handler DNA profile with a high percentage (from 87.6% to 99.24%) at different touch times (2s, 5s, 8s, 10s, 20s, 30s, 45s, 60s) regardless the sampling methods (Sessa et al., 2019). In another experimental study Breathnach et al. report that a handling time of 15 s was enough for successful recovery of handler's DNA profile (Breathnach et al., 2016). So, even if it is commonly thought that the amount of DNA deposited on a surface could be increased with increased handling time, the experimental data suggest that duration of contact is not an essential factor.

In terms of DNA transfer and recovery, the features of the substrate seem to have significant influence. Daly et al. (2012) published results which indicate that deposition of biological material on wooden substrates resulted in the highest DNA yield, followed by fabric and glass.

In the end, there are many studies in the literature which point out that the amount of DNA deposited on the handled object is strongly dependent on individual skin conditions. Some authors considered that individuals can be categorized as "good shedders" and "bad shedders" depending on their ability to deposit DNA traces on handled objects (Lowe et al., 2002; Djuric et al., 2008). In the experiments of Lowe et al. (2002), 18 out of 30 volunteers were classified as "good shedders", who left a full profile on sterile plastic tubes held in the closed fist 15 min after washing. On the other side, there are studies which state that an individual can act as both good or bad shedder depending on the condition of the skin in a moment of sampling (Phipps and Petricevic, 2007). Some skin diseases like atopic dermatitis or psoriasis have a considerable impact on the amplifiable DNA left by skin contact with surfaces (Kamphausen et al., 2012).

Besides the initial amount of biological material deposited on the surface, sampling technique is another factor influencing the overall success of DNA profiling. So called swab technique is one of the most common methods for optimal collection of cellular material which consider using sterile swabs against the surface of the handled object (Sołtyszewski et al., 2015). Swabs can be wet or dry, and many laboratories apply the double swab technique (wet and dry swab) to improve the quality of the resulting DNA profiles (Pang and Cheung, 2007).

This study aimed to investigate the amount of DNA deposited on sterile plastic tubes handled by the employees in our lab. In particular, we wanted to test if there are differences in the amount of DNA deposited by dominant versus non-dominant hand. Moreover, we investigated to what extent hand washing reduces the amount of deposited DNA. In the end, this study compared two sampling techniques, namely wet and dry swabbing, with the aim of defining the technique that guarantees better recovery of touch DNA.

Materials and Methods

Sample collection

Before any sampling, 15 ml plastic tubes were cleaned with 10% bleach, 70% ethanol and sterile water, and placed in UV hood for 15 minutes to improve sterility .

Six employees in our lab (4 females and 2 males, general age range of early 30's to early 40's) held the plastic sterile tubes with moderate (equivalent to shakehand) pressure in their fists for 15 sec, without moving them. On one occasion, volunteers were asked not to wash their hands prior to holding after being involved in everyday activities, mostly doing paper-

work or using computer. Immediately after holding, tubes were sampled with wet swabs (Copan, Brescia, Italy). This sampling procedure was repeated over 3 days. On the other 2 days, volunteers held plastic tube in the same manner, but the sampling was performed using dry swabs (Copan, Brescia, Italy).

On different occasion, volunteers were asked to wash their hands with soap for 15 sec and dry them with cotton towels. Immediately afterwards they held sterile plastic tube in one hand for 15 sec. Half an hour afterwards, the same individuals held sterile tubes for 15 sec but in another fist. The tubes were sampled with wet swabs.

Extraction of DNA

DNA extraction was conducted with *QIAamp DNA mini* kit (Qiagen, Germany), according manufacturer's recommendations with certain modifications. Namely, 400 µl of ATL buffer and 20 µl Proteinase K were added, vortexed and incubated at 56 °C for 1 h. 400 µl of AL buffer was added and incubated for 10 min at 70 °C. 200 µl of ethanol (Merck, Germany) was added, samples were carefully transferred to the columns and centrifuged at 8,000 rpm for 1 min. 500 µl of AW1 buffer was added to the columns and centrifuged at 8,000 rpm for 1 min, afterwards 700 µl of AW2 buffer was added to the columns and centrifuged at 8,000 rpm for 1 min. 700 µl of ethanol was added to the columns and centrifuged at 8,000 rpm for 1 min. In the end, empty columns were centrifuged at 13,200 rpm for 3 min in order to clean the columns from residual buffers and ethanol. The columns were placed into clean 1.5 ml tubes and 100 µl of pre-warmed sterile water (Qiagen) was added, incubated at room temperature for 5 min before centrifugation at 8,000 rpm for 1 min. DNA extracts were quantified on the same day.

Quantification

The *Quantifiler HP* kit assay was used for estimation of the concentration of human DNA present in all samples and this was performed according to the protocol recommended by manufacturer (Applied Biosystems, USA).

Duplicate Quantifler standards ranging from 50ng/µLto 0.005 ng/µL and negative controls were processed together with the reactions. Quantification was carried out on the ABI PRISM 7500 REAL Time PCR System (Applied Biosystems, USA).

Data interpretation and statistical analysis

The total DNA recovered per tube was calculated by multiplying the DNA concentration, determined during quantification, by the volume of the extract (100 µl). Data were analyzed with the software SPSS 22.0 for Windows. The *t*-test and one-way variance analysis (ANOVA) were used to determine any statistically significant differences among the groups.

Results

The concentrations of DNA deposited on sterile plastic tubes by six volunteers during 15 sec contact (unwashed hands, wet *versus* dry swabbing) are summarized in Table 1. For each volunteer there was no significant difference (paired *t*-test, $p < 0.05$) between the amounts of DNA deposited by left and right fist over 3 days (wet swabbing) and 2 days (dry swabbing).

Table 1. Concentrations of DNA deposited by six volunteers during 15 sec contact with plastic tubes (unwashed hands, wet versus dry swabbing)

Volunteer	Wet swabbing				Dry swabbing			
	Left fist (ng/µl)	Right fist (ng/µl)	*t*-test ($p < 0.05$)	Average DNA amount (pg)	Left fist (ng/µl)	Right fist (ng/µl)	*t*-test ($p < 0.05$)	Average DNA amount (pg)
I	0.0139	0.0101			0.0051	0.0029		
	0.0079	0.0039			0.0002	0.0005		
	0.0029	0.0027	t=0.68, p=.27	690	/		t=0.34, p=.38	220
II	0.0040	0.0086			0.0067	0.0054		
	0.0139	0.0040			0.0004	0.0006		
	0.0041	0.0007	t=0.71, p=.26	588	/		t=-0.88, p=.23	330
III	0.0091	0.0045			0.0073	0.0162		
	0.0129	0.0211			0.0005	0.0003		
	0.0074	0.0025	t=0.07, p=.47	959	/		t=-0.5, p=.33	608
IV	0.0029	0.0020			0.0019	0.0027		
	0.0013	0.0016			0.0008	0.0015		
	0.0003	0.0002	t=0.29, p=.39	138	/		t=-0.95, p=.22	171
V	0.0054	0.0043			0.0003	0.0027		
	0.0052	0.0059			0.0005	0		
	0.0069	0.0059	t=0.61, p=.57	559	/		t=-0.71, p=.28	87
VI	0.0025	0.0015			0.0026	0.0018		
	0.0066	0.0036			0.0019	0.0016		
	0.0077	0.0050	t=0.78, p=.24	449	/		t=1.41, p=.15	196

Considering that there was no difference between left and right fist, we used data for each volunteer as six (wet swabbing) and four (dry swabbing) replicates and presented them as box plot diagram (Figure 1). In order to investigate whether there is a difference between volunteers in quantity of deposited biological material, one-way ANOVA ($p < 0.05$) was employed.

As can be seen on Figure 1, volunteer IV deposited significantly lower amount of DNA compared to all other volunteers when wet swabbing technique was employed (one-way ANOVA [F=2.78, p=0.03]). On the other side, one-way ANOVA revealed no differences in quantity of deposited DNA between volunteers when dry swabbing technique was performed ([F=1.12, p=0.4]). After comparing DNA concentrations from samples collected with wet vs dry swabs, the results suggested significant difference in favor of wet swabbing to efficiently collect larger quantities of biological material (one-way ANOVA [F=7.2, p=0.0095]).

The concentrations of DNA deposited on plastic tubes immediately after hand washing are presented as box plot diagram in Figure 2. Four out of six volunteers deposited less than 60 pg of DNA while the maximal deposition was 150 pg. According to paired *t*-test ($p < 0.05$) hand washing significantly reduced the amount of deposited DNA compared to unwashed hands in all volunteers except volunteer IV.

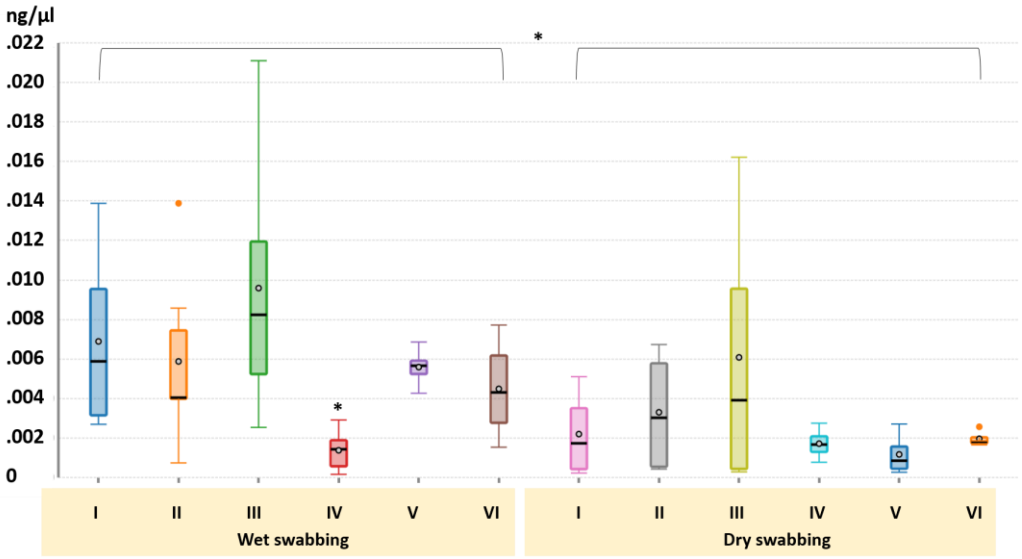

Figure 1. Box plot summarizing the DNA concentrations (ng/µl) deposited on sterile plastic tubes by unwashed hands of six volunteers and sampled with wet and dry swabs. Whiskers extend to min and max values, the bold lines present median values, the dots present mean values. The dots above max value are outliners, while asterisks stand for significant difference.

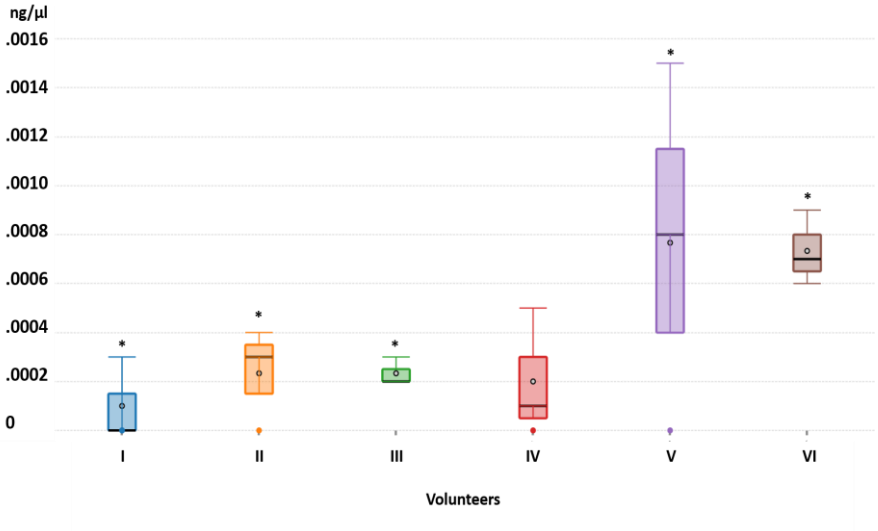

Figure 2. Box plot summarizing the DNA concentrations (ng/µl) deposited on sterile plastic tubes by six volunteers immediately after hand washing. Comparisons were made to depositions left by unwashed hands. Asterisks stand for significant difference (paired *t*-test, $p < 0.05$).

Half an hour after hand washing, three out of six volunteers deposited significantly higher amounts of DNA compared to the amounts deposited immediately after hand washing (Figure 3, pair *t*-test, $p < 0.05$). On the other side, even 30 minutes after hand washing, volunteers I, IV and V deposited amounts of DNA similar to those deposited immediately after hand washing.

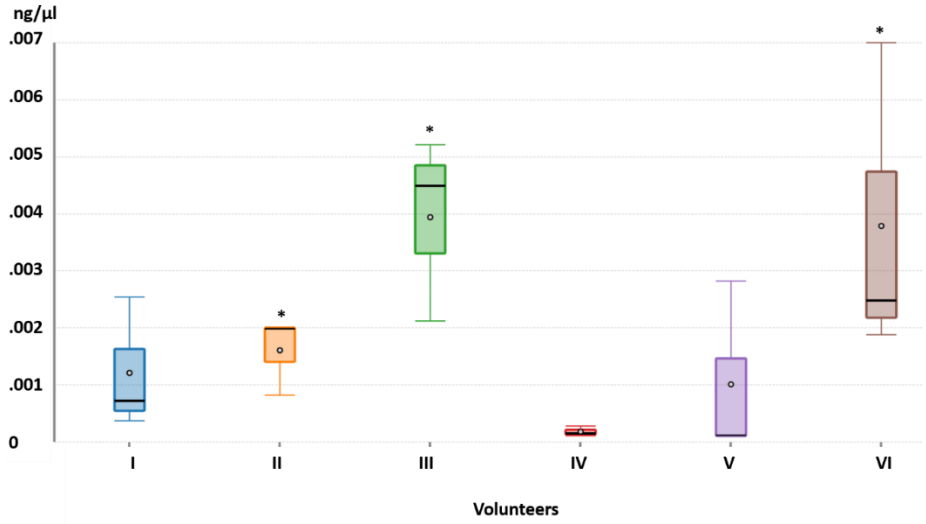

Figure 3. Box plot summarizing the DNA concentrations (ng/µl) deposited on sterile plastic tubes 30 minutes after hand washing. Comparisons were made to depositions left by hands immediately after washing. Asterisks stand for significant difference (paired *t*-test, $p < 0.05$).

Discussion

In our study no significant differences were observed between the amount of DNA deposited by left and right hand of all tested individuals regardless the sampling technique. Such results are consistent with previously published study on 10 individuals and total of 240 handprints on glass plate where the authors have noticed that dominant and non-dominant hand deposited similar amounts of DNA (Goray et al., 2016).

The sampling technique seems to influence the amount of DNA isolated from touch DNA samples. The average amounts of DNA recovered from deposits sampled with wet swabs ranged from 138 to 959 pg while dry swabbing technique resulted in significantly lower amounts of recovered DNA (range from 87 to 608 pg). According to our experience and published data in the literature, the amounts of 100 to 200 pg of DNA (Budowle et al., 2009) are considered as threshold limits for successful PCR reaction. In our study, wet swabbing recovered DNA in threshold limit amount in one out of six individuals, while in five others the amounts of recovered DNA were sufficient for two or even three PCR reactions. Dry swabbing technique recovered DNA in threshold limits in four out of six individuals. Similar results are published by Pang and Cheung (2007) who have compared two sampling techniques against various substrates and have concluded that double swabbing technique is golden standard for collecting touch DNA samples.

In the present study we also tested whether the employees in our lab could be classified as good or bad shedders. One out of six individuals (namely participant IV) regularly left significantly lower DNA depositions on plastic tubes when compared to the others. It seems

that persons differ among each other in ability to shed biological material onto surrounding objects, as previously was suggested by Lowe et al. (2002).

Hand washing reduced the amount of biological material deposited on sterile tubes from 6 up to 40 times, the reduction being significant for all volunteers except volunteer IV. Immediately after hand washing the average amount of deposited DNA ranged from 9 to 77 pg, far below threshold limit for PCR reaction. Zoppis et al. (2014) reported that two hand washing techniques (conventional washing with regular soap and deep washing with antiseptic soap) resulted with no profiles in tested volunteers. Half an hour after hand washing the average amount of recovered DNA ranged from 18 to 394 pg. Although those amounts were lower compared to the amounts deposited by unwashed hands, in volunteers II, III and VI they were sufficient for PCR amplification (range from 160 to 394 pg). On the other side, in volunteers I, IV and V the amounts of DNA 30 minutes after handwashing were similar to those collected immediately after washing (range from 18 to 121 pg), so insufficient for PCR reaction.

According to our results, there are no difference in the amount of DNA deposited by dominant and non-dominant hand. On the other side, sampling techniques matters in a sense that higher amounts of DNA were recovered when wet swabbing was performed. Participant IV constantly deposited low amounts of DNA, regardless of hand washing regime and sampling technique, therefore could be considered as bad shedder. Hand washing significantly reduces the amount of biological material left on handled objects, so can be considered as one of the anticontamination activity in forensic laboratories.

Further studies are needed to elaborate our results. In particular, it would be of interest to determine if individuals deposit consistent quantities of DNA for prolonged period of time considering sex, hand size and hormonal status in women

References

Breathnach, M., Williams, L., McKenna, L., Moore, E. (2016). Probability of detection of DNA deposited by habitual wearer and/or the second individual who touched the garment. *Forensic Sci Int Genet*, 20,53–60.

Burrill, J., Daniel, B., Frascione, N. (2019). A review of trace "Touch DNA" deposits: Variability factors and an exploration of cellular composition. *Forensic Sci Int Genet*, 39,8–18.

Budowle, B., Eisenberg, A.J., van Daal, A. (2009). Validity of low copy number typing and applications to forensic science. *Croat Med J*, 50:207-217.

Daly, D., Murphy, C., McDermott, S.D. (2012). The transfer of touch DNA from hands to glass, fabric and wood. *Forensic Sci Int Genet*, 6,41-46.

Djuric, M., Varljen, T., Stanojevic, A., Stojkovic, O. (2008). DNA typing from handled items. *Forensic Sci Int Genet*, 1:411–412.

Goray, M., Fowler, S., Szkuta, B., van Oorschot, RAH. (2016). Shedder status-An analysis of self and non-self DNA in multiple handprints deposited by the same individuals over time. *Forensic Sci Int Genet*, 23:190–196.

Kamphausen, T., Schadendorf, D., von Wurmb-Schwark, N., Bajanowski, T., Poetsch, M. (2012). Good shedder or bad shedder-the influence of skin diseases on forensic DNA analysis from epithelial abrasions. *Int J Legal Med*, 126:179-183.

Locard, E. (1930). The analysis of dust traces. Part 1. *Am J Police Sci*, 1,276–298.

Lowe, A., Murray, C., Whitaker, J., Tully, G., Gill, P. (2002). The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. *Forensic Sci Int*, 129,25–34.

Pang, B.C.M., Cheung, B.K.K. (2007). Double swab technique for collecting touched evidence. *Leg Med*, 9,181–184.

Phipps, M., Petricevic, S. (2007). The tendency of individuals to transfer DNA to handled items. *Forensic Sci Int*, 168:162–168.

Sessa, F., Salerno, M., Bertozzi, G., Messina, G., Ricci, P., Ledda, C., Rapisarda, V., Cantatore, S., Turillazzi, E., Pomara, C. (2019). Touch DNA: impact of handling time on touch deposit and evaluation of different recovery techniques: An experimental study. *Nature Sci Rep*, 9,9542-9551.

Sołtyszewski, I., Szeremeta, M., Skawrońska, M., Niemcunowicz-Janica, A., Pepiński, W. (2015). Type ability of DNA in touch traces deposited on paper and optical data discs. *Adv Clin Exp Med*, 24,437–440.

Wickenheiser, R.A. (2002). Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. *J Forensic Sci*, 47,442–450.

Zoppis, S., Muciaccia, B., D'Alessio, A., Ziparo, E., Vecchiotti, C., Filippini, A. (2014). DNA fingerprinting secondary transfer from different skin areas: Morphological and genetic studies. *Forensic Sci Int Gen*, 11:137–143.

UTICAJ DOMINANTNOSTI RUKE, PRANJA RUKU I TEHNIKE UZORKOVANJA NA KOLIČINU KONTAKTNE DNK DEPONOVANE NA PLASTIČNE TUBE

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Sažetak. Nekoliko prethodno objavljenih studija ukazuju da različite osobe deponuju manje ili veće količine svoje DNK na predmete koje dodiruju. Cilj ovog istraživanja bio je da se ustanovi količina DNK koju zaposleni u laboratoriji ostavljaju na plastičnim tubama nakon što ih drže 15 sekundi. Posebna pažnja posvećena je testiranju postojanja razlika između količine DNK prilikom deponovanja dominantnom i ne-dominantnom rukom. Pored toga, ispitivana je i količina DNK koja se deponuje pre pranja ruku, odmah nakon i 30 minuta nakon pranja. Na kraju, u ovom istraživanju ispitivane su i dve tehnike za uzorkovanje DNK- uzorkovanje pomoću suvih ili vlažnih briseva sa ciljem da se utvrdi pomoću koje je moguće uzorkovati veću koncentraciju kontaktne DNK. Uzorci su prikupljeni od 6 osoba radi ispitivanja različite koncentracije DNK koju ista osoba deponuje levom i desnom rukom, koncentracije DNK koju deponuju različite osobe, kao i razlika prilikom uzorkovanja DNK različitim tehnikama. Generalno, preliminarni rezultati su pokazali da ne postoji značajna razlika u količini DNK koju ista osoba deponuje dominantnom i ne-dominantnom rukom. Konzistentne razlike su uočene između različitih osoba i količine DNK koju deponuju na objekte. Faktor koji je posebno uticao na koncentraciju uzorkovane DNK je tehnika uzorkovanja, i kao bolja se pokazala tehnika uzorkovanja vlažnim brisevima. Utvrđeno je da je pranje ruku veoma efikasna anti-kontaminacijska mera jer značajno utiče na smanjenje količine deponovane DNK na objektima. Dalja istraživanja su neophodna da bi se potvrdili dobijeni zaključci, a posebno su značajna ispitivanja količine DNK koju deponuju različite osobe tokom dužeg vremenskog perioda.

Ključne reči: deponovanje biološkog materijala, pranje ruku, količina DNK, forenzika.