DNA: transfer and persistence

- A review of current literature
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1 Introduction

The understanding of transfer and persistence of biological traces and DNA is becoming increasingly important in the field of forensics. The development of more sensitive and robust methods for DNA amplification and analysis allows for detection of minute amounts of DNA, but at the same time renders challenges regarding interpretation.

In many cases today, the matching of a crime scene DNA finding to an individual is not the key issue in concluding guilt, regardless of the strength of the match itself. Instead, it is rather the explanations on how the DNA came into place that will be important for the case outcome.

The number of publications on topics like transfer and persistence of DNA, background DNA and ultimately the activity level of DNA findings, are rapidly increasing. This report is a compilation of published papers and reports regarding these and closely related topics. Each paper is followed by a short summary of its content, allowing the reader to get an overview of a selected topic as well as suggestions of papers to dig deeper into.

This report is organized in different sections by selected topics. The publications in each section are in alphabetical order by author. Topics used in this report are:

Activity level  Adhesive tapes  Ammunition & spent cartridges
Background DNA  Case studies  Clothes
Contamination  Court decisions  Explosives
Fingerprints  Finger samples  LCN/LT
Penile swabs  Persistence  Review articles
Touch DNA  Transfer  Washed stains/clothes
Weapons & arms

Naturally, topics and references overlap, which should be taken into account when using the report. Many papers appear more than once depending on the topics covered. The authors claim neither full coverage of the separation of all the papers into the different topics, nor that the compiled publications are exhaustive – additional papers do exist and new ones will be published!

To increase the usability of the report all references are presented with an accurate weblink, often to full texts, but at times only the abstracts are accessible.

2 Materials and methods

References in the report have been retrieved through the internal NFC library and by online searches using scientific portals such as Pubmed, Scopus and ScienceDirect, as well as other more general search sites like Google Scholar.
3 References by topics

3.1 Activity level

Publish or perish – meanwhile, study what’s already out there
Bader S., The Barrister.

Courts in the UK have a tendency to trust “experience” more than science.
“12 hours after digital penetration it was three times more likely to find that the major contributor to a profile was from the woman.” (On the male offenders fingers.)

Environmental DNA monitoring: beware of the transition to more sensitive typing methodologies
http://www.tandfonline.com/doi/abs/10.1080/00450618.2013.788683#.Vd25pfntkXs

Environmental DNA monitoring (EDM): surfaces and tools are sampled on a periodic basis.
Kits used in the study: Profiler Plus, Yfiler, PowerPlex 21.
Washing (hypochlorite + ethanol) did not remove all DNA from items like rulers, pipettes and tube racks etc. However, the detectable DNA after cleaning was present in few alleles with low peak heights.

Assessing the risk of secondary transfer via fingerprint brush contamination using enhanced sensitivity DNA analysis methods

“Detection of secondary transfer of DNA can occur through fingerprint brush contamination and is enhanced using LCN-DNA methods.”
“If a brush were to add DNA-containing material to a surface containing a handprint, the proportion of the added DNA is likely to be less than that retrieved from the depositor of the print. Thus, the minor component of the mixture derived from the brush may not be detectable.” (ref 2 in paper: Proff et al. 2006).
“The dusting of latent prints may dislodge cellular debris from the latent print or substrate. That debris then adheres to the brush. This brush is then used on another item of evidence, or at another crime scene, where it is subject to the same mechanical maneuvering and where it can dislodge cellular debris, leaving traces of biological evidence not pertinent to the evidence being handled. Under LCN conditions, it may be possible to obtain DNA results that are not relevant to the case due to a secondary transfer by fingerprint brush contamination” (ref 16 in paper: Pesari et al. 2003).
“In this study, the risk of false associations through the inclusion of contaminant DNA donors was moderate”.

Persistence of DNA from laundered semen stains: Implications for child sex trafficking cases

Setup: semen stains (one or two (1:1) donors) were placed on clothes (T-shirt, trousers, tights) and stored in a wardrobe for 8 months. Items were washed (together with unstained socks) at 30°C or 60°C and with non-biological or biological detergent.
Results: High quantities of DNA (6-18 µg) were recovered irrespective of washing conditions. The quantity did not decline significantly with repeated washes.
T-shirt was better than trousers when there was more than one donor (trousers --> one major DNA profile).
DNA could be recovered from the unstained socks washed together with the stained clothes.
**Probability of detection of DNA deposited by habitual wearer and/or the second individual who touched the garment**


**Setup:** 63 males wore their own underpants for 12 h, the inside waistband was then touched by one of 11 females for 15 s. The waistband was mini-taped and subjected to DNA profiling.

**Results:** The wearer was detected in 51% of the samples and always as the major contributor.

The test-female was detected in 11% of the samples.

Reportable background DNA (non-wearer, non-test-female) was found in 14% of the samples.

There was no statistical difference in DNA quantification results depending on the time of collection (0 or 12 weeks after wearing and touching).

If a single DNA profile is found on e.g. a pair of underwear, it is 5 times more likely that the person has worn the garment than just touched it. A high total DNA concentration would support wearing the garment.

**Oral intercourse or secondary transfer? A Bayesian approach of salivary amylase and foreign DNA findings**


Oral intercourse leads to salivary-α-amylase in penile swabs. However, salivary-α-amylase in penile swabs does not necessarily mean oral intercourse but may be due to secondary transfer.

**Setup:** This is a study of background salivary-α-amylase in underwear after normal (12 hour) wear (69 male subjects). Masturbation with saliva as lubricant was allowed but oral intercourse was not.

**Test method:** Phadebas press test for screening and RSID-Saliva for confirmation.

**Results:** 44% of the underwear had stains containing amylase on the inside front, 13% of these stains were found to be salivary-α-amylase (5.7% of total). 50% of these produced a mixed DNA profile with a minimum of two donors.

A positive control group with oral intercourse showed larger salivary-α-amylase positive stains in the male underwear than the background-test group. 80% of the underwear in this group gave DNA profiles other than the wearers.

“...the evidence of salivary-α-amylase on male underwear (RSID™-Saliva positive) is 17 times more likely to occur with the allegation of fellatio than the alternative hypothesis of secondary transfer. However the finding of no salivary-α-amylase (RSID™-Saliva negative) upon the male underwear is a 100 times more likely if fellatio did not occur rather than if somebody performed fellatio on Mr B. Likewise, it is evident that if salivary-α-amylase and DNA other than the wearer is present on the inside-front of male underpants that it is 27 times more likely if fellatio occurred rather than fellatio did not occur. In saying that, if salivary-α-amylase is found yet the DNA profile does not contain a profile other than that of the wearer, it is 13 times more likely if fellatio occurred rather than if nobody performed fellatio on an alleged victim”.

This paper also contains a section about the sensitivity and specificity of the Phaebas press test and RSID-Saliva; Phadebas press test cannot distinguish between salivary-α-amylase and pancreatic amylase which leads to false positive results, therefore it should only be used for screening.

**Recovery of trace DNA and its application to DNA profiling of shoe insoles**


0.16-6.4 ng DNA could be recovered by swabbing hands. Even though hands were not washed before the experiment no non-donor alleles could be identified.

Protocol for sampling DNA from shoe insoles and the subsequent sampling and extraction.

Taping was the most efficient method for recovering DNA. Swabbing and soaking methods resulted in dirtier extracts and were likely to include increased amounts of inhibitors.

Recovery from synthetic materials was better than from e.g. leather.
Could secondary DNA transfer falsely place someone at the scene of a crime?
Setup: Hand to hand contact for two minutes, then handling a knife (smooth or rough handled knives). Knives were immediately swabbed for DNA. Secondary transfer was detected in 85% of the samples. In some cases the secondary contributor was the major or the only identified DNA profile. There was no significant difference in concentration of DNA between smooth and rough handled knives. For one smooth-handled knife the major contributor was neither one of the test-subjects nor one of the personnel.

Forensic DNA evidence is not infallible.
This is a one page “personal take” on the subject of secondary transfer of DNA. For more detailed reading see “Could secondary DNA transfer falsely place someone at the scene of a crime?” by Cale et al. J Forensic Sci 2015.

DNA transfer: informed judgement or mere guesswork?
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3872334/
Opinion article
“… the quantity of DNA or the quality of the profile cannot be used “to reliably infer the mode of transfer by which the DNA came to be on the surface of interest.” (Meakin and Jamieson 2013).

The prevalence of mixed DNA profiles in fingernail samples taken from individuals in the general population
Aim: “…examination of the incidence of mixed DNA profiles derived from fingernail samples from members of the general population…”
Setup: Fingernail swabs from 100 volunteers.
Results: Foreign DNA was detected in 13% of the samples, only 6% of these gave reportable mixed DNA profiles. “This study demonstrated a low level of foreign DNA under the fingernails of the general population, suggesting that when a strong mixed DNA profile is obtained from a fingernail swab it is unlikely that it exists only due to previous contact between the suspect and victim.” Mostly male donors had significant amounts of foreign DNA in their nail swabblings.

The transfer of touch DNA from hands to glass, fabric and wood
Setup: Glass/fabric/wood, 100 volunteers per type of material, 60 s contact, objects were mini taped.
Results: Wood gave the best yield of DNA (36% gave handler profile) followed by fabric (23%) and then glass (9%). 10% of the total number of samples gave mixed profiles indicating secondary transfer.

An investigation of the presence of DNA on unused laboratory gloves
DNA was found (up to 20 alleles) on gloves from unopened boxes (vinyl gloves, specific brand). DNA was found (up to 14 alleles) on gloves from opened boxes from different brands and of different materials.
Assesing primary, secondary and tertiary DNA transfer using the Promega ESI-17 Fast PCR chemistry
Setup: Volunteers washed thoroughly, spent an hour doing every day activities, shook hands with one or two other persons in the experiment for 30 s and then gripped a plastic tube. The tube was double swabbed for DNA.
Results: In some samples there was more DNA from secondary transfer than from primary. In some samples there was DNA from a donor not in the study.

Contamination monitoring in the forensic DNA laboratory and a simple graphical model for unbiased EPG classification
There is a need for a standardized, unbiased and independent method for the monitoring of background DNA in the laboratory.
Presentation of a graphical classification model for ranking of electropherograms.

The prevalence of mixed DNA profiles on fingernail swabs
Setup: Fingernail scrapings from 40 laboratory staff and police (right and left hand = 80 samples).
Results: 30 % of the samples were low level mixtures of DNA profiles and 11 % were high level mixtures. 8 samples were 3-person mixtures where one of the profiles was unknown (not partner, children, colleague…). The sex of the test-subject was not significant regarding the occurrence of mixed DNA-profiles but the trend suggests that a high level mixed profile was more likely to be collected from the male test-subjects.
“… intimate contact results in transfer of more cellular material than other types of contact.”

Assessment of individual shedder status and implication for secondary DNA transfer
The shedder status of an individual is very important for recovery of DNA from handled items. If the primary user is a poor shedder and a secondary transfer person is a good shedder the dominating (or only) profile could be from the secondary person.

The transfer and persistence of DNA under the fingernails following digital penetration of the vagina
Setup: eight volunteer couples in transfer study, four couples in persistence study.
Results: Full female profiles from all male samples collected after 0-6 hours after digital penetration and from ¾ of samples collected after 12 hours. For samples collected 18 hours after penetration the majority showed mixed profiles. “… hand washing had a significant effect on the persistence of the female DNA profiles”.

Secondary and subsequent DNA transfer during criminal investigation
Detection of DNA transfer from object to plastic gloves worn by investigator (disposable nitrile-gloves) to new object. The amount of DNA deposited on the first object and the object material-type influenced the transfer.
Analysis and implications of the miscarriages of justice of Amanda Knox and Raffaele Sollecito
LCN-levels of victim-DNA (no detection of blood) on a knife, analyzed without replication and with only a few alleles, was used to convict a suspect. Moreover, the location of the suspect-DNA on the handle was interpreted (without scientific grounds) as a result from stabbing rather than cutting food. The suspect and the victim shared an apartment. Evidence was collected using dirty gloves and in non-sterile containers. Mixed profiles of the two roommates were interpreted as one’s involvement in the murder of the other but was most probably just normal background DNA from two people living together.
“Selective cleaning” was believed to have erased the DNA from two suspects but not the third at the murder-scene. Negative controls during the DNA analysis was interpreted as that the evidence could not have been contaminated in any way, either before the murder, at collection of the evidence or during handling of the evidence by the police officers.

Biological and DNA evidence in 1000 sexual assault cases
Setup: Analysis of what proportion of sexual assault cases (1000 cases) provided interpretable DNA profiles from bodily samples.
Results: Teenagers and adults: 46-56 % success rate, children: 11-14 % success rate. Vaginal swabs: 3 days persistence (maximum 100 hours). For all other bodily samples (anal swabs, skin swabs) the persistence is shorter (24 hours). The persistence of an oral wash is maximum 15 hours. The Acid phosphatase (AP) semen detection test is not a good predictor for whether DNA evidence will be found or not in the sperm fraction.

The complexities of DNA transfer during a social setting
A study on “effects of unstructured social interactions on the transfer of touch DNA”.
Setup: Three individuals were video-recorded while having a drink (one central jug and individual glasses) together around a table for 20 minutes. All relevant surfaces were sampled for DNA and the video was used to identify how the DNA was transferred.
Results: Transfer occurred during everyday interactions and was found to be bi-directional. The last person to touch an object was often but not always the major or the only DNA contributor. The participants often introduced DNA from persons not included in the experiment setup.

DNA transfer during social interactions
Very similar to Goray 2015.
Setup: Three individuals having a drink (one central jug and individual glasses) together around a table for 20 minutes. All relevant surfaces were sampled for DNA. Same results as (Goray 2015).

DNA transfer within forensic exhibit packaging: potential for DNA loss and relocation
Analysis of DNA persistence and transfer during packaging in forensic investigations.
“DNA can be transferred from the deposit area to other parts of the item or to the bag (package) itself and usually to both”. “The effect of bag size on transfer was limited but loose bags can, in certain situations, permit more transfer”.
“DNA was lost to the inside of the container holding bloodied knives”, also a lot of re-distribution of DNA on the knives. Tighter fitting of the container prevented re-distribution of DNA from the tip to the handle.
Cigarette butts should always be packed separately.
Less re-distribution of DNA on gloves when they were packed in paper compared with plastic.

**Evaluation of multiple transfer of DNA using mock case scenarios**
Different experimental case scenarios illustrate the likelihood and expected proportion of DNA transfer. Further work is needed to draw conclusions according to the authors.

**Investigation of secondary DNA transfer of skin cells under controlled test conditions**
“The transfer rates showed that both the primary and secondary type of substrate and the manner of contact are important factors influencing transfer of skin cells… the freshness of the deposit in most instances is not.” “Skin cells deposited on a non-porous primary substrate transferred more readily to subsequent substrates than those deposited on a porous substrate. Porous secondary substrates, however, facilitated transfer more readily than non-porous secondary substrates, from both porous and non-porous surfaces. Friction as the manner of contact significantly increased the rate of transfer.”

**Secondary DNA transfer of biological substances under varying test conditions**
“Porous substrates and/or dry samples diminished transfer… whereas non-porous substrates and/or wet samples facilitated the transfer events”. At secondary transfer porous substrates absorbed transferred samples better than non-porous ones. Friction increased the transfer compared to passive or pressure contact.

**Investigation into ”normal” background DNA on adults necks: Implications for DNA profiling of manual strangulation victims**
**Setup:** Optimization of method for collection, extraction and amplification of DNA from skin.
**Measurements of background DNA on skin (neck) and simulation of an assault to study the transfer of DNA between offender and victim.**
**Results:** The necks of 24 volunteers were swabbed and 23% showed non-donor alleles. After simulated strangulation primary, secondary and zero transfer of victim and/or offender DNA could be detected. The background DNA was abundant enough to interfere with DNA profile interpretation after an assault.

**Investigation into the usefulness of DNA profiling of earprints**
**Setup:** 60 earprints (5 s pressure against surface) collected from 3 adults.
**Results:** Full donor profile from one of 60 earprints. One or more non-donor alleles in one-third of the earprints. Direct swabbing of ears showed high levels of non-donor DNA.
**Conclusion:** DNA results from earprints should be interpreted with great care.

**Semen detection: a retrospective overview from 2010**
46% of the studied cases had microscopically verified spermatozoa even though the acid phosphatase test (AP) was negative. Vulva samples seem to produce more negative AP reactions despite the presence of spermatozoa. AP-test, microscopy verification and DNA analysis correlate the best for textiles.
Timing is of importance for the AP-test and could be used as a determinant for time. If AP is positive and spermatozoa is verified it is likely that the assault took place within 24 hours before sampling.
DNA transfer – a never ending story. A study on scenarios involving a second person as carrier
Setup: The possibility of tertiary transfer onto textile and plastic was investigated. Donor’s neck -->
cotton cloth --> carriers hand (with or without glove) --> plastic bag or cotton cloth.
Results: “… a transfer of donor DNA from epithelial cells through a carrier to a second item is
possible, even if the carrier does not wear gloves.” In 11 % of the samples full donor profiles were
detected and in 8 of these 21 samples the donor was the major profile. In ~50 % of the samples a carrier
profile was detected. Full donor profiles were more frequently found when textile was the final object
compared with plastic.

Kercher trial: How does DNA contamination occur?
Very small amounts of suspect’s and victim’s DNA on a knife without traces of blood and mixed
profiles on a bra clasp led to conviction.

Experience is the name that everyone gives to their mistakes
Jamieson A, Meakin G., Barrister Mag 2010; 45.
Courts are skeptical to experts and prefer experience.
The habitual wearer tends to be the major source of DNA on a garment. DNA can be deposited onto an
object that the donor has never touched through transfer, depending on shedder status the transferred
DNA can provide the major profile.

DNA transfer through nonintimate social contact.
A study on how much female DNA can transfer to male underwear and to the penis through
nonintimate social contact.
Setup: After staged nonintimate contact with females (massaging the female face for 2 min and rubbing
hands for 3 min) and simulated urination, penile swabs and underwear were collected either 5 min or 6
hrs after the experiment. Samples from the underwear were collected by mini-taping five different areas
(waistband, front panels (in- and outside) and back (in- and outside)). Penile swabs and underwear were
also collected after unprotected sexual intercourse (without ejaculation). The underwear used was new
and worn for 5 min after the intercourse before collected.
Results: After nonintimate contact and sample collection after 5 min matching female (partial) profiles
were detected in 5/30 waistbands and 1/30 outside panels (1 matching allele, 56 rfu). The maximum
peak height on the waistbands ranged from 180-816 rfu. 4/30 penile swabs resulted in female DNA
with a maximum of 5 alleles and 166 rfu. When samples and underwear was collected after 6 hrs
female DNA was detected in 1/14 waistbands (maximum peak height 161 rfu) and in no penile swabs.
When unprotected sexual intercourse preceded the sample collection female profiles were found in all
samples. Maximum peak height ranges were: waistband (all full profiles) 1386-1357 rfu, inside front
(all full profiles) 1898-3157 rfu, penile shaft (all full profiles) 958-5835 rfu. Full profiles were also
detected on all samples from outside front as well as inside and outside the back of the underwear.
Conclusion: The authors claim that “if DNA matching the female complainant is found on the
waistband of a male suspect’s underwear, the data obtained in this study suggest that depending on the
time delay before the underpants are seized, a matching female DNA profile below 1000 rfu might be
explained by nonintimate social contact with secondary transfer of female DNA from the male’s
hands.”
Everything clean? Transfer of DNA traces between textiles in the washtub
Transfer of DNA from worn clothing (without bloodstains) to another garment is highly unlikely both during hand- and machine washing. Blood can easily be transferred to other garments during the washing procedures.

Assessment of DNA transfer involving routine human behavior
Kelley, S., Fort Worth, Tx: University of North Texas Health Science Center; thesis 2010.
http://digitalcommons.hsc.unt.edu/cgi/viewcontent.cgi?article=1098&context=theses
A study on secondary transfer in a setup that mimics normal day behaviour regarding saliva and sweat. Setup: Volunteers were instructed to e.g. lick their thumb (as when turning a page) and after 5-30 minutes shake hands with a person B who would then grip a plastic tube, samples were collected from the tube. Experiments involving pen-licking and simulated sweat were also included in different combinations.
Results: 28 amplification cycles on 208 samples resulted in 10 samples with one allele or more. Five of these 10 were setup as follows: person A bites a pen for 2 minutes, after 30 minutes the the palm of person B is sprayed with water to stimulate sweat, then person B holds the pen and after different time intervals person B grips a plastic tube which is swabbed for analysis. 12 samples were reanalysed with 34 amplification cycles and the profiles were interpreted, all but one of the profiles were partial. The profiles were dominantly from person A and in two samples only from person A.

The recovery and persistence of salivary DNA on human skin
Setup: Saliva from men was deposited on female skin and left there for 96 hours.
Results: Full male profiles were obtained after 96 hours in 8 of 9 cases. Tapelifting was the most efficient method to recover the DNA (compared to wet and dry swabbing). Dried salivary DNA on skin transferred to fabric, cotton was most efficient and leather least efficient for DNA transfer.

IPV – Bridging the juridical gap between scratches and DNA detection under fingernails of cohabitating partners
(IPV = Intimate partner violence)
The respective partners DNA will be under the fingernails in 17 % of couples that live together.
Setup: Female vigorously scratched male back.
Results: There was a highly significant difference in absolute and relative male DNA under scratching and non-scratching fingers. The mean difference was 16-22-fold depending on finger. However, a negative result for male DNA did not mean the nail was not involved in the scratching.

A systematic analysis of secondary DNA transfer
http://projects.nfstc.org/workshops/resources/literature/A%20Systematic%20Analysis%20of%20Secondary%20DNA.pdf
The data in this paper does not suggest that secondary transfer can interfere with DNA typing results under typical forensic conditions.
Setup: 1) Handshaking and holding of object (rubbing in both instances), 2) Coffee mugs were handled for 2 hours and then handled by another user. Palms and objects were swabbed. 3) Commonly handled objects were swabbed for primary transfer.
Results: Secondary transfer was not detected. Primary transfer could yield interpretable results.
Following the transfer of DNA: How does the presence of background DNA affect the transfer and detection of a target source of DNA?

Setup: wet blood, dried blood or touch DNA in five transfer steps on glass or on cotton fabric. Background biological material was deposited on the substrates, either from the same individual on all substrates or from different individuals on different substrates in the transfer series.

Results: In most cases the deposited background DNA affected the DNA profiling of the target sample. Background blood (wet and dry) affected more than touch DNA and if the substrate was cotton background DNA from blood made DNA profiling impossible. If the background DNA was touch DNA full target DNA profiles could mostly be obtained, even after multiple transfer steps. It seemed like the transfer of the target sample was inhibited by the presence of background DNA on the substrates. Moreover, detection of the target DNA decreased with an increased number of background donors.

See paper for specific results regarding different combinations of samples and substrates.

Following the transfer of DNA: How far can it go?

Setup: Wet blood, dry blood or Touch DNA was transferred (15 s, 1.4 kg) on glass or cotton substrates.

Results: Wet blood could transfer full profiles to the fourth cotton and sixth glass substrate. Dry blood and Touch DNA gave full profiles on the first cotton substrate only. Dry blood on glass could transfer full profiles to the sixth substrate while Touch DNA on glass gave partial profiles from the second to the fifth substrate.

Use of low copy number DNA in forensic inference
http://www.isfg.org/files/31f9316afbc584bc0befdd454d6cd38c4f064f3a.02004843_693490260903.pdf
Discussion about shedder status and the likelihood of getting DNA profiles from Touch DNA and used garments.
E.g. a good shedder can be the major profile on a garment worn by a poor shedder. Also, a good shedder can be the major profile after a secondary transfer event with a poor shedder.

The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces
Individuals differ in their tendency to deposit DNA. Hand washing seemed to be of importance. A good shedder could deposit DNA close after hand washing while a poor shedder required longer time after washing. If the time period after hand washing is between 2 – 6 hours the shedder status may not be of big importance. Secondary transfer is a real possibility in forensic case work.

The prevalence of mixed DNA profiles in fingernail samples taken from couples who co-habit using autosomal and Y-STRs
Setup: Fingernail swabs from 12 cohabiting couples.
Results: Mixed profiles in 17 % of the samples. The majority on non-donor alleles belonged to the partner. Mixture ratios ranged from 20:1 to 1:2. Female swabs were analyzed with Y-filer and in 63 % of the samples gave full or partial Y-chromosome profiles even though no foreign profile was detected during regular analysis.
Prevalence and persistence of foreign DNA beneath fingernails
Setup: Casework data, general population and experimental scratching setup.
Results: 33 % of casework fingernail samples contained foreign DNA of which 63 % had 5 or more loci. 19 % of the general population fingernail samples contained foreign DNA of which 35 % had 5 or more loci. 33 % of fingernail samples after deliberate scratching contained DNA from the scratched person but only 7 % retained the donor DNA after 6 hours.
Conclusion: “… more than just casual contact is required for fingernails to acquire and retain foreign DNA and that it generally will not persist for long periods.

Understanding DNA results within the case context: importance of the alternative proposition
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3853867/
Examples and probabilities of how a bloodstain from the victim ended up on the suspect and how likely it is that a person’s DNA ends up on a handled item (weapon).
The likelihood is 0.3 that DNA is transferred from shooter to gun and 0.7 that DNA is not transferred.
Thus, it is only slightly more likely to transfer DNA to the gun than the chance of finding background DNA on the gun (35 % mixed profiles and 24 % single background profiles). Conclusively, not finding a suspects DNA on a gun does not eliminate the suspect as the shooter.

DNA transfer: Review and implications for casework
Review on factors (shedder status, surfaces, time aspects…) and probabilities of transfer.

Exploring the relative DNA contribution of first and second object’s users on mock touch DNA mixtures
Setup: First user handled an object (plastic, metal, fabric or nitrile) for 8-10 days. Second user handled the object for 5 min, 30 min or 120 min. Hands were washed at least two hours before handling the object. The % contribution of the second user increased in proportion to the time the object was handled.
Results: A plastic bracelet gave 40 % second user even after 5 minutes handling. The bracelet also showed 10 % profile that did not belong to either the first or second user. In one third of the evaluated samples the second user was eventually the major contributor.

DNA profiling of trace DNA recovered from bedding
Setup: Five volunteers slept one night (in their own, freshly laundered night wear) on a new lower bed sheet (the rest of the sheets were unwashed) in their own bed and then one night in a foreign bed. Samples were collected from upper shoulder, mid body and foot areas on the sheets.
Results: Own bed, new lower sheet – “DNA from a second individual was obtained from at least one of the sheet samples collected from three of the five volunteers” (from member of household). Foreign bed, new lower sheet, same nightwear as in own bed – “At least one of the three samples collected from each volunteer’s sheet provided the DNA profile of the volunteer sleeper after one night in a bed foreign to them. In addition, DNA profiles from at least one sample collected from sheets of four of the volunteers showed the presence of additional DNA resulting in mixed DNA profiles.”
An investigation of DNA recovery from firearms and cartridge cases

Weapons setup: Different types of weapons were cleaned and then fired in environments similar to a crime scene. Each weapon was shot by two persons without cleaning in between. 128 samples (32 from each firearm).

Cartridge setup: Samples were collected from both fired and unfired cartridges. Also a test with swabbed-on saliva on cartridges.

Results weapons: The most successful DNA recovery was from the grips of the firearms (80% of the samples had enough quantity of DNA) and slide serrations of the pistol (87.5%).
30 of 47 samples gave mixed DNA profiles. 24 of these 30 had a clear major profile, however, the major profile was not always the designated shooter. 8 of 19 samples resulted in mixed profiles after shooter one only, some mixtures were investigated and could be attributed to boyfriend and office partner.

Results cartridges: results indicate DNA from fired/unfired cartridges or the magazine holding cartridges. There seemed to be a considerable loss of DNA by the movement through the weapon, even when saliva was swabbed-on.

Conclusion: A DNA profile from a weapon does not mean it was the last person to touch/use the weapon. Furthermore, it does not indicate that direct contact has occurred between the weapon and the individual.

Trace DNA presence, origin and transfer within a forensic biology laboratory and its potential effect on casework

Gloves were at high risk of acting as vectors for DNA transfer.
Two other high contamination risk objects were a plastic ruler and a drying line for clothes.
DNA could be found on most investigated vectors in the whole forensic laboratory chain but the risk of transferring a profile that would interfere with analysis were medium to low.

Beware; gloves and equipment used during the examination of exhibits are potential vectors for transfer of DNA-containing material
http://www.isfg.org/files/91a748919b016087a260b2ab392c88f79a21c0f.05014093_845812755086.pdf

A paper very similar to the other paper by Poy 2006.

Conclusions: Regularly change gloves during examinations. Avoid contact with areas of the exhibit that are likely to be sampled for DNA analysis. Regularly clean tools and objects that may come in contact with an exhibit.

Experiments on the DNA contamination risk via latent fingerprint brushes

Setup 1: Analysis of 51 used fingerprint brushes.
Results 1: 86% of the brushes gave full or partial profiles.

Setup 2: A selection of brushes and some artificially contaminated brushes to test secondary transfer
Results 2: “The larger the brushed area, the greater is the risk for contamination; single fingerprints are less affected.” “Contact of the brush with body fluids such as blood or saliva makes secondary transfer highly likely.”

Trace evidence characteristics of DNA: a preliminary investigation of the persistence of DNA at crime scenes

Casework data gave no clear correlation between the amount of DNA recovered and time of the object spent outdoors.
Experimental setup with human buffy coat showed that two weeks outdoors halved the amount of recovered DNA while the amount was negligible after 6 weeks. Storage at dark and cold locations could preserve the DNA for at least 6 weeks.

Trace DNA: An underutilized resource or Pandora's box? A review of the use of trace DNA analysis in the investigation of volume crime
Since touch DNA cannot be derived to a specific source the activity level of the evidence might be problematic.
Secondary transfer reviewing.
Discussion of the problems with LCN in court.

Regina v. Peter Weller (court trial on “digital penetration”)”
How did the DNA from the girl end up on the man’s fingers? Different scenarios. Expert witness drawing poorly based conclusions? The clash between science and experience in court.

The urban myths & conventional wisdom of transfer: DNA as trace evidence
Review of trace DNA and transfer − believers and non-believers of secondary transfer, the occurrence (or not) of good and bad shedders…

An investigation into the transference and survivability of human DNA following simulated manual strangulation with consideration of the problem of third party contamination

Setup: A man “strangled” a woman for 1 minute 29 separate times. Samples were collected by moist swabbing between 1 minute and 10 days after the “assault”. Samples were taken from man´s fingertips and woman´s neck, the neck was not touched or washed after the assault, the man’s fingertips were allowed normal daily routine. The pair worked in the same building but was not allowed direct contact during the experiment.
Results: Neck – 7 of 29 neck-swabs showed full offender profile up to 6 hours after the assault. By LCN offender alleles were present up to 10 days.
Fingertips – 7 of 29 showed an offender + victim profile. 6 of 7 were partial victim profiles detected up to 24 hours after the assault.
Caution: Control areas of neck and control fingertips also showed profiles from the other person in some cases up to 10 and 5 days after assault resp. Third party profiles were found on both control sites and test sites.

DNA contamination of mortuary instruments and work surfaces: a significant problem in forensic practice?
"Of the 20 mortuaries studied, 50 % were found to have material containing quantifiable human DNA on the instruments and surfaces sampled (after routine cleaning). This DNA was amplified and found, in some cases, to have been derived from at least three people.”
“…autoclaving does not necessarily remove the risk of instrument contamination by human DNA.”
Trace DNA analysis: If your DNA is on the evidence, did you really touch it?
Ryan SR., Posted on LinkedIn 2014; June 26.
https://www.linkedin.com/pulse/20140626163650-13967252-trace-dna-analysis-if-your-dna-is-on-the-evidence-did-you-really-touch-it

“…simply touching an object can leave anywhere from 0-169 ng of DNA” (Meakin and Jamieson 2013).
“In 14 of 24 individuals tested, non-self DNA was found on their necks” (Graham and Rutty 2008).
“41 % of fingernail samples tested showed some evidence of a mixed DNA profile” (Dowlman et al. 2010).
“Touch DNA is known to last for up to 2 weeks outside and 6 weeks or longer inside” (Raymond et al. 2009).

Touch DNA. What is it? Where is it? How much can be found? And, how can it impact my case?
A question and answer guide to all things touch DNA
Ryan SR., Ryan Forensic DNA Consulting, January 2012.
http://www.ryanforensicdna.com/yahoo_site_admin/assets/docs/Touch_DNA_article.59101908.pdf

Review: On definition of touch DNA, how much DNA is left behind when an object is touched, is DNA always left on an object via touch? Factors that increase the amount of shed DNA, persistence of touch DNA, secondary transfer, can we tell who handled the item last? Which items can contain touch DNA?

Yield of male contact DNA evidence in an assault simulation model

Grab setup: Man with washed hands grabbed the washed wrist and washed upper arm of a female for 5 seconds (females did not resist or struggle). Wrists were double swabbed within 30 minutes.
Struggle setup: Man with washed hands grabbed the washed wrist and washed upper arm of a female for 10 seconds while she was struggling.

Results: “The maximum concentration of total DNA extracted was 40 pg/µL in “grab” situations and 90 pg/µL in “struggle” situations, whereas estimates of male DNA from the female’s skin were up to 20 pg/µL in the “grab” situations and 40 pg/µL in the “struggle” Situations”. “… the difference in DNA transferred during grab and struggle situations were not statistically significant in this study.” “Our results showed that no full or usable profiles were attainable with the AmpFLSTR® Identifiler® PCR Amplification kit. Even the Yfiler™, which was designed to detect trace male DNA in a predominantly female background and has been shown to generate complete profiles with less than 60 pg of DNA, did not yield usable profiles.”

Phantoms in the mortuary - DNA transfer during autopsies

Surfaces and instruments used during autopsies was swabbed after routine cleaning procedures and analyzed for contaminations. Most samples (almost 100 %) contained alleles that could be connected to bodies that had been handled there. Six bodies were investigated and four of them had alleles transferred from the autopsy environment.
A bleach containing cleaner (DAN Klorix) was shown to provide clean enough surfaces and instruments.

Yield of male touch DNA from fabrics in an assault model

Setup: Grab and struggle for 15 seconds was used to deposit male touch DNA to cotton, polyester or cotton/polyester blend on a female (hands were washed 15 minutes before the test). Sampling was done by cutting the fabric after 12 hours or 7 days.
Results: A maximum of 7 pg/µL DNA was extracted after 12 hours. No significant difference after 7 days. Fabric types could not be compared due to the low yield (Quantifiler Duo was used which has a limit of 23 pg/µL).

Recovery of DNA and fingerprints from touched documents
Comparison of two extraction methods: DNeasy plant mini kit (QIAGEN) worked better than QIAamp mini kit.
Fingerprint enhancement by DFO and/or Ninhydrin significantly decreased the amount of recovered DNA but did not seem to interfere with the PCR amplification.
DNA was better recovered from magazine and filter paper compared with office paper and white card. LCN (32 cycles) increased the number of full profiles obtained but also increased the incidence of PCR artefacts.

Caution must be taken regarding contamination from fingerprint powder and brushes when extracting DNA from fingerprints.

DNA transfer by examination tools – a risk for forensic casework?
High risk vectors: scissors, forceps, gloves.
Setup: Substrates: glass and cotton. “Dried blood or touch DNA, deposited on the primary substrate, was transferred via the vector to the secondary substrate, which was either DNA-free or contained a target sample (dried blood or touch DNA).”
Results: Touch DNA transferred less than blood. Transfer did not interfere with the target profile on the second substrate but could complicate the analysis when no suspect is known.

The potential transfer of trace DNA via high risk vectors during exhibit examination
High risk vectors: scissors, forceps, gloves.
Results: “DNA was transferred for all vectors in both heavy and light contamination scenarios”. Acceptable profiles were observed for all scenarios except forceps light contamination.

Persistence of DNA deposited by the original user on objects after subsequent use by a second person
“Our findings show that the profile, percentage contribution of the 1st user relative to the 2nd user of an object declines in a linear manner, over time.”
Setup: a) Non-porous, hard, flat surface object (pens and pen lids). Person 1 rubbed 49 new, cleaned pens between his/her hands and the lids was removed and replaced once a day for four days (30 s/60 s/60 s/60 s rubbing/day). Five of the rubbed pens were taken as controls. The rest of the 44 pens were given to Person 2 (one person/pen). Person 2 used the pen as normally when writing and documented the
duration, the activity and details about e.g. contact with skin and other materials. Sample collection made by wet+dry swab.
b) Porous worn object (bracelets of elastic fabric). Person 1 wore rubber-band bracelets for 34 hours (divided in five days) and also rubbed the bracelets with his/her hand for 30s before removing them every day. 4x17 of these bracelets were extra rubbed and given to a person 2. Six were also rubbed again and stored as control. Person 2 used the bracelets and documented when they were temporarily removed, total usage for person 2 was 5, 10, 20, 40 h and 1, 2, 4, 8 days. Sample collection made by cutting the bracelets into small pieces.
c) Wide range of every day personal objects used by a second person. Sample collection by wet+dry swab on hard surfaces and tape lifting on soft/porous surfaces.

Results:
a) The 2\textsuperscript{nd} user was the dominant profile on the pen after 30 minutes of use (50/50 after 1 minute) and on the lid after 3 times of removing/replacing the lid (50/50 after 1 time).
b) A large number of “1\textsuperscript{st} wearer derived unknown alleles” in the control bracelets (15 %). The 2\textsuperscript{nd} user was the dominant profile on the bracelet after 29 hours of use, the increase of the 2\textsuperscript{nd} user was linear over time.
c) Many details to consider in this part of the experiment. Well-worn watches retained the 1\textsuperscript{st} user as dominant after 10 days of use by 2\textsuperscript{nd} user. E.g. sunglasses, perfume bottles, lighters etc. were dominated by the 2\textsuperscript{nd} user relatively quickly. A well-worn cap used by 2\textsuperscript{nd} user for 2 weeks (12 hours in total) was dominated by the 2\textsuperscript{nd} users profile but the 1\textsuperscript{st} user was still a full profile.

Forensic trace DNA: a review
http://investigativegenetics.biomedcentral.com/articles/10.1186/2041-2223-1-14

History and discussion on STRs and PCR.
Touch/trace DNA should not be confused with LCN.
Touch DNA = collection of minute biological samples. Touched objects does not always result in low amounts of DNA.
LCN = often used to describe increased cycle numbers.
Trace DNA is herein defined as “any sample which may fall below the recommended thresholds at any stage of the process - detection, collection, extraction, amplification and interpretation.” Usually less than 100 pg DNA.
A sample defined as trace DNA in the recovery phase does not necessarily have to be trace DNA in later stages of the process.
The precise location of where to recover the trace DNA is very important.
Double swabbing (not necessarily wet + dry, can be wet+wet if all the moisture is recaptured by the first swab) is recommended for collection of trace DNA.
If the swab is allowed to dry before extraction less DNA will be extracted, if the dried swab is frozen before extraction the DNA recovery will be similar to if it would still have been wet.
LMD can be useful to differentiate relevant cells from other cell types which allow for efficient DNA analysis from the minor cell type in a sample. Flow cytometry can be used to separate sperm cells from vaginal wash fluid.
Chelex and organic extraction can lead to up to 75 % loss of DNA. However, this loss is often not relevant.
Partial or complete profiles have been obtained from samples that did not pass the quantification limit.
Trace DNA samples should not always be discarded due to low quantification.
The paper reviews different amplification methods for LCN samples, the detection of the amplified products and interpretation of results.
Common problems with trace DNA amplification: 1) allele drop-out, 2) decreased heterozygote balance, 3) allele dop-in (stutter), 4) allele drop-in (contamination).
In analysis it can be useful with detection thresholds such as LOD (limit of detection), T (low template DNA threshold), MIT (match interpretation threshold) and LOQ (limit of quantification). However, the peak intensity should be included in the exclusion calculation and continuous measures should be used instead of definite thresholds.
Replicates should be employed for trace DNA analysis, four replicates with reported alleles detected in at least two of the replicates seems to be the most accurate. Bayesian based likelihood ratios of drop-outs can also be employed.

Mixed profiles can be challenging due to e.g. biased drop-outs, complicating drop-ins and over-amplification of some alleles.

Contamination of trace-DNA can be a serious problem. The contamination can be the major profile in the sample. Contamination can occur before the crime is committed (background DNA), between the crime is committed and the crime scene is secured, during the crime scene investigation and in the forensic laboratory.

**Impact of relevant variables on the transfer of biological substances**


Setup: Calculations based on data from Goray et al (2009) on how much biological material would have had to be deposited at the original source to yield enough DNA (1 ng) after up to 5 transfer steps.

Results: “We demonstrate that, in many scenarios incorporating multiple transfer steps, unrealistically large amounts of biological material would need to be present at source to generate a detectable level of DNA from the targeted crime scene surface.”

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**An evaluation of the transfer of saliva-derived DNA**


Moisture and a smooth surface were shown to increase transfer. Moisture in the first event of transfer was more significant than moisture in the following events. When saliva is the source of DNA the primary depositor was found to be the major profile.

Substantial loss of DNA was observed in each transfer event.

**Trace DNA: A review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact**


The use of trace-DNA is possible in forensic applications. Examples and guidelines.

**DNA fingerprinting secondary transfer from different skin areas: morphological and genetic studies**


Touch DNA is traditionally considered to come from shed keratinocytes. This study shows that sebaceous fluid represents an important vector responsible for DNA transfer.

“…we confirmed that in DNA secondary transfer, under the “ideal” conditions of clean objects (glass slides) and washed hands, the full profile of an individual can be recovered from an item that he/she had not touched while the profile of the person (vector) having contact with that item was not observed. Thus the “single full profile” may be misleading in terms of determining who actually had contact with an item.”

“The results obtained indicate that “touch DNA” secondary transfer is indeed an important phenomenon but we should consider the specific touched cutaneous area in the evaluation of the genetic results.” …” we show that secondary transfer of DNA traces originates from sebum rather than from keratinocytes, following contact with different skin areas.”
3.2 Adhesive tapes

The use of adhesive tape for recovery of DNA from crime scene items

Adhesive tape has shown to be superior in recovering shed epithelial cells (over swabbing/cutting) for e.g. shoe insoles, baseball caps, jackets and cadavers (refs in paper).
Tape inside and outside of gloves separately to avoid contamination.
Taping large areas might require several tapes. However, the need to pool and concentrate the samples increased the risk for both DNA loss and contamination.

Revision of the tape used in a tape-lift protocol for DNA recovery

13 tapes were evaluated, Scapa (4405) was found to be the best.
Scensafe was discarded due to the unpracticality of getting it into the test-tube, otherwise it seemed to fulfil the criteria.

Recovery of trace DNA and its application to DNA profiling of shoe insoles

0.16-6.4 ng DNA could be recovered by swabbing hands. Even though hands were not washed before the experiment no non-donor alleles could be identified.
Protocol for sampling DNA from shoe insoles and the subsequent sampling and extraction.
Taping was the most efficient method for recovering DNA. Swabbing and soaking methods resulted in dirtier extracts and were likely to include increased amounts of inhibitors.
Recovery from synthetic materials was better than from e.g. leather.

Comparison of stubbing and the double swab method for collecting offender epithelial material from a victim’s skin

Setup: Manual strangulation by a man on a woman was simulated. DNA was secured by double swab or by a tape-lifting method called stubbing.
Results: Fewer alleles from the victim were detected after double swabbing compared with stubbing.
There was no difference in the number of alleles from the offender between swabbing and stubbing.

Success rate of a forensic tape-lift method for DNA recovery
http://www.forensicscience.pl/component/option,com_jbook/task,view/Itemid,2/catid,67/id,628/lang,en/

For trace DNA recovery mini taping provided a lower degree of inhibited DNA extracts and a higher proportion of usable DNA results than conventional techniques. However, the proportion of mixed profiles found was also increased.

A single approach to the recovery of DNA and firearm discharge residue evidence
http://www.scienceandjusticejournal.com/article/S1355-0306(04)71680-9/abstract

The mini-tape used for recovery of firearm discharge residue (FDR) in this paper was successfully used for extraction of DNA after the FDR identification was done.

Trace DNA collection - performance of minitape and three different swabs

Comparison of the recovery of DNA using three different swabs (two synthetic and one cotton) or mini tape.

Nationellt forensiskt centrum, NFC – Biologisektionen
There was no major difference between the three kinds of swabs but the mini tape was the best option for recovery of DNA from absorbent materials (like textiles). (No statistics calculated).

**DNA extraction of forensic adhesive tapes – A comparison of two different methods**

Setup: Scenesafe FAST minitapes were extracted with two different methods; 1) PrepFiler Express BTA (Applied Biosystems) and 2) Chelex/iPrep (Invitrogen).

Results: BTA yielded more DNA than Chelex/iPrep for both blood and touch DNA. The profiling results were also better for BTA than for Chelex/iPrep.

Drawback with BTA: only 230 µL lysis buffer is used, which does not cover the entire tape. The experiments were performed on halved mini tapes.

**Using hydrophilic adhesive tape for collection of evidence for forensic DNA analysis**

When collecting human samples (blood, saliva and semen) there is a risk of DNA degradation due to the moisture. A hydrophilic adhesive tape (HAT) was examined for non-invasive collection (taping of ankle, arm, behind ear, behind fingers and back of the neck) of human cells for DNA analysis. Authors claim it is a good method and the samples were stable for a month. Ears resulted in the highest success-rate.

**Nondestructive biological evidence collection with alternative swabs and adhesive lifters**

Setup: blood and fingerprints were collected from glass, painted drywall, 100 % cotton and copy paper with different types of dry swabs and adhesive lifters.

Results: Adhesive tapes were better than dry swabs at collecting DNA from fingerprints on 100 % cotton and painted drywall. There was no statistically significant difference between the different methods regarding fingerprints on the other materials or regarding blood on any material. Moreover, the recovery of DNA was in general higher from fingerprints than from blood, indicating that wet swabbing is the better option regarding collection of blood. Interestingly, all methods resulted in 100 % profile coverage from fingerprints on glass and copy paper.

**Comparison of collection methods from touch samples on metal and wearer samples from simulated mixtures on clothing**

Puritan sterile cotton swabs can contain up to 23 pg of human DNA.

Touch DNA on metal: comparison between DNA sterile cotton swabs, DNA free cotton swabs and foam tipped DNA free swabs (organic extraction, qPCR, PCR STR multiplex amplifications and capillary electrophoresis). The foam tipped DNA free swabs worked poorly on both stainless steel and brass. DNA sterile cotton swabs worked the best for stainless steel and DNA free cotton swabs worked best for brass.

Touch DNA on clothes (different types): Comparison between swabbing, scraping and adhesive taping (Gel-Pak ‘0’). All three methods resulted in mixed profiles. Swabbing and Gel-Pak ‘0’ were comparable in that the last wearers profile was the most distinct one. Gel Pak ‘0’ gave generally less DNA. Scraping recovered more DNA from the habitual wearers.

**The hand on the shoulder. A case report where low copy number (LCN) DNA analysis was vital for solving a robbery**
DNA from suspect was successfully recovered from victims clothing using adhesive tape even though the time of physical contact was short.
Epidermal cells on stubs used for detection of GSR with SEM-EDX: analysis of DNA polymorphisms
DNA was successfully extracted from “gunshot residue stubs” used on the back of hands. Only a small piece of the adhesive tape was used.

Preliminary investigation of differential tapelifting for sampling forensically relevant layered deposits
Evaluation of differential tape-lifting to separate different layers of DNA.
Setup touch/touch: Donor A rubbed a hand over a piece of polyester-cotton for 60 s three times. Donor B rubbed the same way on plastic. The fabric and the plastic were put together after 24 hours, either touchside against touchside or touchside of plastic against backside of fabric.
Setup saliva/touch: Saliva from donor C was applied to fabric. Donor A rubbed a hand on plastic. The fabric and the plastic were put together after 24 hours, either touchside against touchside or touchside of plastic against backside of fabric.
Setup: touch/saliva: Donor A rubbed a hand on fabric. Saliva from donor C was applied on the touchside or the backside of the fabric and allowed to dry for 24 hours.
Setup sample collection: Light tapping = one taping /area on the substrate. Maximum collection = 16 tapings/substrate. Tapes used: Scotch® Magic™, Scenesafe FAST™. Taped substrate was also cut for extraction of DNA.
Results: “This study has demonstrated that there is no clear preference of sampling method when attempting to differentially sample deposits of touch DNA layered over a pre-existing DNA background.” “…the selective collection of saliva from a background of touch DNA is not enhanced by tapelifting; rather, it seems that the resulting profile reflects the ratio of the respective deposits.” “…DNA is easily transferred through the polyester–cotton fabric to the other side.”

Evaluation of tapelifting as a collection method for touch DNA
Comparison between Scotch® Magic™, Scenesafe FAST™ and wet/dry-swabbing for sampling touch DNA on different fabric types.
“Significantly more DNA was extracted, and a higher proportion of alleles detected, from Scenesafe FAST tape than from Scotch Magic tape.” Additionally, profiles were of higher quality with Scenesafe compared with Scotch. Tapelifting was more efficient than swabbing for all fabric types examined except flannelette where swabbing was as efficient.

A possible source of reference DNA from archived treated adhesive lifters
“In fatal shooting cases where a bullet has been fired through a body surface, an adhesive lifter is applied directly to the entrance wound.” “…archived adhesive lifters can be used as a future source of reference DNA from cadavers where no other sample is available.” (after the lifter had been treated for GSR pattern)

Applicability of DNA analysis on adhesive tape in forensic casework
Retrospective case study.
“In conclusion, DNA profiling of adhesive tape samples can be useful in a variety of forensic cases”. Of 150 tapes that were processed for DNA profiling, between 1999 and 2010 in Switzerland, 98 yielded DNA profiles.
“In some cases, such as immobilization or gagging, DNA profiling is not applicable owing to the overload of the victim’s cells masking the perpetrator’s contribution to the sample.”
3.3 Ammunition & spent cartridges

A sensitive method to extract DNA from biological traces present on ammunition for the purpose of genetic profiling
Description of a sensitive method for extraction of DNA from cartridges, bullets and casings, including the usage of cell lysis buffer on the subsequent investigation of striation patterns.

Development of STR profiles from firearms and fired cartridge cases
Test of different kits for DNA recovery from firearms and fired cartridge cases and where it is useful or not to swab for DNA (hull vs. head of shotshell cases and firearm surface areas).

Evaluation of methodology for Low Copy Number (LCN) DNA analysis - on spent cartridge cases - after enhancement of latent fingerprints
Johansson C., Linköpings Universitet, magisteruppsats, LiU-IFM-Ex-08/1892-SE.
“The report includes a discussion on the definition of LCN, different applications, risks and limitations, optimizations of the procedure and how to deal with the interpretation. Finally there are some words on LCN in perspective of the justice system.”

Analysis of DNA from fired cartridge casings
http://waset.org/publications/10002182/analysis-of-dna-from-fired-cartridge-casings
Even though cartridges are subjected to high temperatures at firing the weapon full DNA profiles can be obtained.

Mitochondrial DNA recovery and analysis from spent cartridge casings
Metchikian, M., Michigan State University, 2013 masters thesis.
http://etd.lib.msu.edu/islandora/object/etd%3A2258/datastream/OBJ/view
“…mtDNA analysis is a reliable method to generate genetic profiles recovered from spent cartridge casings.”
The introduction can be read as a review on cartridges in forensic work.

Optimization of recovery and analysis of touch DNA from spent cartridge casings
Mottar AM., Michigan State University, 2014 masters thesis, publication number 1563498.
http://gradworks.umi.com/15/63/1563498.html
Optimization of DNA extraction from fired casings. Best method: “…double swabbing with organic extraction and amplification with Fusion…”

The recovery and analysis of DNA from fired cartridge casings
No association found between DNA yield and profile quality. Cumulative swabbing of several casings resulted in higher quantities of DNA but not better profile quality. However, the author suggests that cumulative swabbing is the best approach.
Introduction and discussion can be read as a review.
An investigation of DNA recovery from firearms and cartridge cases

Weapons setup: Different types of weapons were cleaned and then fired in environments similar to a crime scene. Each weapon was shot by two persons without cleaning in between. 128 samples (32 from each firearm).

Cartridge setup: Samples were collected from both fired and unfired cartridges. Also a test made with swabbed-on saliva on cartridges.

Results weapons: The most successful DNA recovery was from the grips of the firearms (80 % of the samples had enough quantity of DNA) and slide serrations of the pistol (87.5 %).

30 of 47 samples gave mixed DNA profiles. 24 of these 30 had clear major profiles. However, the major profile was not always the designated shooter. 8 of 19 samples resulted in mixed profiles after shooter one only, some mixtures were investigated and could be attributed to boyfriend and office partner.

Results cartridges: results indicate DNA from fired/unfired cartridges or the magazine holding cartridges. There seemed to be a considerable loss of DNA by the movement through the weapon, even when saliva was swabbed-on.

Conclusion: A DNA profile from a weapon does not mean it was the last person to touch/use the weapon. Furthermore, it does not indicate that direct contact has occurred between the weapon and the individual.

Examination of factors that affect the recovery and analysis of DNA from spent cartridge casings
Ray R, Mottar AM, Foran DR., Abstract AAFS, Criminalistics section 2015, B68.
http://www.aafs.org/sites/default/files/2015/abstracts/B68.pdf

“Cyanoacrylate fuming had a negative effect on DNA yields”.
Cumulative swabbing yielded more DNA than single-swabbing. Overall, all tested variables had an effect on the yield (cyanoacrylate, swabbing approach, cartridge size).

Fingerprints & cartridge cases: How often are fingerprints found on handled cartridge cases and can these fingerprints be successfully typed for DNA?
https://www.cacnews.org/

Setup: “Three types of fingerprints were placed on these cartridges: bloody fingerprints, eccrine/sweat prints and oily prints”. “The cartridge cases were made of brass, nickel-plated brass, and aluminum.”

“The bloody fingerprints were developed with amido black while the eccrine and oily prints were processed by cyanoacrylate fuming followed by rhodamine 6G dye and illumination with laser light. After the cartridges/cases were processed for fingerprints, DNA was collected from the cartridges/cases with dampened swabs.”

Results: DNA profiles were obtained from 3 of 48 cartridges. All three were from bloody prints, two from fired cartridges and one from an unfired.

DNA profiles from fingermarks: A mock case study

Uncleaned substrates were handled for less than 15 s to create a fingerprint and left outdoors (a glass door was not left outdoors but kept in place indoors).
Samples were recovered after 24 hours and 8 days.
“Direct PCR” (without extraction) was used.

Results: Glass had the highest rate of DNA recovery, masking tape was second best. Mixed DNA profiles were recovered but the donor profile was always the major one.
The retrieval of a DNA profile from spent cartridge cases
www.staffs.ac.uk/assets/cartridge_cases_tcm44-12721.pdf
Firing a weapon could cause a reduction in the quality of the DNA profile from saliva seeded cartridge cases. Higher caliber weapons produced poorer profiles than lower caliber. Gunshot residues may negatively affect the quality of the DNA profile.

3.4 Background DNA

Environmental DNA monitoring: beware of the transition to more sensitive typing methodologies
http://www.tandfonline.com/doi/abs/10.1080/00450618.2013.788683#.Vd25pfntkXs
Environmental DNA monitoring (EDM): surfaces and tools are sampled on a periodic basis.
Kits used in the study: Profiler Plus, Yfiler, PowerPlex 21.
Washing (hypochlorite + ethanol) did not remove all DNA from items like rulers, pipettes and tube racks etc. However, the detectable DNA after cleaning was present in few alleles with low peak heights.

Analysis of DNA from fingernail samples in criminal cases
Setup: 164 fingernail samples from 71 cases, collection by extraction, clipping or swabbing.
Results: Extraction of DNA from fingernails showed only the donor profile while clipping and surface swabbing were equally efficient in recovering mixed profiles.
The victim profile was detected in ~75 % of the cases and in ~13 % of these cases a second contributor was detected.
In an expert testimony it should be clearly stated that foreign DNA can be found under the fingernails of volunteer donors as well (6-24 %).

Probability of detection of DNA deposited by habitual wearer and/or the second individual who touched the garment
Setup: 63 males wore their own underpants for 12 h, the inside waistband was then touched by one of 11 females for 15 s. The waistband was mini-taped and subjected to DNA profiling.
Results: The wearer was detected in 51% of the samples and always as the major contributor.
The test-female was detected in 11% of the samples.
Reportable background DNA (non-wearer, non-test-female) was found in 14% of the samples.
There was no statistical difference in DNA quantification results depending on the time of collection (0 or 12 weeks after wearing and touching).
If a single DNA profile is found on e.g. a pair of underwear, it is 5 times more likely that the person has worn the garment than just touched it. A high total DNA concentration would support wearing the garment.

Oral intercourse or secondary transfer? A Bayesian approach of salivary amylase and foreign DNA findings
Oral intercourse leads to salivary-α-amylase in penile swabs. However, salivary-α-amylase in penile swabs does not necessarily mean oral intercourse but may be due to secondary transfer.
Setup: This is a study of background salivary-α-amylase in underwear after normal (12 hour) wear (69 male subjects). Masturbation with saliva as lubricant was allowed but oral intercourse was not.
Test method: Phadebas press test for screening and RSID-Saliva for confirmation.
Results: 44% of the underwear had stains containing amylase on the inside front, 13% of these stains were found to be salivary-α-amylase (5.7% of total). 50% of these produced a mixed DNA profile with a minimum of two donors.

A positive control group with oral intercourse showed larger salivary-α-amylase positive stains in the male underwear than the background-test group. 80% of the underwear in this group gave DNA profiles other than the wearers.

“…the evidence of salivary-α-amylase on male underwear (RSID™-Saliva positive) is 17 times more likely to occur with the allegation of fellatio than the alternative hypothesis of secondary transfer. However the finding of no salivary-α-amylase (RSID™-Saliva negative) upon the male underwear is a 100 times more likely if fellatio did not occur rather than if somebody performed fellatio on Mr B. Likewise, it is evident that if salivary-α-amylase and DNA other than the wearer is present on the inside-front of male underpants that it is 27 times more likely if fellatio occurred rather than fellatio did not occur. In saying that, if salivary-α-amylase is found yet the DNA profile does not contain a profile other than that of the wearer, it is 13 times more likely if fellatio occurred rather than if nobody performed fellatio on an alleged victim”

This paper also contains a section about the sensitivity and specificity of the Phaebas press test and RSID-Saliva; Phadebas press test cannot distinguish between salivary-α-amylase and pancreatic amylase which leads to false positive results, therefore it should only be used for screening.

Recovery of trace DNA and its application to DNA profiling of shoe insoles

0.16-6.4 ng DNA could be recovered by swabbing hands. Even though hands were not washed before the experiment no non-donor alleles could be identified.

Protocol for sampling DNA from shoe insoles and the subsequent sampling and extraction.
Taping was the most efficient method for recovering DNA. Swabbing and soaking methods resulted in dirtier extracts and were likely to include increased amounts of inhibitors.
Recovery from synthetic materials was better than from e.g. leather.

The prevalence of mixed DNA profiles in fingernail samples taken from individuals in the general population

Aim: “…examination of the incidence of mixed DNA profiles derived from fingernail samples from members of the general population…”

Setup: Fingernail swabs from 100 volunteers.
Results: Foreign DNA was detected in 13 % of the samples, only 6 % of these gave reportable mixed DNA profiles.
“…This study demonstrated a low level of foreign DNA under the fingernails of the general population, suggesting that when a strong mixed DNA profile is obtained from a fingernail swab it is unlikely that it exists only due to previous contact between the suspect and victim.”
Mostly male donors had significant amounts of foreign DNA in their nail swabbings.

Contamination monitoring in the forensic DNA laboratory and a simple graphical model for unbiased EPG classification

There is a need for a standardized, unbiased and independent method for the monitoring of background DNA in the laboratory.
Presentation of a graphical classification model for ranking of electropherograms.

The prevalence of mixed DNA profiles on fingernail swabs

Setup: Fingernail scrapings from 40 laboratory staff and police (right and left hand = 80 samples).
Results: 30% of the samples were low level mixtures of DNA profiles and 11% were high level mixtures. 8 samples were 3-person mixtures where one of the profiles was unknown (not partner, children, colleague…). The sex of the test-subject was not significant regarding the occurrence of mixed DNA-profiles but the trend suggests that a high level mixed profile was more likely to be collected from the male test-subjects.

“…intimate contact results in transfer of more cellular material than other types of contact.”

Analysis and implications of the miscarriages of justice of Amanda Knox and Raffaele Sollecito

LCN-levels of victim-DNA (no detection of blood) on a knife, analyzed without replication and with only a few alleles, were used to convict a suspect. Moreover, the location of the suspect-DNA on the handle was interpreted (without scientific grounds) as a result from stabbing rather than cutting food. The suspect and the victim shared an apartment. Evidence was collected using dirty gloves and in non-sterile containers. Mixed profiles of the two roommates were interpreted as one’s involvement in the murder of the other but was most probably just normal background DNA from two people living together.

“Selective cleaning” was believed to have erased the DNA from two suspects but not the third at the murder-scene.

Negative controls during the DNA analysis was interpreted as that the evidence could not have been contaminated in any way, either before the murder, at collection of the evidence or during handling of the evidence by the police officers.

Defining background DNA levels found on the skin of children aged 0-5 years

Setup: swabbing of 50 children (under the age of 5) on face/neck and body, in total 944 samples.
Results: 3,4% of the samples showed six or more alleles that did not belong to the child. 31,6% of the samples showed 1-5 alleles that did not belong to the child. Large individual differences, both between children and between sites were observed. “There was a significant association between the amount of child and non-child DNA recovered; when non-child DNA was observed, it tended to be on swabs where a greater proportion of the child’s own DNA profile was also observed.”

Investigation into ”normal” background DNA on adults necks: Implications for DNA profiling of manual strangulation victims

Setup: swabbing of 50 children (under the age of 5) on face/neck and body, in total 944 samples.
Results: 3,4% of the samples showed six or more alleles that did not belong to the child. 31,6% of the samples showed 1-5 alleles that did not belong to the child. Large individual differences, both between children and between sites were observed. “There was a significant association between the amount of child and non-child DNA recovered; when non-child DNA was observed, it tended to be on swabs where a greater proportion of the child’s own DNA profile was also observed.”

Investigation into the usefulness of DNA profiling of earprints
Setup: 60 earprints (5 s pressure against surface) collected from 3 adults.
Results: Full donor profile from one of 60 earprints. One or more non-donor alleles in one-third of the earprints. Direct swabbing of ears showed high levels of non-donor DNA.
Conclusion: DNA results from earprints should be interpreted with great care.
IPV – Bridging the juridical gap between scratches and DNA detection under fingernails of cohabitating partners
(IPV = Intimate partner violence)
The respective partners DNA will be under the fingernails in 17 % of couples that live together.
Setup: Female vigorously scratched male back.
Results: There was a highly significant difference in absolute and relative male DNA under scratching and non-scratching fingers. The mean difference was 16-22-fold depending on finger. However, a negative result for male DNA did not mean the nail was not involved in the scratching.

The prevalence of mixed DNA profiles in fingernail samples taken from couples who co-habit using autosomal and Y-STRs
Setup: Fingernail swabs from 12 cohabiting couples.
Results: Mixed profiles in 17 % of the samples. The majority on non-donor alleles belonged to the partner. Mixture ratios ranged from 20:1 to 1:2.
Female swabs were analyzed with Y-filer and in 63 % of the samples gave full or partial Y-chromosome profiles even though no foreign profile was detected during regular analysis.

Prevalence and persistence of foreign DNA beneath fingernails
Setup: Casework data, general population and experimental scratching setup.
Results: 33 % of casework fingernail samples contained foreign DNA of which 63 % had 5 or more loci. 19 % of the general population fingernail samples contained foreign DNA of which 35 % had 5 or more loci. 33 % of fingernail samples after deliberate scratching contained DNA from the scratched person but only 7 % retained the donor DNA after 6 hours.
Conclusion: “… more than just casual contact is required for fingernails to acquire and retain foreign DNA and that it generally will not persist for long periods.

Understanding DNA results within the case context: importance of the alternative proposition
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3853867/
Examples and probabilities of how a bloodstain from the victim ended up on the suspect and how likely it is that a person’s DNA ends up on a handled item (weapon).
The likelihood is 0.3 that DNA is transferred from shooter to gun and 0.7 that DNA is not transferred. Thus, it is only slightly more likely to transfer DNA to the gun than the chance of finding background DNA on the gun (35 % mixed profiles and 24 % single background profiles). Conclusively, not finding a suspects DNA on a gun does not eliminate the suspect as the shooter.

Evaluating the prevalence of DNA mixtures found in fingernail samples from victims and suspects in homicide cases
Claims that “The amount of biological material transferred under the fingernails during “casual” activities is not sufficient to genotype reportable mixtures.”
After a homicide the majority (78 %) of the studied fingernail samples (Israeli retrospective study of 137 DNA-profiles) are single profiles from the donor. DNA mixtures were found mostly from the victims’ fingernail samples.
Sample collection by clipping and swabbing.
25 % of murder victim fingernail samples in the study contained foreign DNA.
Trace DNA and street robbery: a criminalistic approach to DNA evidence
DNA background levels on handbags and wallets were determined as well as the probability of transfer during and after a robbery.
Both owner and non-owner DNA could be recovered from “un-robbed” items. Simulated robberies resulted in majority mixtures or single profiles of the robber in 40 % of the cases.

Assessing trace DNA evidence from a residential burglary: abundance, transfer and persistence.
Both background DNA and deposited DNA at sites of burglary entry points were low. 29 residences were double swabbed (doors, windows) resulting in 150 swabbed locations. 20 different residences (39 different swabbings) produced alleles after amplification.
DNA was deposited by grabbing a pre-cleaned door-frame for 1 minute (without prior handwashing). 40 % of the surfaces yielded 10-200 pg DNA.
DNA (solution) was deposited on wooden window-frames and sampled between 0-6 weeks, after 2 weeks no DNA could be recovered.

Trace DNA analysis: If your DNA is on the evidence, did you really touch it?
Ryan SR., Posted on LinkedIn 2014; June 26.
https://www.linkedin.com/pulse/20140626163650-13967252-trace-dna-analysis-if-your-dna-is-on-the-evidence-did-you-really-touch-it
“…simply touching an object can leave anywhere from 0-169ng of DNA” (Meakin and Jamieson 2013).
“In 14 of 24 individuals tested, non-self DNA was found on their necks” (Graham and Rutty 2008).
“41% of fingernail samples tested showed some evidence of a mixed DNA profile” (Dowlman et al. 2010).
“Touch DNA is known to last for up to 2 weeks outside and 6 weeks or longer inside” (Raymond et al. 2009).

Forensic trace DNA: a review
http://investigativegenetics.biomedcentral.com/articles/10.1186/2041-2223-1-14
History and discussion on STRs and PCR.
Touch/trace DNA should not be confused with LCN.
Touch DNA = collection of minute biological samples. Touched objects does not always result in low amounts of DNA.
LCN = often used to describe increased cycle numbers.
Trace DNA is herein defined as “any sample which may fall below the recommended thresholds at any stage of the process - detection, collection, extraction, amplification and interpretation.” Usually less than 100 pg DNA.
A sample defined as trace DNA in the recovery phase does not necessarily have to be trace DNA in later stages of the process.
The precise location of where to recover the trace DNA is very important.
Double swabbing (not necessarily wet + dry, can be wet+wet if all the moisture is recaptured by the first swab) is recommended for collection of trace DNA.
If the swab is allowed to dry before extraction less DNA will be extracted, if the dried swab is frozen before extraction the DNA recovery will be similar to if it would still have been wet.
LMD can be useful to differentiate relevant cells from other cell types which allow efficient DNA analysis from the minor cell type in a sample. Flow cytometry can be used to separate sperm cells from vaginal wash fluid.
Chelex and organic extraction can lead to up to 75 % loss of DNA. However, this loss is often not relevant.
Partial or complete profiles have been obtained from samples that did not pass the quantification limit. Trace DNA samples should not always be discarded due to low quantification. Reviews amplification methods for LCN samples, detection of the amplified product and interpretation. Common problems with trace DNA amplification: 1) allele drop-out 2) decreased heterozygote balance 3) allele dop-in (stutter) 4) allele drop-in (contamination).

In analysis it can be useful with detection thresholds such as LOD (limit of detection), T (low template DNA threshold), MIT (match interpretation threshold) and LOQ (limit of quantification). However, the peak intensity should be included in the exclusion calculation and continuous measures should be used instead of definite thresholds.

Replicates should be employed for trace DNA analysis, four replicates with reported alleles detected in at least two of the replicates seems to be the most accurate. Bayesian based likelihood ratios of drop-outs can also be employed.

Mixed profiles can be challenging due to e.g. biased drop-outs, complicating drop-ins and over-amplification of some alleles.

Contamination of trace-DNA can be a serious problem. The contamination can be the major profile in the sample. Contamination can occur before the crime is committed (background DNA), between the crime is committed and the crime scene is secured, during the crime scene investigation and in the forensic laboratory.

**Preliminary investigation of differential tape-lifting for sampling forensically relevant layered deposits**


Evaluation of differential tape-lifting to separate different layers of DNA.

Setup touch/touch: Donor A rubbed a hand over a piece of polyester-cotton for 60 s three times. Donor B rubbed the same way on plastic. The fabric and the plastic were put together after 24 hours, either touchside against touchside or touchside of plastic against backside of fabric.

Setup saliva/touch: Saliva from donor C was applied to fabric. Donor A rubbed a hand on plastic. The fabric and the plastic were put together after 24 hours, either touchside against touchside or touchside of plastic against backside of fabric.

Setup: touch/saliva: Donor A rubbed a hand on fabric. Saliva from donor C was applied on the touchside or the backside of the fabric and allowed to dry for 24 hours.

Setup sample collection: Light tapping = one taping /area on the substrate. Maximum collection = 16 tapings/substrate. Tapes used: Scotch® Magic™, Scenesafe FAST™. Taped substrate was also cut for extraction of DNA.

Results: “This study has demonstrated that there is no clear preference of sampling method when attempting to differentially sample deposits of touch DNA layered over a pre-existing DNA background.” “…the selective collection of saliva from a background of touch DNA is not enhanced by tapelifting; rather, it seems that the resulting profile reflects the ratio of the respective deposits.” “…DNA is easily transferred through the polyester–cotton fabric to the other side.”

**3.5 Case studies**

**Analysis of DNA from fingernail samples in criminal cases**


Setup: 164 fingernail samples from 71 cases, collection by extraction, clipping or swabbing.

Results: Extraction of DNA from fingernails showed only the donor profile while clipping and surface swabbing were equally efficient in recovering mixed profiles.

The victim profile was detected in ~75 % of the cases and in ~13 % of these cases a second contributor was detected.

In an expert testimony it should be clearly stated that foreign DNA can be found under the fingernails of volunteer donors as well (6-24 %).
DNA profiling success and relevance of 1739 contact stains from casework
Statistics on 1739 real cases with contact stains.
More DNA was recovered when stains were collected with the double swab (wet swab + dry swab) technique (both for contact stains and saliva stains).
Clothes has the highest success-rate (61%) (the proportion of DNA profiles that were suitable, 1 person > 5 loci, 2 persons > 7 loci), car items second best (37 %), plugs and cables third (29 %). Stones were most difficult (7 %).
Apparently the relevance of car-contact-stains was high suggesting that the “borrowing” persons DNA covers the car owners DNA

Another phantom from the morgue - A case of instrument-born sample contamination in the course of identifying an unknown deceased
The paper focuses on instrument/equipment contamination.

Persistence of biological traces at inside parts of a firearm from a case of multiple familial homicide
Backspatter on firearms.
Profileable DNA could be found in both the barrel and in other places inside the firearm.

Analysis and implications of the misscarriages of justice of Amanda Knox and Raffaele Sollecito
LCN-levels of victim-DNA (no detection of blood) on a knife, analyzed without replication and with only a few alleles, were used to convict a suspect. Moreover, the location of the suspect-DNA on the handle was interpreted (without scientific grounds) as a result from stabbing rather than cutting food. The suspect and the victim shared an apartment. Evidence was collected using dirty gloves and in non-sterile containers. Mixed profiles of the two roommates were interpreted as one´s involvement in the murder of the other but was most probably just normal background DNA from two people living together.
“Selective cleaning” was believed to have erased the DNA from two suspects but not the third at the murder-scene.
Negative controls during the DNA analysis was interpreted as that the evidence could not have been contaminated in any way, either before the murder, at collection of the evidence or during handling of the evidence by the police officers.

Leading-edge forensic DNA analyses and the necessity of including crime scene investigators, police officers and technicians in a DNA elimination database
On the importance of an employee DNA-database to prevent misleading “unknown” DNA profiles to interfere with crime-investigations. This paper comes from Canada where forensic elimination databases are built on voluntarily basis.

DNA analysis of fingernail debris using different multiplex systems: a case report
Evaluation of different kits for the efficiency of recovering DNA from fingernail debris two days after the reported incident. “…we recommend that fingernail debris should be recovered in cases of violent crimes, even after a time lapse of some days between incident and examination.”
Female criminals - it’s not always the offender
DNA from female found on several crime scenes in Austria turned out to be from a woman working in the cotton-swab kit production.
A follow-up study showed that sterile swabs (by radiation) from different manufacturers were often contaminated with DNA.

Forensic identification of a murderer by LCN DNA collected from the inside of the victim’s car
http://www.isfg.org/files/1a528d0fcee17e821a369c61d28d759fbaed8d30.03018557_1 29265714647.pdf
They collected samples with FTA-paper from steering wheel, gear leaver and handbrake from a car and successfully matched the profiles from a suspect’s profile. Quantity of DNA from the extractions were less than 100 pg, 16 markers were analyzed.
“...it is possible to type even low copy number (LCN) DNA if a proper and immediate collection of biological evidence is performed.”

Ninhydrin-dyed latent fingerprints as a DNA source in a murder case
“Preliminary tests prove that ninhydrin-dyed material still can be useful for STR typing”.

Recovery of DNA from latent fingerprint tape lifts archived against matte acetate
Extraction of DNA from fingerprints is problematic for several reasons. The amount of DNA is often very low and the brushes used to enhance the fingerprints are often contaminated with DNA from other crime scenes. Also, the collection of DNA will destroy the fingerprint for further comparison analysis.
This study aimed to develop a technique to recover DNA from latent fingerprints caught between a tape and matte acetate.
No experimental setup in this paper yielded enough DNA for a reliable DNA STR-profile and some even contained non-donor alleles.

New developments and challenges in the use of the UK DNA database: addressing the issue of contaminated consumables
http://www.fsijournal.org/article/S0379-0738(04)00535-3/abstract
An employee of a tube manufacturer had contaminated tubes for years, leading to minor profiles in several cases. Follow-ups showed that other employees had also contaminated tubes resulting in partial or full profiles in several cases. Similar contaminations had later been observed for plates and swabs.

Analysis of salivary DNA evidence from a bite mark on a body submerged in water
A body was recovered after 5.5 hours in water and the perpetrator was found as the minor profile along with the victims profile from a swabbed bite mark (4 single swabs, 4 different parts of the bite mark). Less than 1.5 ng/µL was recovered from each swab so the extracts were pooled for typing. The minor profile yielded a frequency of 1:220 of the Canadian Caucasian population but together with the characteristics of the bite-mark the perpetrator was identified.

Germany’s phantom serial killer. A DNA blunder
Time Magazine Online, 2009. Himmelreich C.
www.time.com/time/world/article/0,8599,1888126,00.html
Cotton swab factory worker was thought to be “phantom serial killer”.

Nationellt forensiskt centrum, NFC – Biologisektionen
Evaluating forensic DNA evidence – essential elements of a competent defense review
Thomson WC, Ford S, Doom T, Raymer M, Krane DE.
http://www.americanbar.org/content/dam/aba/events/legal_aid_indigent_defendants/2015/lssclaid_summit_03c_champion2.authcheckdam.pdf
Tertiary transfer in trial. “He and his wife had shared a towel the morning of the murder - perhaps his DNA was transferred from his face to the towel, and from the towel to his wife’s face. His wife was later attacked by a glove-wearing stranger who struck her on the face, strangled her, and stabbed her, in the process transferring husband’s DNA from his wife’s face to the gloves and the knife.” The scenario was tested and found plausible.

DNA typing of fingerprints using capillary electrophoresis: Effect of dactyloscopic powders
Some dactyloscopic powders used for fingerprint enhancement can be used prior to DNA recovery but precautions should be taken to avoid contamination.

Report: Inquiry into the circumstances that led to the conviction of Mr Farah Abdulkadir Jama
Rape conviction (girl unconscious) based on a slide and a swab collected in the medical examination of the victim. The slide and the swab had been collected in the same unit as samples from another woman 28 hour earlier who undisputedly had had sexual contact with the accused.

Forensic lab error led to miscarriage of justice
Walter P., Chemistry world 5 October 2012.
http://www.rsc.org/chemistryworld/2012/10/lgc-forensics-lab-error-rape-accused
Cross-contamination during extraction in a forensic lab in the UK led to 5 months arrest of an innocent man. Apparently a disposable plastic microtiterplate had been reused.

Trace DNA: A review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact
The use of trace-DNA is possible in forensic applications. Examples and guidelines.

DNA typing of epithelial cells after strangulation
Experimental study of DNA typing after strangulation. Success rate was > 70 %. Profiles were often a mix of the “suspect” and the “victim”.
Setup: 16 pairs. Upper arm used for strangulation for 1 min including arm movements by the victim. Collection of DNA by 1) glass fibre pieces or 2) moistened cotton swabs. Polyacrylamide gel electrophoresis for visualization of the alleles.
Results: 0.5-1 ng DNA extracted for 14 of the 16 samples. For the other 2 samples ~2 ng was extracted. Case study: Strangled victim found after 48 h. The neck was swabbed and the suspect could be identified.

Touch DNA: Forensic collection and application to investigations
“… the scraping/tape lift methods are ideal in situations where the scientist can locate areas on the item which are most likely to contain the perpetrator’s skin cells.”
“...an increased chance of obtaining mixed DNA profiles containing DNA from individuals that may have come into contact with the victim/evidence item near the time of the crime. Contributors to these mixtures could include the victim’s spouse or children, and again, elimination samples may need to be collected from these individuals.”

“Some evidence items are also not recommended for the collection of Touch DNA samples. Such items include those that are severely degraded (for example, moldy clothing), have been exposed to extreme environmental conditions (such as weapons left outside for months or years), have been washed, or are heavily soaked in the victim’s body fluids. Also, items that are likely to have been touched by many people, such as a public pay phone or store counter are usually not good sources for probative or interpretable Touch DNA profiles.”

### 3.6 Clothes

#### PCR DNA typing of washed stains


http://link.springer.com/chapter/10.1007%2F978-3-642-78782-9_90#page-1

Large amounts of semen, blood and saliva could be recovered after washing. The yield was inversely proportional to washing temperature (several washes at 95°C did not yield any DNA). Addition of detergent substantially reduced the amount of recovered DNA. Cotton and denim yielded the highest amounts of DNA. E.g. a 10 µL semen stain on cotton could easily be extracted after 95°C wash with detergent.

#### Persistence of DNA from laundered semen stains: Implications for child sex trafficking cases


Setup: semen stains (one or two (1:1) donors) were placed on clothes (T-shirt, trousers, tights) and stored in a wardrobe for 8 months. Items were washed (together with unstained socks) at 30°C or 60°C and with non-biological or biological detergent.

Results: High quantities of DNA (6-18 µg) were recovered irrespective of washing conditions. The quantity did not decline significantly with repeated washes. T-shirt was better than trousers when there was more than one donor (trousers --> one major DNA profile).

DNA could be recovered from the unstained socks washed together with the stained clothes.

#### Probability of detection of DNA deposited by habitual wearer and/or the second individual who touched the garment


Setup: 63 males wore their own underpants for 12 h, the inside waistband was then touched by one of 11 females for 15 s. The waistband was mini-taped and subjected to DNA profiling.

Results: The wearer was detected in 51 % of the samples and always as the major contributor. The test-female was detected in 11 % of the samples. Reportable background DNA (non-wearer, non-test-female) was found in 14 % of the samples. There was no statistical difference in DNA quantification results depending on the time of collection (0 or 12 weeks after wearing and touching).

If a single DNA profile is found on e.g. a pair of underwear, it is 5 times more likely that the person has worn the garment than just touched it. A high total DNA concentration would support wearing the garment.
Oral intercourse or secondary transfer? A Bayesian approach of salivary amylase and foreign DNA findings

Oral intercourse leads to salivary-α-amylase in penile swabs. However, salivary-α-amylase in penile swabs does not necessarily mean oral intercourse but may be due to secondary transfer.

Setup: This is a study of background salivary-α-amylase in underwear after normal (12 hour) wear (69 male subjects). Masturbation with saliva as lubricant was allowed but oral intercourse was not.

Test method: Phadebas press test for screening and RSID-Saliva for confirmation.

Results: 44 % of the underwear had stains containing amylase on the inside front, 13 % of these stains were found to be salivary-α-amylase (5,7 % of total). 50 % of these produced a mixed DNA profile with a minimum of two donors.

A positive control group with oral intercourse showed larger salivary-α-amylase positive stains in the male underwear than the background-test group. 80 % of the underwear in this group gave DNA profiles other than the wearers.

“…the evidence of salivary-•-amylase on male underwear (RSID™-Saliva positive) is 17 times more likely to occur with the allegation of fellatio than the alternative hypothesis of secondary transfer. However the finding of no salivary-•-amylase (RSID™-Saliva negative) upon the male underwear is a 100 times more likely if fellatio did not occur rather than if somebody performed fellatio on Mr B. Likewise, it is evident that if salivary-•-amylase and DNA other than the wearer is present on the inside-front of male underpants that it is 27 times more likely if fellatio occurred rather than fellatio did not occur. In saying that, if salivary-•-amylase is found yet the DNA profile does not contain a profile other than that of the wearer, it is 13 times more likely if fellatio occurred rather than if nobody performed fellatio on an alleged victim”

This paper also contains a section about the sensitivity and specificity of the Phaebas press test and RSID-Saliva; Phadebas press test cannot distinguish between salivary-α-amylase and pancreatic amylase which leads to false positive results, therefore it should only be used for screening.

Recovery of trace DNA and its application to DNA profiling of shoe insoles

0.16-6.4 ng DNA could be recovered by swabbing hands. Even though hands were not washed before the experiment no non-donor alleles could be identified.

Protocol for sampling DNA from shoe insoles and the subsequent sampling and extraction.
Taping was the most efficient method for recovering DNA. Swabbing and soaking methods resulted in dirtier extracts and were likely to include increased amounts of inhibitors.

Recovery from synthetic materials was better than from e.g. leather.

DNA profiling success and relevance of 1739 contact stains from casework

Statistics on 1739 real cases with contact stains.
More DNA was recovered when stains were collected with the double swab (wet swab + dry swab) technique (both for contact stains and saliva stains).

Clothes has the highest success-rate (61 %) (the proportion of DNA profiles that were suitable, 1 person > 5 loci, 2 persons > 7 loci), car items second best (37 %), plugs and cables third (29 %). Stones were most difficult (7 %).

Apparently the relevance of car-contact-stains was high suggesting that the “borrowing” persons DNA covers the car owners DNA

The effect of laundering on the detection of acid phosphatase and spermatozoa on cotton T-shirts
http://www.tandfonline.com/doi/abs/10.1080/00085030.2000.10757498#.VeU7APntkXs

Setup: 12 cotton T-shirts were stained with 500 µL seminal fluid from one donor. The T-shirts were taken to different dry-cleaners for “normal treatment” or washed in a domestic washing machine at
18°C or 30°C with normal amount of detergent, two T-shirts were treated with “spot cleaner” before washing.

Results: Results from dry-cleaning: seminal fluid was detected after dry-cleaning if no spot-cleaner was used. Results from washing machine: Only 18°C without detergent gave positive result with the AP-test (acid phosphatase), however, even if the AP-test was negative there was still spermatozoa and STR DNA profiles could be identified after all different washing procedures.

**Spermatozoa recovered on laundered clothing**

Setup: Cotton briefs treated with semen were washed by different programmes. 40°C or 60°C washes with detergent or 60°C with detergent and softener.

Results: None of the samples were positive for acid phosphatase (AP) and all 60°C samples were negative for prostate specific antigen (PSA). Spermatozoa was detected in all 40°C washed samples and in ~50 % of the 60°C washed samples (could depend on longer storage before analysis). DNA was recovered from all the randomly selected samples, detection of twice the amount of DNA after 40°C wash compared to 60°C wash.

**DNA typing of trace DNA recovered from different areas of sandals found at a homicide crime scene investigation: A comparative study**

A pair of used sandals (flip-flops) was swabbed at 10 different places after 5 months of recovery from crime scene. The amount of DNA varied between the spots but full DNA profiles were obtained from all. Highest recovery from “toe-pin” and under the heal.

**DNA transfer within forensic exhibit packaging: potential for DNA loss and relocation**

Analysis of DNA persistence and transfer during packaging in forensic investigations.
“DNA can be transferred from the deposit area to other parts of the item or to the bag (package) itself and usually to both”. “The effect of bag size on transfer was limited but loose bags can, in certain situations, permit more transfer”.
“DNA was lost to the inside of the container holding bloodied knives”, also a lot of re-distribution of DNA on the knives. Tighter fitting of the container prevented re-distribution of DNA from the tip to the handle.
Cigarette butts should always be packed separately.

Less re-distribution of DNA on gloves when they were packed in paper compared with plastic.

**A single approach to the recovery of DNA and firearm discharge residue evidence**
The mini-tape used for recovery of firearm discharge residue (FDR) in this paper was successfully used for extraction of DNA after the FDR identification was done.

**DNA transfer – a never ending story. A study on scenarios involving a second person as carrier**

Setup: The possibility of tertiary transfer onto textile and plastic was investigated. Donor’s neck --> cotton cloth --> carriers hand (with or without glove) --> plastic bag or cotton cloth.

Results: “… a transfer of donor DNA from epithelial cells through a carrier to a second item is possible, even if the carrier does not wear gloves.” In 11 % of the samples full donor profiles were detected and in 8 of these 21 samples the donor was the major profile. In ~50 % of the samples a carrier profile was detected. Full donor profiles were more frequently found when textile was the final object compared with plastic.
Experience is the name that everyone gives to their mistakes
Jamieson A, Meakin G., Barrister Mag 2010; 45.
Courts are skeptical to experts and prefer experience.
The habitual wearer tends to be the major source of DNA on a garment. DNA can be deposited onto an object that the donor has never touched through transfer, depending on shedder status the transferred DNA can provide the major profile.

One method of collecting fallen off epithelial cell
Vacuum method to collect skin cells from clothes.

The Persistence of seminal constituents on panties after laundering. Significance to investigations of sexual assault
http://www.tandfonline.com/doi/abs/10.1080/00085030.2003.10757551#.VeU6VfntkXs
Test-methods: 1) Blue Test for acid phosphatase, 2) microscopic identification of spermatozoa and 3) PSA ANAcard Test for detection of prostate specific antigen (PSA).
Results: Laundered clothing with semen stains could still produce DNA profiles, the efficiency depended on the type of fabric (cotton retained spermatozoa/DNA better than nylon). Negative AP-test was not reliable.

Everything clean? Transfer of DNA traces between textiles in the washtub
Transfer of DNA from worn clothing (without bloodstains) to another garment is highly unlikely both during hand- and machine washing. Blood can easily be transferred to other garments during the washing procedures.

Use of low copy number DNA in forensic inference
http://www.isfg.org/files/31f9316afbc584bc0be4d445d6cd38c4f064f3a_02004843_693490260903.pdf
Discussion about shedder status and the likelihood of getting DNA profiles from Touch DNA and used garments. E.g. a good shedder can be the major profile on a garment worn by a poor shedder. Also, a good shedder can be the major profile after a secondary transfer event with a poor shedder.

Comparison of collection methods from touch samples on metal and wearer samples from simulated mixtures on clothing
Puritan sterile cotton swabs can contain up to 23 pg of human DNA.
Touch DNA on metal: comparison between DNA sterile cotton swabs, DNA free cotton swabs and foam tipped DNA free swabs (organic extraction, qPCR, PCR STR multiplex amplifications and capillary electrophoresis). The foam tipped DNA free swabs worked poorly on both stainless steel and brass. DNA sterile cotton swabs worked the best for stainless steel and DNA free cotton swabs worked best for brass.
Touch DNA on clothes (different types): Comparison between swabbing, scraping and adhesive taping (Gel-Pak `0´). All three methods resulted in mixed profiles. Swabbing and Gel-Pak ´0´ were comparable in that the last wearers profile was the most distinct one. Gel Pak ´0´ gave generally less DNA. Scraping recovered more DNA from the habitual wearers.
Yield of male touch DNA from fabrics in an assault model

Setup: Grab and struggle for 15 seconds was used to deposit male touch DNA to cotton, polyester or cotton/polyester blend on a female (hands were washed 15 minutes before the test). Sampling was done by cutting the fabric after 12 hours or 7 days.
Results: A maximum of 7 pg/µL DNA was extracted after 12 hours. No significant difference after 7 days. Fabric types could not be compared due to the low yield (Quantifiler Duo was used which has a limit of 23 pg/µL).

The effect of washing on the detection of blood and seminal stains
http://www.tandfonline.com/doi/abs/10.1080/00085030.1971.10757279#.VeRiUfntkXs

Setup: Washing of semen or blood or semen+blood -stains was planted on new underwear (cellulose acetate fabric or cotton fabric). Detergents used were 1) for hot water 2) for cold water 3) for hot or cold water with enzymatic activity. 12 different washing procedures were used combining different lengths of soaking before washing and different detergents.
Results: No visible blood stains on cellulose acetate underwear were detected after washing and most washing procedures resulted in negative detection of blood with benzidine. The blood on cotton underwear was however more resistant and all washing procedures resulted in positive reaction with benzidine even though no visible stain was detected. Seminal stains were visibly not detected after any washing procedure on either cellulose acetate or cotton and the “fast blue” seminal stain was negative for most washing procedures, however, only the detergent containing enzymatic activity washed away the spermatozoa as detected by microscopy (spermatozoa could be found in some samples even after washing with the enzymatic detergent as well).

3.7 Contamination

Fingerprints as evidence for genetic profile: morphological study on fingerprints and analysis of exogenous and individual factors affecting DNA typing
http://www.crime-scene-investigator.net/fingerprintDNAextraction.pdf

Setup: 347 fingerprints, 11 persons, glass/wood/metal, 30s, with/without handwashing.
Results: Recovery: 0.04-0.2 ng.
Handwashing reduced the amount of recovered DNA. “Shredder status” of donor is very important regarding how much DNA can be recovered from a fingerprint.
“…LCN method enhances the amplification of spurious alleles present in fingerprints, but laboratory-based contamination cannot be excluded, since standard negative controls of amplification cannot reveal lower levels of contamination.”
“When fingerprints were made after subjects had touched objects of routine use, including computer keyboards used by many people, mixed alleles from multiple donors were observed. In many cases, the peaks of accessory alleles reached those of true alleles, hindering definite allele typing.”

Low copy number: Interpretation of evidence results
Laboratory contamination can be a problem when the number of amplification cycles is high.

Internal quality control in forensic DNA analysis
Review on the importance of quality controls.
Validation of a dual cycle ethylene oxide treatment technique to remove DNA from consumables used in forensic laboratories
Ethylene oxide is an organic gas that can be used for sterilization. The EO-technique was optimized for removal of DNA from disposable plastic consumables.
“…DNA from up to 50 µl of blood and saliva can be removed to a level where the consumable can be considered DNA free. DNA from semen was more resilient…”

Reducing contamination in forensic science
Balk C., Justice Studies and Forensic Science: 3, Article 12 (student essay).
http://scholarworks.sjsu.edu/themis/vol3/iss1/12
200 pg DNA/ 33 cells is enough for analysis.
LCN: risk of drop-in, drop-out alleles.
Sterilization: UV-, gamma-, electron beam radiation and ethylene oxide.
UV was found to be useless, gamma and EB were OK for small amounts of DNA, Ethylene oxide (ethylene glycol) was best.
PCR-related contaminants: After a selected fragment of DNA is amplified, it will have dUTPs in it; something unamplified DNA does not possess. UNGcoupled PCR is an effective technique to reduce risk of contamination during the amplification process, and should be utilized as a preventative measure whenever possible in the forensic laboratory.
Sources of contamination: Manufacturer contamination, DNA transfer at crime scene, Evidence packaging and transport, Exhibit examination (forceps, scissors, gloves, speaking without facemask).

DNA contamination minimisation – finding an effective cleaning method
http://www.tandfonline.com/doi/abs/10.1080/00450618.2015.1004195#.VeVMD_ntkXs
1 % sodium hypochlorite 5 min + 70 % EtOH wiping was able to remove DNA, saliva, blood, semen and skin cells from both smooth and pitted surfaces.

Environmental DNA monitoring: beware of the transition to more sensitive typing methodologies
http://www.tandfonline.com/doi/abs/10.1080/00450618.2013.788683#.Vd25pfntkXs
Environmental DNA monitoring (EDM): surfaces and tools are sampled on a periodic basis.
Kits used in the study: Profiler Plus, Yfiler, PowerPlex 21.
Washing (hypochlorite + ethanol) did not remove all DNA from items like rulers, pipettes and tube racks etc. However, the detectable DNA after cleaning was present in few alleles with low peak heights.

Assessing the risk of secondary transfer via fingerprint brush contamination using enhanced sensitivity DNA analysis methods
“Detection of secondary transfer of DNA can occur through fingerprint brush contamination and is enhanced using LCN-DNA methods.”
“If a brush were to add DNA-containing material to a surface containing a handprint, the proportion of the added DNA is likely to be less than that retrieved from the depositor of the print. Thus, the minor component of the mixture derived from the brush may not be detectable.” (ref 2 in paper; Proff et al. 2006).
“The dusting of latent prints may dislodge cellular debris from the latent print or substrate. That debris then adheres to the brush. This brush is then used on another item of evidence, or at another crime scene, where it is subject to the same mechanical maneuvering and where it can dislodge cellular debris, leaving traces of biological evidence not pertinent to the evidence being handled. Under LCN conditions, it may be possible to obtain DNA results that are not relevant to the case due to a secondary transfer by fingerprint brush contamination.” (ref 16 in paper; Pesari et al. 2003).
“In this study, the risk of false associations through the inclusion of contaminant DNA donors was moderate.”

**UV light irradiation of plastic reaction tubes inhibits PCR**

“When DNA contamination in PCR reagents is treated with UV light, it produces pyrimidine dimer adducts and prevents them from being effective templates in subsequent PCR.” (ref 5 in paper).

“…UV irradiation of plastic microcentrifuge tubes inhibits PCRs and is dose-dependent.”

**A review of the science of low template DNA analysis**

Budlowe (2009) is critical to the conclusions of this review: “The reviewers found that LCN typing as practiced specifically in the United Kingdom was “robust” and “fit for purpose” but offered a number of recommendations to improve the methodology. The findings of the commission seem inconsistent with the nature of LCN typing and LCN typing warrants a more in-depth evaluation by the greater scientific community.”

This review address concerns about Low template DNA analyses (LTDNA) for legal purposes, instigated by the Forensic Science Regulator and contains guidelines, recommendations and validation for use of LTDNA.

Transfer of cellular material:  
Individuals vary in their propensity to transfer their cellular material to an object.  
Time and pressure affects the efficiency of the transfer.  
(Person→ object = primary transfer. To next object/person = secondary transfer.)

**Another phantom from the morgue - A case of instrument-born sample contamination in the course of identifying an unknown deceased**

The paper focuses in instrument/equipment contamination.

**An investigation of the presence of DNA on unused laboratory gloves**

DNA was found (up to 20 alleles) on gloves from unopened boxes (vinyl gloves, specific brand).  
DNA was found (up to 14 alleles) on gloves from opened boxes from different brands and of different materials.

**Contamination monitoring in the forensic DNA laboratory and a simple graphical model for unbiased EPG classification**

There is a need for a standardized, unbiased and independent method for the monitoring of background DNA in the laboratory.  
Presentation of a graphical classification model for ranking of electropherograms.

**Contamination during criminal investigation: Detecting police contamination and secondary DNA transfer from evidence bags**

More sensitive analyses require more attention regarding contamination.

Set up: 1) Environmental DNA monitoring of commonly touched surfaces in examination rooms at two large police units.
3) Study of whether DNA from the outside of evidence bags can transfer to the material (swabs and fabric).

Results: 1) High risk- low risk- and medium risk surfaces (rulers, chairs, glove boxes, sides of roles with bench paper…) contained significant amounts of DNA from the police officers working in that room. Only 12 of the analyzed 45 areas had no or few peaks above threshold (200 rfu).
2) 16 incidents of police officer contamination were found in the retrospective matching of DNA and case work. In 6 of these cases the detected police officer was not directly involved in the case work. 12 of the contaminations were from swabs collected by the police officers and 4 were from exhibits sent to the laboratory for DNA collection. However, the number of contaminated samples related to the total amount of samples is < 1 %. The laboratory caused significantly less contamination incidents than police officers.
3) Evidence bags were handled without gloves on the outer surface and then handled as usual. 60 swabs were analyzed and one contamination event was detected (full profile). 20 pieces of fabric were analyzed and one full profile and 11 partial profiles (0-6 alleles >200 rfu, 10-29 alleles >30 rfu) were recovered. There was a correlation between the amount of DNA on the bags and the contamination events.

Conclusion: Secondary transfer can occur during examination. Shaking a contaminated evidence bag over the material can lead to contamination of the material. Contaminated opening edges of the evidence bags can also contaminate the material. The authors present guidelines on how to avoid contamination.

Secondary and subsequent DNA transfer during criminal investigation

Detection of DNA transfer from object to plastic gloves worn by investigator (disposable nitrile-gloves) to new object. The amount of DNA deposited on the first object and the object material-type influenced the transfer.

UV irradiation and autoclave treatment for elimination of contaminating DNA from laboratory consumables
Test of cleaning procedures for removing DNA from saliva.
“Autoclaving is more effective than UV irradiation…”

Assessment of the possibility of DNA accumulation and transfer in a superglue chamber
DNA can be transferred from one object to another when treated in a superglue chamber (fingerprint technique) where multiple objects are often treated together or one after the other.

Analysis and implications of the misscarriages of justice of Amanda Knox and Raffaele Sollecito
LCN-levels of victim-DNA (no detection of blood) on a knife, analyzed without replication and with only a few alleles, were used to convict a suspect. Moreover, the location of the suspect-DNA on the handle was interpreted (without scientific grounds) as a result from stabbing rather than cutting food. The suspect and the victim shared an apartment. Evidence was collected using dirty gloves and in non-sterile containers. Mixed profiles of the two roommates were interpreted as one’s involvement in the murder of the other but was most probably just normal background DNA from two people living together.
“Selective cleaning” was believed to have erased the DNA from two suspects but not the third at the murder-scene.
Negative controls during the DNA analysis was interpreted as that the evidence could not have been contaminated in any way, either before the murder, at collection of the evidence or during handling of the evidence by the police officers.

**Manufacturer contamination of disposable plastic-ware and other reagents – an agreed position statement by ENFSI, SWGDAM and BSAG**


There has been a report that estimates that approximately 1 of 25,000 consumable items in the forensic lab is contaminated enough to give a full DNA profile. This could be a problem since we cannot test that many objects for possible contaminations.

“Sterile” does not necessarily mean DNA-free.

**DNA transfer within forensic exhibit packaging: potential for DNA loss and relocation**


Analysis of DNA persistence and transfer during packaging in forensic investigations.

“DNA can be transferred from the deposit area to other parts of the item or to the bag (package) itself and usually to both”. “The effect of bag size on transfer was limited but loose bags can, in certain situations, permit more transfer”.

“DNA was lost to the inside of the container holding bloodied knives”, also a lot re-distribution of DNA on the knives. Tighter fitting of the container prevented re-distribution of DNA from the tip to the handle.

Cigarette butts should always be packed separately.

Less re-distribution of DNA on gloves when they were packed in paper compared with plastic.

**DNA typing of human dandruff**


Dandruff contains nuclei-free cells but also enough nucleated cells to provide a DNA-profile. The amount of DNA in a dandruff particle has been calculated to be 0.8-1.5 ng. In the paper they could find between 0.8-16.6 ng DNA in a single particle.

Experiments in the paper showed that 10 dandruff particles were enough to detect alleles from the dandruff donor in a blood stain. Dandruff mixed with semen showed that 3-5 dandruff particles was enough to detect donor alleles and could even result in the major band (polyacrylamide gel electrophoresis analysis).

**Kercher trial: How does DNA contamination occur?**


Very small amounts of suspect’s and victim’s DNA on a knife without traces of blood and mixed profiles on a bra clasp led to conviction.

**Error rates in forensic DNA analysis: Definition, numbers, impact and communication**


Statistics regarding quality issues and non-conformance reporting at the NFI. The most common cause of failure in forensic DNA casework was found to be contamination and human error. The human errors could mostly be corrected for. Contaminations were mainly between samples from different crimes.
Leading-edge forensic DNA analyses and the necessity of including crime scene investigators, police officers and technicians in a DNA elimination database
On the importance of an employee DNA-database to prevent misleading “unknown” DNA profiles to interfere with crime-investigations. This paper comes from Canada where forensic elimination database are built on voluntarily basis.

Risk of DNA transfer by gloves in forensic casework
Setup: All gloves used by 4 lab workers during a day were analyzed for DNA from the lab worker or from other samples.
Results: No alleles were observed from 12.5 % of the gloves. Lab worker related alleles from 10 % of the gloves. Lab worker and test sample from 12.5 % of the gloves. Test subject and unknown donor from 50 % of the gloves.
DNA was also found on unused gloves.
Guidelines: Clean the gloves before use (UV irradiation is not sufficient). Change gloves after touching items or surfaces prior to touching an exhibit. Use multiple layers of gloves to avoid skin exposure during the changing of gloves.

The DNA lab and contamination
Minor J., Forensic Magazine 05/15/2014.
http://www.forensicmag.com/articles/2014/05/dna-lab-and-contamination
Examples on how to avoid contamination in the forensic laboratory setting.

Touch DNA: From the crime scene to the crime laboratory
Minor J., Forensic Magazine 04/12/2013.
A review on touch DNA and contamination, and guidelines on how to avoid the contaminations.

Female criminals - it’s not always the offender
DNA from female found on several crime scenes in Austria turned out to be from a woman working in the cotton-swab kit production.
A follow-up study showed that sterile swabs (by radiation) from different manufacturers were often contaminated with DNA.

Contamination when collecting trace evidence – An issue more relevant than ever?
Austrian study on contamination and on the importance of a elimination database. About 8 % of the 1491 police officers registered in their database caused a contamination at least once between 2009 and 2015.

How long does it take a static speaking individual to contaminate the immediate environment?
http://link.springer.com/article/10.1007%2Fs12024-006-0004-z#page-1
Setup: Experiment in a room without air-conditioning, no open windows and no people in there for 1 hour before. Benchkote was placed at different places in the room and the test person repeated the same phrase for 15 minutes while kneeling or standing, samples were taken at 30 sec, 1 min, 5 min, 15 min.
A sitting experiment was conducted with the same setup but at a table at time points ~2 s, 30 s, 1 min, 5 min.

**Results:**
- Results kneeling: Full or acceptable profiles up to 69 cm away for all time points, donor alleles found up to 184 cm away.
- Results standing: Full or acceptable profiles up to 92 cm away after 1 minute, donor alleles up to 184 cm away for all time points.
- Results sitting: Full or acceptable profiles after 30 seconds over the whole table.

**Conclusion:** use face masks!

**Beware; gloves and equipment used during the examination of exhibits are potential vectors for transfer of DNA-containing material**


[http://www.isfg.org/files/91a748919b016087a260b2ab392c8c8f79a21c0f.05014093_845812755086.pdf](http://www.isfg.org/files/91a748919b016087a260b2ab392c8c8f79a21c0f.05014093_845812755086.pdf)

A paper very similar to the other paper by Poy 2006.

**Conclusions:** Regularly change gloves during examinations. Avoid contact with areas of the exhibit that are likely to be sampled for DNA analysis. Regularly clean tools and objects that may come in contact with an exhibit.

**Trace DNA presence, origin and transfer within a forensic biology laboratory and its potential effect on casework**


Gloves were at high risk of acting as vectors for DNA transfer.

Two other high contamination risk objects were a plastic ruler and a drying line for clothes.

DNA could be found on most investigated vectors in the whole forensic laboratory chain but the risk of transferring a profile that would interfere with analysis were medium to low.

**The problem of DNA contamination in forensic case work—How to get rid of unwanted DNA?**


The authors have tried UV-irradiation and other decontamination procedures on artificially contaminated slides but found no one eliminated the contamination completely. They found that the distance to the UV source and the wavelength is important for the results and that isolated DNA is more susceptible to UV irradiation than saliva.

**Experiments on the DNA contamination risk via latent fingerprint brushes**


**Setup 1:** Analysis of 51 used fingerprint brushes.

**Results 1:** 86 % of the brushes gave full or partial profiles.

**Setup 2:** A selection of brushes and some artificially contaminated brushes to test secondary transfer

**Results 2:** “The larger the brushed area, the greater is the risk for contamination; single fingerprints are less affected.” “Contact of the brush with body fluids such as blood or saliva make secondary transfer highly likely.”

**The forensic science regulator: response to professor Brian Caddy’s review of the science of low template DNA analysis**

Rennison A., FSR, Home Office, UK. 2008


Proposals on how to follow recommendations from Caddy’s review in the UK.
The effectiveness of protective clothing in the reduction of potential DNA contamination of the scene crime
http://link.springer.com/article/10.1007%2Fs00414-002-0348-1#page-1

Setup: no movement, movement, talking (15 minutes) and coughing (10 seconds) was performed in a test-zone with or without protective clothing. Samples were collected by swabbing.

Results: No alleles were detected after no movement, regardless of protective clothing. Normal movement without protective clothing introduced a high rate of contamination. Vigorous movement with protective clothing could cause contamination. Standing and talking did not cause contamination on the floor regardless of face-mask. Kneeling led to heavy contamination and not even the face-mask could completely contain the contamination. Face mask also reduced contamination from coughing.

DNA contamination of mortuary instruments and work surfaces: a significant problem in forensic practice?
http://link.springer.com/article/10.1007/s004140000142#page-1

“Of the 20 mortuaries studied, 50% were found to have material containing quantifiable human DNA on the instruments and surfaces sampled (after routine cleaning). This DNA was amplified and found, in some cases, to have been derived from at least three people.”
“…autoclaving does not necessarily remove the risk of instrument contamination by human DNA.”

Phantoms in the mortuary - DNA transfer during autopsies

Surfaces and instruments used during autopsies was swabbed after routine cleaning procedures and analyzed for contaminations. Most samples (almost 100%) contained alleles that could be connected to bodies that had been handled there. Six bodies were investigated and four of them had alleles transferred from the autopsy environment.
A bleach containing cleaner (DAN Klorix) was shown to provide clean enough surfaces and instruments.

Comparison of the effects of sterilisation techniques on subsequent DNA profiling

“This work indicated that the most successful technique to reduce DNA contamination was ethylene oxide treatment. Of the radiation techniques tested in this study, gamma was the most successful at eradicating DNA and UV radiation was the least.”
“Ethylene oxide is a poisonous gas and therefore can only be used for sterilisation by approved companies. Items sterilised by the ethylene oxide must also go through a decontamination stage to ensure all residual gas is removed.”

New developments and challenges in the use of the UK DNA database: addressing the issue of contaminated consumables
http://www.fsijournal.org/article/S0379-0738(04)00535-3/abstract

An employee of a tube manufacturer had contaminated tubes for years, leading to minor profiles in several cases. Follow-ups showed that other employees had also contaminated tubes resulting in partial or full profiles in several cases. Similar contaminations had later been observed for plates and swabs.
Potential degrading effect of sodium hypochlorite on exhibits containing DNA
http://www.fsigeneticssup.com/article/S1875-1768(15)30033-0/pdf
Sodium hypochlorite can be used as a cleaning reagent to minimize contaminations. When a surface was cleaned with 10 % sodium hypochlorite and air dried the residual sodium hypochlorite showed a degrading effect on DNA. If the surface was wiped dry after cleaning with 10 % sodium hypochlorite or washed with 1 % sodium hypochlorite no degrading effects were observed.

Residual DNA on examination tools following use
High risk vectors: scissors, forceps, gloves.
Dried blood or touch DNA, deposited on the primary substrate of cotton or glass, was transferred via the vector to the secondary DNA-free substrate. The vector was sampled directly after contact with the secondary substrate.
Results: The vectors contained DNA after touching the second substrate which means that further contamination can occur.

DNA transfer by examination tools – a risk for forensic casework?
High risk vectors: scissors, forceps, gloves.
Setup: Substrates: glass and cotton. “Dried blood or touch DNA, deposited on the primary substrate, was transferred via the vector to the secondary substrate, which was either DNA-free or contained a target sample (dried blood or touch DNA).”
Results: Touch DNA transferred less than blood. Transfer did not interfere with the target profile on the second substrate but could complicate the analysis when no suspect is known.

The potential transfer of trace DNA via high risk vectors during exhibit examination
High risk vectors: scissors, forceps, gloves.
Light contamination: single touch/cut for 2-3 seconds with vector.
Heavy contamination: multiple cuts/touches.
Results: “DNA was transferred for all vectors in both heavy and light contamination scenarios”. Acceptable profiles were observed for all scenarios except forceps light contamination.

The application of ultraviolet irradiation to exogenous sources of DNA in plasticware and water for the amplification of low copy number DNA
An optimization of UV-irradiation with Stratalinker 2400 for cleaning plastics and water used in LCN-work.
Lining the area around the samples with aluminum foil decreased the irradiation time needed. An inch raise in the UV source improved the efficiency of the irradiation for PCR-plates. Precise guidelined presented in paper.

Germany’s phantom serial killer. A DNA blunder
Time Magazine Online, 2009. Himmelreich C.
www.time.com/time/world/article/0,8599,1888126,00.html
Cotton swab factory worker was thought to be “phantom serial killer”.

Nationellt forensiskt centrum, NFC – Biologisektionen
Sources of DNA contamination and decontamination procedures in the forensic laboratory
Analysis of air, surfaces, tools and equipment in a pre-PCR laboratory. Decontamination by sodium hypochlorite and DNA ZAP (commercially available solution).
Air was found to be an unlikely source of contamination.
DNA ZAP was found to decontaminate more effectively than sodium hypochlorite.
Conclusion: To avoid contamination equipment should be cleaned and gloves changed often.

DNA typing of fingerprints using capillary electrophoresis: Effect of dactyloscopic powders
Some dactyloscopic powders used for fingerprint enhancement can be used prior to DNA recovery but precautions should be taken to avoid contamination.

Forensic trace DNA: a review
http://investigativegenetics.biomedcentral.com/articles/10.1186/2041-2223-1-14
History and discussion on STRs and PCR.
Touch/trace DNA should not be confused with LCN.
Touch DNA = collection of minute biological samples. Touched objects does not always result in low amounts of DNA.
LCN = often used to describe increased cycle numbers.
Trace DNA is herein defined as “any sample which may fall below the recommended thresholds at any stage of the process - detection, collection, extraction, amplification and interpretation.” Usually less than 100 pg DNA.
A sample defined as trace DNA in the recovery phase does not necessarily have to be trace DNA in later stages of the process.
The precise location of where to recover the trace DNA is very important.
Double swabbing (not necessarily wet + dry, can be wet+wet if all the moisture is recaptured by the first swab) is recommended for collection of trace DNA.
If the swab is allowed to dry before extraction less DNA will be extracted, if the dried swab is frozen before extraction the DNA recovery will be similar to if it would still have been wet.
LMD can be useful to differentiate relevant cells from other cell types which allow efficient DNA analysis from the minor cell type in a sample. Flow cytometry can be used to separate sperm cells from vaginal wash fluid.
Chelex and organic extraction can lead to up to 75 % loss of DNA. However, this loss is often not relevant.
Partial or complete profiles have been obtained from samples that did not pass the quantification limit. Trace DNA samples should not always be discarded due to low quantification.
Reviews amplification methods for LCN samples, detection of the amplified product and interpretation. Common problems with trace DNA amplification: 1) allele drop-out, 2) decreased heterozygote balance, 3) allele dop-in (stutter), 4) allele drop-in (contamination).
In analysis it can be useful with detection thresholds such as LOD (limit of detection), T (low template DNA threshold), MIT (match interpretation threshold) and LOQ (limit of quantification). However, the peak intensity should be included in the exclusion calculation and continuous measures should be used instead of definite thresholds.
Replicates should be employed for trace DNA analysis, four replicates with reported alleles detected in at least two of the replicates seems to be the most accurate. Bayesian based likelihood ratios of drop-outs can also be employed.
Mixed profiles can be challenging due to e.g. biased drop-outs, complicating drop-ins and over-amplification of some alleles.
Contamination of trace-DNA can be a serious problem. The contamination can be the major profile in the sample. Contamination can occur before the crime is committed (background DNA), between the crime is committed and the crime scene is secured, during the crime scene investigation and in the forensic laboratory.

Beware of the possibility of fingerprinting techniques transferring DNA
http://www.crime-scene-investigator.net/fingerprintingtechniqscontamination.pdf
Fingerprint brushes can accumulate DNA from surfaces they come into contact with and might cause contamination. However, the proportion compared to the studied DNA should be low.

Report: Inquiry into the circumstances that led to the conviction of Mr Farah Abdulkadir Jama
Rape conviction (girl unconscious) based on a slide and a swab collected in the medical examination of the victim. The slide and the swab had been collected in the same unit as samples from another woman 28 hour earlier who undisputedly had had sexual contact with the accused.

Forensic lab error led to miscarriage of justice
Walter P., Chemistry world 5 October 2012.
http://www.rsc.org/chemistryworld/2012/10/lec-forensics-lab-error-rape-accused
Cross-contamination during extraction in a forensic lab in the UK led to 5 months arrest of an innocent man. Apparently a disposable plastic microtiter plate had been reused.

Transfer of biological stains from different surfaces
An experimental contamination study on blood and saliva.
Setup: 50 µL saliva or blood was placed on paper/cotton/plastic and allowed to dry.
  a) 2 s pressing a thumb on the stain with or without gloves
  b) 10 s rubbing a thumb on the stain with or without gloves
  c) 10 s rubbing a thumb on the stain with or without gloves and then the thumb was placed on a piece of paper.
Sample collection by swabbing.
Results: DNA from saliva was transferred from paper and cotton in low but detectable amounts with both setup a) and b), < 10pg/µL. Setup c) resulted in very low amounts of DNA (<0.1 pg/µL) in the 50% of the cases where DNA was detected at all. The transfer from plastic resulted in much higher DNA yields in all experimental setups (primary transfer up tp 100 pg/µL and secondary transfer 0-10 pg/µL). DNA from blood was transferred poorly from fabric (~0.1 pg/µL) but higher yields were detected from paper and plastic (in some cases more than 100 pg/µL, even in secondary transfer).

Touch DNA: Forensic collection and application to investigations
“…the scraping/tape lift methods are ideal in situations where the scientist can locate areas on the item which are most likely to contain the perpetrator’s skin cells.”
“…an increased chance of obtaining mixed DNA profiles containing DNA from individuals that may have come into contact with the victim/evidence item near the time of the crime. Contributors to these mixtures could include the victim’s spouse or children, and again, elimination samples may need to be collected from these individuals.”
“Some evidence items are also not recommended for the collection of Touch DNA samples. Such items include those that are severely degraded (for example, moldy clothing), have been exposed to extreme environmental conditions (such as weapons left outside for months or years), have been washed, or are
heavily soaked in the victim’s body fluids. Also, items that are likely to have been touched by many people, such as a public pay phone or store counter are usually not good sources for probative or interpretable Touch DNA profiles.

### 3.8 Court decisions

**Publish or perish – meanwhile, study what’s already out there**

Bader S., The Barrister.  

Courts in the UK have a tendency to trust “experience” more than science. “12 hours after digital penetration it was three times more likely to find that the major contributor to a profile was from the woman.” (On the offender’s fingers.)

**Validity of low copy number typing and applications to forensic science**

[http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2702736/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2702736/)

“LCN typing simply can be defined as the analysis of any DNA sample where the results are below the stochastic threshold for reliable interpretation.” “Some touch DNA samples do not qualify as LCN samples in that they contain sufficient DNA for routine conventional analyses. Conversely, many crime scene samples do not meet the criteria of LCN samples. Such samples should be clearly distinguished and analyzed and interpreted accordingly.” If LCN samples are not handled correctly they can be useless in trial. Difficult (not possible) to use LCN for exculpatory purposes. LCN can basically only be used to identify human remains/missing persons according to the authors.

**A review of the science of low template DNA analysis**


Budlowe (2009) is critical to the conclusions of this review: “The reviewers found that LCN typing as practiced specifically in the United Kingdom was “robust” and “fit for purpose” but offered a number of recommendations to improve the methodology. The findings of the commission seem inconsistent with the nature of LCN typing and LCN typing warrants a more in-depth evaluation by the greater scientific community.”

This review address concerns about Low template DNA analyses (LTDNA) for legal purposes, instigated by the Forensic Science Regulator and contains guidelines, recommendations and validation for use of LTDNA.

**Transfer of cellular material:**

Individuals vary in their propensity to transfer their cellular material to an object. Time and pressure affects the efficiency of the transfer. (Person--> object = primary transfer. To next object/person = secondary transfer.)

**DNA transfer: informed judgement or mere guesswork?**

[http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3872334/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3872334/)

Opinion article  
“…the quantity of DNA or the quality of the profile cannot be used “to reliably infer the mode of transfer by which the DNA came to be on the surface of interest.” (Meakin and Jamieson 2013).

**Investigation into the usefulness of DNA profiling of earprints**


Setup: 60 earprints (5 s pressure against surface) collected from 3 adults.  
Results: Full donor profile from one of 60 earprints. One or more non-donor alleles in one-third of the earprints. Direct swabbing of ears showed high levels of non-donor DNA.  
Conclusion: DNA results from earprints should be interpreted with great care.
Low Copy Number DNA: Reality vs Jury Expectations
Hoffman Wulff P., Silent Witness Newsletter 2006; 10(3)
Discussion about the limitations and the future of LCN.

Kercher trial: How does DNA contamination occur?
Very small amounts of suspect’s and victim’s DNA on a knife without traces of blood and mixed profiles on a bra clasp lead to conviction.

Anything you touch may be used against you
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2892330/
How reliable is DNA evidence?
Care must be taken when analyzing DNA from cold cases since the sampling procedures were not the same as now and contaminations are likely.

Experience is the name that everyone gives to their mistakes
Jamieson A, Meakin G., Barrister Mag 2010; 45.
Courts are skeptical to experts and prefer experience.
The habitual wearer tends to be the major source of DNA on a garment. DNA can be deposited onto an object that the donor has never touched through transfer, depending on shedder status the transferred DNA can provide the major profile.

Regina v. Peter Weller (court trial on “digital penetration”)
England and Wales Court of Appeal (Criminal Division) Decisions, EWCA Crim 1085, 4 March 2010.
http://www.forensicdna.com/assets/weller_decision.pdf
How did the DNA from the girl end up on the man’s fingers? Different scenarios. Expert witness drawing poorly based conclusions? The clash between science and experience in court.

Recovery of DNA from latent fingerprint tape lifts archived against matte acetate
Extraction of DNA from fingerprints is problematic for several reasons. The amount of DNA is often very low and the brushes used to enhance the fingerprints are often contaminated with DNA from other crime scenes. Also, the collection of DNA will destroy the fingerprint for further comparison analysis. This study aimed to develop a technique to recover DNA from latent fingerprints caught between a tape and matte acetate.
No experimental setup in this paper yielded enough DNA for a reliable DNA STR-profile and some even contained non-donor alleles.

Report: Inquiry into the circumstances that led to the conviction of Mr Farah Abdulkadir Jama
Rape conviction (girl unconscious) based on a slide and a swab collected in the medical examination of the victim. The slide and the swab had been collected in the same unit as samples from another woman 28 hour earlier who undisputedly had had sexual contact with the accused.
3.9 Explosives

Systematic study for DNA recovery and profiling from common IED substrates: From laboratory to casework
Method development for recovery of DNA from IED (Improvised Explosive Devices, made from household items).
“All factors - swab types, moistening agents, substrates, and their second- and third-order interactions - affected the amount of DNA recovered to a varying degree”. Swab type influenced the most, moistening agent affected the least. “In general, we were able to recover more DNA from PVC sections, batteries, and electrical tape as opposed to copper wire”. “There was no single best swab type or moistening agent for all substrates.”
Tape lifting could be successfully used when PVC was the substrate.

3.10 Fingerprints

Fingerprints as evidence for genetic profile: morphological study on fingerprints and analysis of exogenous and individual factors affecting DNA typing
http://www.crime-scene-investigator.net/fingerprintDNAextraction.pdf
Setup: 347 fingerprints, 11 persons, glass/wood/metal, 30s, with/without handwashing.
Results: Recovery: 0.04-0.2 ng.
Handwashing reduced the amount of recovered DNA. “Shedder status” of donor is very important regarding how much DNA can be recovered from a fingerprint.
 “… LCN method enhances the amplification of spurious alleles present in fingerprints, but laboratory-based contamination cannot be excluded, since standard negative controls of amplification cannot reveal lower levels of contamination.”
“When fingerprints were made after subjects had touched objects of routine use, including computer keyboards used by many people, mixed alleles from multiple donors were observed. In many cases, the peaks of accessory alleles reached those of true alleles, hindering definite allele typing.”

Fingerprints from fingerprints
http://www.isfg.org/files/8113b53efe142f5fac93aab44ea82e1cfdece0456.02002303_961657393440.pdf
Latent fingerprints usually contain a small number of epithelial cells. The cells may also be corneocytes only (no nuclei).
Tested on white paper.
Minimal incidence of nucleated cells (microscopic inspection), but enough for analysis.

STR genotyping and mtDNA sequencing of latent fingerprint on paper
Setup: Fingerprints were deposited on ordinary white paper for 1-60 s.
Results: Most cells deposited on the paper were nuclei-free corneocytes but enough nucleated cells for DNA extraction were found.
Assessing the risk of secondary transfer via fingerprint brush contamination using enhanced sensitivity DNA analysis methods
“Detection of secondary transfer of DNA can occur through fingerprint brush contamination and is enhanced using LCN-DNA methods.”
“If a brush were to add DNA-containing material to a surface containing a handprint, the proportion of the added DNA is likely to be less than that retrieved from the depositor of the print. Thus, the minor component of the mixture derived from the brush may not be detectable.” (ref 2 in paper; Proff et al. 2006).
“The dusting of latent prints may dislodge cellular debris from the latent print or substrate. That debris then adheres to the brush. This brush is then used on another item of evidence, or at another crime scene, where it is subject to the same mechanical maneuvering and where it can dislodge cellular debris, leaving traces of biological evidence not pertinent to the evidence being handled. Under LCN conditions, it may be possible to obtain DNA results that are not relevant to the case due to a secondary transfer by fingerprint brush contamination.” (ref 16 in paper; Pesari et al. 2003).
“In this study, the risk of false associations through the inclusion of contaminant DNA donors was moderate.”

Analysis of cellular and extracellular DNA in fingerprints
Cellular DNA – from skin cells.
Extracellular DNA – from body fluids (s.a. sweat)
Setup: Glass slides and plastic pens were wet swabbed (TE buffer).
Preparation methods: centrifugation, acoustofluidics.
Analysis method: qPCR, 45 cycles.
Results: Extracellular fractions contain more DNA than cellular fractions. Some people are better “shedders” than others.
Some contaminations through carryover in blank runs.

Assessment of the possibility of DNA accumulation and transfer in a superglue chamber
DNA can be transferred from one object to another when treated in a superglue chamber (fingerprint technique) where multiple objects are often treated together or one after the other.

Science in court: DNA’s identity crisis
Discussion about the reliability of LCN (by increased number of cycles) in forensic investigations.

Investigation of secondary DNA transfer of skin cells under controlled test conditions
“The transfer rates showed that both the primary and secondary type of substrate and the manner of contact are important factors influencing transfer of skin cells… the freshness of the deposit in most instances is not.”“Skin cells deposited on a non-porous primary substrate transferred more readily to subsequent substrates than those deposited on a porous substrate. Porous secondary substrates, however, facilitated transfer more readily than non-porous secondary substrates, from both porous and non-porous surfaces. Friction as the manner of contact significantly increased the rate of transfer.”
Evaluation of methodology for Low Copy Number (LCN) DNA analysis - on spent cartridge cases - after enhancement of latent fingerprints
Johansson C., Linköpings Universitet, magisteruppsats, LiU-IFM-Ex-08/1892-SE.
“The report includes a discussion on the definition of LCN, different applications, risks and limitations, optimizations of the procedure and how to deal with the interpretation. Finally there are some words on LCN in perspective of the justice system.”

Touch DNA – The prospect of DNA profiles from cables.
This study was performed with metal theft in mind. Cable sheaths are often removed and left behind when the metal inside is stolen. The material is often made of black plastic polymers and both DNA and fingerprint investigations are of interest. For fingerprint visualization on this type of material cyanoacrylate (CAN) fuming or wet powder suspensions (WPS) are often used.
Setup: Sweat, extracted DNA or touch DNA was deposited on smooth non-porous black cable (0.02 m diameter). Sweat and extracted DNA were from stock solutions collected from volunteers and touch DNA was deposited with unwashed hands by rolling the top-half of each finger on the cable. DNA was recovered by either double swabbing or mini-taping, before or after fingerprint visualization by CAN fuming or WPS treatment.
Results: There was no difference in DNA yield between double swabbing and mini-taping. Treatment with CAN fuming before swabbing or taping resulted in a significant increase in amount of recovered DNA (~8 times more). However, treatment with WPS before collection of DNA decreased the recovered yield when samples were collected by swabbing and increased the yield when mini-tape was used. Pre-treatment by CAN fuming resulted in significantly higher amounts of DNA than WPS treatment.
Conclusions: Full profiles could be recovered from touch DNA on the cables. CAN fuming significantly increased the amount of recovered DNA, possibly because the collection area was clear and thus limited to only the stained parts.

New optimized DNA extraction protocol for fingerprints deposited on a special self-adhesive security seal and other latent samples used for human identification
Evaluation and protocol improvements of a new kit (Fingerprint DNA Finder (FDF Kit)) for noninvasive extraction of DNA from fingerprints. The fingerprints in the study were placed on the special “Self-Adhesive Security Seal Sticker” or on metallic guns.

Effects of latent fingerprint development reagents on subsequent forensic DNA typing: A review
Most fingerprint enhancement methods can be used without interfering with DNA extraction. The DNA extraction efficiency can in some cases depend on the time between development and extraction.
The type of surface the fingerprint is placed on can be of importance.

Evaluation of methodology for the isolation and analysis of LCN-DNA before and after dactyloscopic enhancement of fingerprints
http://www.isfg.org/files/6834bde1ff72de292232127e2a49d99fa400b26e.05013919_810758811900.pdf
Setup: Cellotape, swab or gauze for recovery of DNA from fingerprints. Persistence of DNA on swabs used for fingerprint sampling.
Results: Swabbing fingerprints for DNA is more efficient (in quantity) than cellotape or gauze. Room temp storage (several weeks) of the swabs did not lead to significant degradation of DNA. The dactyloscopic enhancement of fingerprints was tested on one good shedder and two poor shedders, there was no conclusive data on whether the method influences DNA recovery.
Evaluation of collection and extraction methodologies of latent fingerprints for military application
A method for DNA collection and extraction from fingerprints on glass and metal. Swabbing with 0.9 % NaCl and lysis extraction was OK for both substrates.

Qualitative and quantitative assessment of single fingerprints in forensic DNA analysis
Setup: collection and analysis of DNA from 700 fingerprints. Comparison of different methods and kits.
Results: Regardless of method, most profiles were partial.
HighSens and Zygem extraction was better than the one-tube method. For one-tube method the Identifiler improved the results.
Some persons were found to be better shedders than others but a lot of skin flakes in the fingerprint was not an indication of a high quality profile.

Qualitative and quantitative analysis of DNA recovered from fingerprints
http://www.sciencedirect.com/science/article/pii/S0531513102005721
Setup: Fingerprints from 11 persons were applied on glass, metal and wood by either pressing for 30 s or by rolling friction.
Results: When hands were unwashed 63 % were mixed profiles while washed hands gave single profiles but also no profiles. Dactyloscopic powders did not seem to influence the DNA typing (no brush was used to apply the powder).

Nondestructive biological evidence collection with alternative swabs and adhesive lifters
Setup: blood and fingerprints were collected from glass, painted drywall, 100 % cotton and copy paper with different types of dry swabs and adhesive lifters.
Results: Adhesive tapes were better than dry swabs at collecting DNA from fingerprints on 100 % cotton and painted drywall. There was no statistically significant difference between the different methods regarding fingerprints on the other materials or regarding blood on any material. Moreover, the recovery of DNA was in general higher from fingerprints than from blood, indicating that wet swabbing is the better option regarding collection of blood. Interestingly, all methods resulted in 100 % profile coverage from fingerprints on glass and copy paper.

ESDA®-Lite collection of DNA from latent fingerprints on documents
Evaluation of the Electrostatic Detection Apparatus (ESDA), a non-destructive method to collect DNA from latent fingerprints.  
Results: Both ESDA and dry swabbing yielded more DNA than cutting. More full or partial profiles were produced by dry swabbing (93 %) than ESDA (65 %).  
Conclusion: Dry swabbing was better at the moment but ESDA is worth more evaluation since it is completely non-destructive.
Maximization of STR DNA typing success for touched objects
Evaluation of sample recovery, extraction, amplification and separation of DNA from fingerprints on different substrates (credit cards, keys and pens). Development of a “specialized swab” that produced at least 2.5 times more DNA than ordinary swabs. Single tube digestion with SDS followed by purification and concentration with Micron column was more efficient than more complicated extraction methods. Most of the samples contained less than 100 pg DNA. ~60 % of the samples contained less than 40 pg DNA. Suggests that samples containing at least 20 pg of DNA may generate database-acceptable DNA profiles. 10-20 pg DNA could be enough for direct comparison of profiles.

Experiments on the DNA contamination risk via latent fingerprint brushes
Setup 1: Analysis of 51 used fingerprint brushes.
Results 1: 86 % of the brushes gave full or partial profiles.
Setup 2: A selection of brushes and some artificially contaminated brushes to test secondary transfer
Results 2: “The larger the brushed area, the greater is the risk for contamination; single fingerprints are less affected.” “Contact of the brush with body fluids such as blood or saliva make secondary transfer highly likely.”

Trace DNA: An underutilized resource or Pandora’s box? A review of the use of trace DNA analysis in the investigation of volume crime
Since touch DNA cannot be derived to a specific source the activity level of the evidence might be problematic. Secondary transfer reviewing.
Discussion of the problems with LCN in court.

The effect of common fingerprint detection techniques on the DNA typing of fingerprints deposited on different surfaces
The recovery of DNA from a fingerprint was determined to be more dependent on the surface type than the method used to develop the fingerprint. Fingerprints on paper and aluminum foil did not give any DNA profiles. Fingerprints on plastic bags, glass slides and adhesive tape resulted in DNA profiles.

A further study to investigate the effect of fingerprint enhancement techniques on the DNA analysis of bloodstains
Setup: Bloodstains of varying ages on different surfaces were treated with different fingerprint-enhancement techniques and then DNA was recovered.
Results: “…magnetic powder, multimetal deposition (MMD) and ultraviolet (UV) radiation are not recommended for use in a sequence of analyses involving DNA typing.” “Strong white light, white and aluminum fingerprint powders, physical developer (PD) after 1,8-diazao-9-fluorenone (DFO), PD after ninhydrin with cadmium (Cd) salt treatment, and cyanoacrylate with gentian violet or Ardrox stains may be used successfully in a sequence of analyses involving DNA typing. Ninhydrin with secondary metal salt treatment, DFO, amido black, diaminobenzidine (DAB), black powder, Stickyside Powder, cyanoacrylate with rhodamine stain, and luminol may be used before DNA analysis but care must be taken to ensure that sufficient DNA is extracted and analyzed.”
Ninhydrin-dyed latent fingerprints as a DNA source in a murder case
“Preliminary tests prove that ninhydrin-dyed material still can be useful for STR typing”.

Archived or directly swabbed latent fingerprints as a DNA source for STR typing
“Magnetic powder, soot powder and scotch tape used for visualization and archiving fingerprints in Germany were tested for their PCR inhibitory characteristics.” “Partly it proved possible to type fingerprints taken directly from the surface as well as fingerprints removed from the surface with scotch tape.”

Recovery of DNA and fingerprints from touched documents
Comparison of two extraction methods: DNeasy plant mini kit (QIAGEN) worked better than QIAamp mini kit.
Fingerprint enhancement by DFO and/or Ninhydrin significantly decreased the amount of recovered DNA but did not seem to interfere with the PCR amplification.
DNA was better recovered from magazine and filter paper compared with office paper and white card.
LCN (32 cycles) increased the number of full profiles obtained but also increased the incidence of PCR artefacts.

The recovery of latent fingermarks and DNA using a silicone-based casting material
Setup: Recovery of fingerprints by Isomark (silicone based) and enhanced by cyanoacrylate fuming.
DNA was extracted by double swabbing from the casts. Surfaces were aluminium can, plastic bottle, coin and glass light bulb, cup and mobile phone cover.
Results: > 0.1 ng/µL DNA was recovered from 42 % of the samples and full DNA profiles were recovered from 82 % of these (34 % of all the samples). The recovery of fingerprints using Isomark was not very efficient for the cup and the mobile phone cover (semi-porous surfaces). The quality of the fingerprint was not important for the DNA recovery.

Simplified Low-Copy-Number DNA analysis by post-PCR purification
“In mock case type samples with dermal ridge fingerprints, genetic profiles were obtained by amplification with 28 cycles followed by post-PCR purification whereas no profiles were obtained without purification of the PCR product. Allele dropout, increased stutter, and sporadic contamination typical of LCN analysis were observed; however, no contamination was observed in negative amplification controls. Post-PCR purification of the PCR product can increase the sensitivity of capillary electrophoresis to such an extent that DNA profiles can be obtained from <100 pg of DNA using 28-cycle amplification.”

Fingerprints & cartridge cases: How often are fingerprints found on handled cartridge cases and can these fingerprints be successfully typed for DNA?
https://www.cacnews.org/
Setup: “Three types of fingerprints were placed on these cartridges: bloody fingerprints, eccrine/sweat prints and oily prints”. “The cartridge cases were made of brass, nickel-plated brass, and aluminum.” “The bloody fingerprints were developed with amido black while the eccrine and oily prints were processed by cyanoacrylate fuming followed by rhodamine 6G dye and illumination with laser light.”
After the cartridges/cases were processed for fingerprints, DNA was collected from the cartridges/cases with dampened swabs.”

**Results:** DNA profiles were obtained from 3 of 48 cartridges. All three were from bloody prints, two from fired cartridges and one from an unfired.

**Recovery of DNA from latent fingerprint tape lifts archived against matte acetate**  
Extracted DNA from fingerprints is problematic for several reasons. The amount of DNA is often very low and the brushes used to enhance the fingerprints are often contaminated with DNA from other crime scenes. Also, the collection of DNA will destroy the fingerprint for further comparison analysis. This study aimed to develop a technique to recover DNA from latent fingerprints caught between a tape and matte acetate. No experimental setup in this paper yielded enough DNA for a reliable DNA STR-profile and some even contained non-donor alleles.

**DNA typing of fingerprint treated biological stains**  
Fingerprint reagents did not interfere with subsequent DNA analysis. Reagents tested: carbon, cyanoacrylate and ninhydrin.

**Commentary on:** Wickenheiser RA. Trace DNA: a review, discussion of theory and application of the transfer of trace quantities of DNA through skin contact. J Forensic Sci 2002;47(3):442-50  
http://www.astm.org/DIGITAL_LIBRARY/JOURNALS/FORENSIC/PAGES/JFS2002315.htm  
Caution must be taken regarding contamination from fingerprint powder and brushes when extracting DNA from fingerprints.

**DNA profiles from fingermarks: A mock case study**  
Setup: Substrates: wooden knife handle, glass, masking tape, brass cartridge case, aluminum cartridge case, nickel cartridge case. Uncleaned substrates were handled for less than 15 s to create a fingerprint and left outdoors (a glass door was not left outdoors but kept in place indoors). Samples were recovered after 24 hours and 8 days. “Direct PCR” (without extraction) was used.  
**Results:** Glass had the highest rate of DNA recovery, masking tape was second best. Mixed DNA profiles were recovered but the donor profile was always the major one.

**The influence of swabbing solutions on DNA recovery from touch samples**  
Fingerprints were collected by swabs moistened with water or different detergents followed by a dry swab. All detergent based moisteners yielded higher amounts of DNA than water. SDS (2 % optimal conc.) and Triton X-100 (0.1 %) swabs yielded significantly more DNA than water. Yields from different individuals and different fingers differed significantly.

**DNA typing of fingerprints using capillary electrophoresis: Effect of dactyloscopic powders**  
Some dactyloscopic powders used for fingerprint enhancement can be used prior to DNA recovery but precautions should be taken to avoid contamination.
Beware of the possibility of fingerprinting techniques transferring DNA
http://www.crime-scene-investigator.net/fingerprintingtechniqscontamination.pdf
Fingerprint brushes can accumulate DNA from surfaces they come into contact with and might cause contamination. However, the proportion compared to the studied DNA should be low.

Are you collecting all the available DNA from touched objects?
http://www.isfg.org/files/31f9316afbc584bc0befd4454d6cd38c4f064f3a02004983_748131740352.pdf
Single swabbing might not be enough to retrieve all DNA. Extraction methods may not be as efficient as needed.
Fingerprint powder affected the DNA recovery by 25%. Moreover, the powder can inhibit the DNA amplification.

DNA fingerprints from fingerprints
http://www.nature.com/scitable/content/DNA-fingerprints-from-fingerprints-11782
DNA can easily be extracted from the palm of a hand with a water-moistened cotton cloth. Dry hands or recently washed hands yielded less DNA.
Swabbing of regularly used objects all provided user-matched profiles. Washed objects used for a limited time also produced user profiles.
Indications of secondary transfer were observed.
The strongest profile on an object handled by several users was not always from the last user.

Effect of 1,2-indanedione on PCR-STR typing of fingerprints deposited on thermal and carbonless paper
“Indanedione did not adversely affect the DNA profiles obtained from the treated fingerprints. Partial DNA profiles were obtained at all post-development time frames.”
Both Chelex and Qiamp were used and “Both extraction methods produced comparable profiles although more “drop-ins” were observed with the Qiamp method.”

Fingerprints and DNA: STR typing of DNA extracted from adhesive tape after processing for fingerprints
“In this work, various donors left fingerprints on the adhesive side of tapes. The tapes were then sequentially processed for fingerprints using an alternate light source, cyanoacrylate fuming, and staining with BY-40 and then crystal violet. DNA was subsequently successfully extracted, amplified and typed for six STR loci.”

3.11 Finger samples
Application of victims’ fingernails in forensic DNA analysis
http://medind.nic.in/jal/t10/i4/jalt10i4p289.pdf
DNA was extracted from fingernail clippings by mild digestion. The protocol was developed on fingernails from real cases. For example epithelial cells and blood can be found under the fingernails.
Fingernails are useful to study according to the authors.
Publish or perish – meanwhile, study what’s already out there
Bader S., The Barrister
Courts in the UK have a tendency to trust “experience” more than science.
“12 hours after digital penetration it was three times more likely to find that the major contributor to a profile was from the woman.” (On the offenders fingers.)

Analysis of DNA from fingernail samples in criminal cases
Setup: 164 fingernail samples from 71 cases, collection by extraction, clipping or swabbing.
Results: Extraction of DNA from fingernails showed only the donor profile while clipping and surface swabbing were equally efficient in recovering mixed profiles.
The victim profile was detected in ~75 % of the cases and in ~13 % of these cases a second contributor was detected.
In an expert testimony it should be clearly stated that foreign DNA can be found under the fingernails of volunteer donors as well (6-24 %).

The fingernails of Mary Sullivan: developing reliable methods for selectively isolating endogenous and exogenous DNA from evidence
Developed protocol to recover DNA from nails (on the nail/from the nail).

The prevalence of mixed DNA profiles in fingernail samples taken from individuals in the general population
Aim: “…examination of the incidence of mixed DNA profiles derived from fingernail samples from members of the general population…”
Setup: Fingernail swabs from 100 volunteers.
Results: Foreign DNA was detected in 13 % of the samples, only 6 % of these gave reportable mixed DNA profiles. “This study demonstrated a low level of foreign DNA under the fingernails of the general population, suggesting that when a strong mixed DNA profile is obtained from a fingernail swab it is unlikely that it exists only due to previous contact between the suspect and victim.” Mostly male donors had significant amounts of foreign DNA in their nail swabblings.

The prevalence of mixed DNA profiles on fingernail swabs
Setup: Fingernail scrapings from 40 laboratory staff and police (right and left hand = 80 samples).
Results: 30 % of the samples were low level mixtures of DNA profiles and 11 % were high level mixtures. 8 samples were 3-person mixtures where one of the profiles was unknown (not partner, children, colleague…). The sex of the test-subject was not significant regarding the occurrence of mixed DNA-profiles but the trend suggests that a high level mixed profile was more likely to be collected from the male test-subjects.
 “…intimate contact results in transfer of more cellular material than other types of contact.”

Genetic analysis of fingernail debris: application to forensic casework
Setup: 106 samples of fingernail material from 40 forensic cases. Mostly clippings but also debris scrapes, wood or plastic sticks and sterile swabs.
Results: Eight samples (6 cases) showed a mixture of victim and offender DNA.
The transfer and persistence of DNA under the fingernails following digital penetration of the vagina
Setup: eight volunteer couples in transfer study, four couples in persistence study.
Results: Full female profiles from all male samples collected after 0-6 hours after digital penetration and from ¾ of samples collected after 12 hours. For samples collected 18 hours after penetration the majority showed mixed profiles. “…hand washing had a significant effect on the persistence of the female DNA profiles”.

Collecting and analyzing DNA evidence from fingernails: A comparative study
http://www.forensic.msu.edu/documents/FSPub-
Collecting%20and%20Analyzing%20DNA%20Evidence%20from%20Fingernails%20A%20Comparative%20Study.pdf
Comparison of soaking, swabbing and scraping nails following scratching.
Soaking gave a lot of exogenous DNA, swabbing and scraping did not. All three methods recovered DNA from the nail itself. Soaking gave the most nail-DNA while scraping gave the least, but scraping also gave the most drop-out alleles in the exogenous profile. Should nails be processed individually or in group? Light scratching in the experiment gave enough DNA from one single finger nail for exogenous profiles. However, in some cases the profile was only partial, which could have been improved would the fingers have been cumulated.

IPV – Bridging the juridical gap between scratches and DNA detection under fingernails of cohabitating partners
(IPV = Intimate partner violence)
The respective partners DNA will be under the fingernails in 17 % of couples that live together.
Setup: Female vigorously scratched male back.
Results: There was a highly significant difference in absolute and relative male DNA under scratching and non-scratching fingers. The mean difference was 16-22-fold depending on finger. However, a negative result for male DNA did not mean the nail was not involved in the scratching.

DNA analysis of fingernail debris using different multiplex systems: a case report
Evaluation of different kits for the efficiency of recovering DNA from fingernail debris two days after the reported incident. “…we recommend that fingernail debris should be recovered in cases of violent crimes, even after a time lapse of some days between incident and examination.”

The prevalence of mixed DNA profiles in fingernail samples taken from couples who co-habit using autosomal and Y-STRs
Setup: Fingernail swabs from 12 cohabiting couples.
Results: Mixed profiles in 17 % of the samples. The majority on non-donor alleles belonged to the partner. Mixture ratios ranged from 20:1 to 1:2. Female swabs were analyzed with Y-filer and in 63 % of the samples gave full or partial Y-chromosome profiles even though no foreign profile was detected during regular analysis.

Prevalence and persistence of foreign DNA beneath fingernails
Setup: Casework data, general population and experimental scratching setup.
Results: 33 % of casework fingernail samples contained foreign DNA of which 63 % had 5 or more loci. 19 % of the general population fingernail samples contained foreign DNA of which 35 % had 5 or more loci. 33 % of fingernail samples after deliberate scratching contained DNA from the scratched person but only 7 % retained the donor DNA after 6 hours.

Conclusion: “…more than just casual contact is required for fingernails to acquire and retain foreign DNA and that it generally will not persist for long periods.

DNA transfer: Review and implications for casework.
Review on factors (shedder status, surfaces, time aspects…) and probabilities of transfer.

Evaluating the prevalence of DNA mixtures found in fingernail samples from victims and suspects in homicide cases
Claims that “The amount of biological material transferred under the fingernails during “casual” activities is not sufficient to genotype reportable mixtures.”.

After a homicide the majority (78 %) of the studied fingernail samples (Israeli retrospective study of 137 DNA-profiles) are single profiles from the donor. DNA mixtures were found mostly from the victims’ fingernail samples.

Sample collection by clipping and swabbing.
25 % of murder victim fingernail samples in the study contained foreign DNA.

DNA profiles from fingernails using direct PCR.
http://link.springer.com/article/10.1007%2Fs12024-014-9626-8

mm² nail clippings were subjected to PCR without any prior treatment using the NGM™ kit.
17 of 40 samples resulted in full profiles. Alleles from non-donor profiles were detected in 9/40 samples.

An evaluation of the relevance of routine DNA typing of fingernail clippings for forensic casework

Setup: aggressive scratching by and on volunteers without bloodshed but with skin flakes. Nails were cut.

Results: “In this study, the profile detected from each volunteer’s clippings was the same before and after scratching, and matched the profile of the corresponding volunteer (donor) as defined by typing each volunteer’s reference buccal swab.” Nail clippings should according to the authors not be sent routinely for forensic analysis since the chances of useful results are small.

Trace DNA analysis: If your DNA is on the evidence, did you really touch it?
Ryan SR., Posted on LinkedIn 2014; June 26.
https://www.linkedin.com/pulse/20140626163650-13967252-trace-dna-analysis-if-your-dna-is-on-the-

evidence-did-you-really-touch-it

“…simply touching an object can leave anywhere from 0-169ng of DNA” (Meakin and Jamieson 2013).
“14 of 24 individuals tested, non-self DNA was found on their necks” (Graham and Rutty 2008).
41% of fingernail samples tested showed some evidence of a mixed DNA profile” (Dowlman et al. 2010).
“Touch DNA is known to last for up to 2 weeks outside and 6 weeks or longer inside” (Raymond et al. 2009).
DNA typing of debris from fingernails
http://link.springer.com/article/10.1007%2FBF01225045#page-1
Scratching experiments with and without prior cleaning of the nails.
Setup: 4 series, 121 samples. Sample collection made with a small pair of scissors.
Results: When the scraping of the nail was done very carefully: “In 71% of these cases DNA patterns of the person who had been scratched or mixed DNA patterns of both persons could be detected.”

3.12  LCN/LT

Fingerprints as evidence for genetic profile: morphological study on fingerprints and analysis of exogenous and individual factors affecting DNA typing
http://www.crim-scene-investigator.net/fingerprintDNAextraction.pdf
Setup: 347 fingerprints, 11 persons, glass/wood/metal, 30s, with/without handwashing.
Results: Recovery 0.04-0.2 ng.
Handwashing reduced the amount of recovered DNA. “Shedder status” of donor is very important regarding how much DNA can be recovered from a fingerprint.
“… LCN method enhances the amplification of spurious alleles present in fingerprints, but laboratory-based contamination cannot be excluded, since standard negative controls of amplification cannot reveal lower levels of contamination.”
“When fingerprints were made after subjects had touched objects of routine use, including computer keyboards used by many people, mixed alleles from multiple donors were observed. In many cases, the peaks of accessory alleles reached those of true alleles, hindering definite allele typing.”

Specific quantification of human genomes from Low Copy Number DNA samples in forensic and ancient DNA studies
Sometimes the quantification is not accurate enough rendering false negative results. Negative results can also arise from high concentrations of inhibitor(s).
Interpret the results with care in regard to adventitious DNA transfer. Independent replication from a second DNA extract could be useful.
Mix of high and low copy number profiles leads to complicated analysis. RT-PCR can target the number of X and Y chromosome copies and lead to accurate detection of each contribution (sexual assault cases).

Low copy number: Interpretation of evidence results
Laboratory contamination can be a problem when the number of amplification cycles is high.

Validation of a dual cycle ethylene oxide treatment technique to remove DNA from consumables used in forensic laboratories
Ethylene oxide is an organic gas that can be used for sterilization. The EO-technique was optimized for removal of DNA from disposable plastic consumables.
“…DNA from up to 50 µl of blood and saliva can be removed to a level where the consumable can be considered DNA free. DNA from semen was more resilient…”
Reducing contamination in forensic science
Balk C., Justice Studies and Forensic Science: 3, Article 12 (student essay).
http://scholarworks.sjsu.edu/themis/vol3/iss1/12
200 pg DNA/33 cells is enough for analysis.
LCN: risk of drop-in, drop-out alleles.
Sterilization: UV-, gamma-, electron beam radiation and ethylene oxide.
UV was found to be useless, gamma and EB were OK for small amounts of DNA, Ethylene oxide (ethylene glycol) was best.
PCR-related contaminants: After a selected fragment of DNA is amplified, it will have dUTPs in it; something unamplified DNA does not possess. UNGcoupled PCR is an effective technique to reduce risk of contamination during the amplification process, and should be utilized as a preventative measure whenever possible in the forensic laboratory.
Sources of contamination: Manufacturer contamination, DNA transfer at crime scene, Evidence packaging and transport, Exhibit examination (forceps, scissors, gloves, speaking without facemask).

LCN DNA typing from touched objects
http://www.isfg.org/files/91a748919b016087a260b2ab392c8e8f79a21c0f.05014020_311586137609.pdf
Marking pen and comb swabbing (1x PBS) --> less than 100 pg DNA --> full DNA profile with LCN.

Evaluation of samples comprising minute amounts of DNA
Setup: Single donor and two- and three- person samples, 3-12 pg DNA/contributor. Standard and enhanced CE-settings.
Conclusion: “…an assessment of the criminalistic relevance of a sample carrying minute amounts of DNA is essential prior to applying enhanced interrogation techniques and/or calculating a weight of evidence statistic.”

Assessing the risk of secondary transfer via fingerprint brush contamination using enhanced sensitivity DNA analysis methods
“Detection of secondary transfer of DNA can occur through fingerprint brush contamination and is enhanced using LCN-DNA methods.”
“If a brush were to add DNA-containing material to a surface containing a handprint, the proportion of the added DNA is likely to be less than that retrieved from the depositor of the print. Thus, the minor component of the mixture derived from the brush may not be detectable.” (ref 2 in paper; Proff et al. 2006).
“The dusting of latent prints may dislodge cellular debris from the latent print or substrate. That debris then adheres to the brush. This brush is then used on another item of evidence, or at another crime scene, where it is subject to the same mechanical maneuvering and where it can dislodge cellular debris, leaving traces of biological evidence not pertinent to the evidence being handled. Under LCN conditions, it may be possible to obtain DNA results that are not relevant to the case due to a secondary transfer by fingerprint brush contamination” (ref 16 in paper; Pesari et al. 2003).
“In this study, the risk of false associations through the inclusion of contaminant DNA donors was moderate”

Validity of low copy number typing and applications to forensic science
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2702736/
“LCN typing simply can be defined as the analysis of any DNA sample where the results are below the stochastic threshold for reliable interpretation.” “Some touch DNA samples do not qualify as LCN samples in that they contain sufficient DNA for routine conventional analyses. Conversely, many crime scene samples do meet the criteria of LCN samples. Such samples should be clearly distinguished and analyzed and interpreted accordingly.” If LCN samples are not handled correctly they can be useless in...
trial. Difficult (not possible) to use LCN for exculpatory purposes. LCN can basically only be used to identify human remains/missing persons according to the authors.

**Low Copy Number - consideration and caution**

Stochastic amplification may occur --> imbalance of two alleles at a given heterozygous locus, or allelic dropout/dropin.
A stutter allele can have larger height/area that the true allele in LCN.
Contaminations can occur even though the negative control shows no contamination.
Considerations with LCN:
Difficult to exclude an individual after LCN.
Drop-in, contamination
Causal contact between suspect and victim must be considered.
Heterozygous peak imbalance.
Increased stutter.
Difficult to separate contributions in a mixture.

**Analysis of cellular and extracellular DNA in fingerprints**
[https://e-reports-ext.llnl.gov/pdf/781140.pdf](https://e-reports-ext.llnl.gov/pdf/781140.pdf)

Cellular DNA – from skin cells.
Extracellular DNA – from body fluids (s.a. sweat)
**Setup:** Glass slides and plastic pens were wet swabbed (TE buffer).
**Preparation methods:** centrifugation, acoustofluidics.
**Analysis method:** qPCR, 45 cycles.
**Results:** Extracellular fractions contain more DNA than cellular fractions. Some people are better “shedders” than others.
Some contaminations through carryover in blank runs.

**A review of the science of low template DNA analysis**

Budlowe (2009) is critical to the conclusions of this review: “The reviewers found that LCN typing as practiced specifically in the United Kingdom was “robust” and “fit for purpose” but offered a number of recommendations to improve the methodology. The findings of the commission seem inconsistent with the nature of LCN typing and LCN typing warrants a more in-depth evaluation by the greater scientific community.”

This review address concerns about Low template DNA analyses (LTDNA) for legal purposes, instigated by the Forensic Science Regulator and contains guidelines, recommendations and validation for use of LTDNA.
Transfer of cellular material:
Individuals vary in their propensity to transfer their cellular material to an object.
Time and pressure affects the efficiency of the transfer.
(Person--> object = primary transfer. To next object/person = secondary transfer.)

**LMD as a forensic tool in a sexual assault casework: LCN DNA typing to identify the responsible**

Optimization of a technique to use LMD for analysis of a mixed semen and blood sample from a car seat.
Science in court: DNA’s identity crisis
Discussion about the reliability of LCN (by increased number of cycles) in forensic investigations.

Analysis and implications of the miscarriages of justice of Amanda Knox and Raffaele Sollecito
LCN-levels of victim-DNA (no detection of blood) on a knife, analyzed without replication and with only a few alleles, were used to convict a suspect. Moreover, the location of the suspect-DNA on the handle was interpreted (without scientific grounds) as a result from stabbing rather than cutting food. The suspect and the victim shared an apartment. Evidence was collected using dirty gloves and in non-sterile containers. Mixed profiles of the two roommates were interpreted as one´s involvement in the murder of the other but was most probably just normal background DNA from two people living together.
“Selective cleaning” was believed to have erased the DNA from two suspects but not the third at the murder-scene.
Negative controls during the DNA analysis was interpreted as that the evidence could not have been contaminated in any way, either before the murder, at collection of the evidence or during handling of the evidence by the police officers.

Low Copy Number DNA: Reality vs Jury Expectations
Hoffman Wulff P., Silent Witness Newsletter 2006; 10(3)
Discussion about the limitations and the future of LCN.

Application of Low Copy Number STR Typing to the identification of aged, degraded skeletal remains
Identification of very old remains (80 years).

Evaluation of methodology for Low Copy Number (LCN) DNA analysis - on spent cartridge cases - after enhancement of latent fingerprints
Johansson C., Linköpings Universitet, magisteruppsats, LiU-IFM-Ex-08/1892-SE.
“The report includes a discussion on the definition of LCN, different applications, risks and limitations, optimizations of the procedure and how to deal with the interpretation. Finally there are some words on LCN in perspective of the justice system.”

Assessment of DNA transfer involving routine human behavior
Kelley, S., Fort Worth, Tx: University of North Texas Health Science Center; thesis 2010.
http://digitalcommons.hsc.unt.edu/cgi/viewcontent.cgi?article=1098&context=theses
A study on secondary transfer in a setup that mimics normal day behaviour regarding saliva and sweat. Setup: Volunteers were instructed to e.g. lick their thumb (as when turning a page) and after 5-30 minutes shake hands with a person B who would then grip a plastic tube, samples were collected from the tube. Experiments involving pen-licking and simulated sweat were also included in different combinations.
Results: 28 amplification cycles on 208 samples resulted in 10 samples with one allele or more. Five of these 10 were setup as follows: person A bites a pen for 2 minutes, after 30 minutes the the palm of person B is sprayed with water to stimulate sweat, then person B holds the pen and after different time intervals person B grips a plastic tube which is swabbed for analysis. 12 samples were reanalysed with 34 amplification cycles and the profiles were interpreted, all but one of the profiles were partial. The profiles were dominantly from person A and in two samples only from person A.
Evaluation of methodology for the isolation and analysis of LCN-DNA before and after dactyloscopic enhancement of fingerprints
http://www.isfg.org/files/6834bde1f72de292232127e2a49d99fa490b26e.05013919_810758811900.pdf
Setup: Cellotape, swab or gauze for recovery of DNA from fingerprints. Persistence of DNA on swabs used for fingerprint sampling.
Results: Swabbing fingerprints for DNA is more efficient (in quantity) than cellotape or gauze. Room temp storage (several weeks) of the swabs did not lead to significant degradation of DNA. The dactyloscopic enhancement of fingerprints was tested on one good shedder and two poor shedders, there was no conclusive data on whether the method influences DNA recovery.

Use of low copy number DNA in forensic inference
http://www.isfg.org/files/31f9316afbc584bc0be6d4454d6cd38c4f6e4f3a.02004843_693490260903.pdf
Discussion about shedder status and the likelihood of getting DNA profiles from Touch DNA and used garments. E.g. a good shedder can be the major profile on a garment worn by a poor shedder. Also, a good shedder can be the major profile after a secondary transfer event with a poor shedder.

The recovery and analysis of DNA from fired cartridge casings
No association found between DNA yield and profile quality. Cumulative swabbing of several casings resulted in higher quantities of DNA but not better profile quality. However, the author suggests that cumulative swabbing is the best approach. Introduction and discussion can be read as a review.

DNA analysis and document examination: the impact of each technique in respective analyses
DNA can transfer from the hand and arm when a letter is handwritten. Areas of a document subjected to most touch were fold lines, center top face and middle face. DNA could be successfully recovered from a document by dry/dry-swabbing only, a method that allows other types of document analyses to be performed on the document afterwards. Most fold lines contained detectable DNA probably due to the applied pressure when folding.

Forensic identification of a murderer by LCN DNA collected from the inside of the victim’s car
http://www.isfg.org/files/1a528d0fcee17e821a369c61d28d759fbaed8d30.03018557_129265714647.pdf
They collected samples with FTA-paper from steering wheel, gear lever and handbrake from a car and successfully matched the profiles from a suspect’s profile. Quantity of DNA from the extractions were less than 100 pg, 16 markers were analyzed.
“…it is possible to type even low copy number (LCN) DNA if a proper and immediate collection of biological evidence is performed.”

Maximization of STR DNA typing success for touched objects
Evaluation of sample recovery, extraction, amplification and separation of DNA from fingerprints on different substrates (credit cards, keys and pens). Development of a “specialized swab” that produced at least 2.5 times more DNA than ordinary swabs. Single tube digestion with SDS followed by purification and concentration with Micron column was more efficient than more complicated extraction methods. Most of the samples contained less than 100 pg DNA. ~60 % of the samples
contained less than 40 pg DNA. Suggests that samples containing at least 20 pg of DNA may generate database-acceptable DNA profiles. 10-20 pg DNA could be enough for direct comparison of profiles.

**Trace DNA: An underutilized resource or Pandora’s box? A review of the use of trace DNA analysis in the investigation of volume crime**
Since touch DNA cannot be derived to a specific source the activity level of the evidence might be problematic.
Secondary transfer reviewing.
Discussion of the problems with LCN in court.

**Regina v. Peter Weller (court trial on “digital penetration”)**
England and Wales Court of Appeal (Criminal Division) Decisions, EWCA Crim 1085, 4 March 2010.
http://www.forensicdna.com/assets/weller_decision.pdf
How did the DNA from the girl end up on the man’s fingers? Different scenarios. Expert witness drawing poorly based conclusions? The clash between science and experience in court.

**The forensic science regulator: response to professor Brian Caddy’s review of the science of low template DNA analysis**
Proposals on how to follow recommendations from Caddy’s review in the UK.

**Maximising DNA profiling success form sub-optimal quantities of DNA: A staged approach.**
“….a flexible, staged approach using 28 or 30 thermocycle PCR in combination with the enhancement techniques described here (the amount of PCR product injected during capillary electrophoresis was increased) is proposed for processing samples with sub-optimal quantities of DNA.”

**A novel approach for genotyping of LCN-DNA recovered from highly degraded samples**
http://www.isfg.org/files/91a748919b016087a260b2ab392c8c8f79a21c0f.05017577_537759952848.pdf
“A new kind of STRs profiling system is based on the amplification of shorter fragments compared to the conventional STR multiplexes; because of their length, these MiniQ STR amplicons could be obtained even from extremely degraded DNA and/or from few copies of template DNA.”…….
“MiniSTRs turned out to be more robust and sensitive markers than the traditional ones.”

**Yield of male contact DNA evidence in an assault simulation model**
Grab setup: Man with washed hands grabbed the washed wrist and washed upper arm of a female for 5 seconds (females did not resist or struggle). Wrists were double swabbed within 30 minutes.
Struggle setup: Man with washed hands grabbed the washed wrist and washed upper arm of a female for 10 seconds while she was struggling.

Results: “The maximum concentration of total DNA extracted was 40 pg/*L in “grab” situations and 90 pg/*L in “struggle” situations, whereas estimates of male DNA from the female’s skin were up to 20 pg/*L in the “grab” situations and 40 pg/*L in the “struggle” Situations”. “… the difference in DNA transferred during grab and struggle situations were not statistically significant in this study.” “Our results showed that no full or usable profiles were attainable with the AmpFiSTR® Identifiler® PCR Amplification kit. Even the Yfiler™, which was designed to detect trace male DNA in a predominantly female background and has been shown to generate complete profiles with less than 60 pg of DNA, did not yield usable profiles.”
The hand on the shoulder. A case report where low copy number (LCN) DNA analysis was vital for solving a robbery
DNA from suspect was successfully recovered from victims clothing using adhesive tape even though the time of physical contact was short.

Simplified Low-Copy-Number DNA analysis by post-PCR purification
“In mock case type samples with dermal ridge fingerprints, genetic profiles were obtained by amplification with 28 cycles followed by post-PCR purification whereas no profiles were obtained without purification of the PCR product. Allele dropout, increased stutter, and sporadic contamination typical of LCN analysis were observed; however, no contamination was observed in negative amplification controls. Post-PCR purification of the PCR product can increase the sensitivity of capillary electrophoresis to such an extent that DNA profiles can be obtained from <100 pg of DNA using 28-cycle amplification.”

Reliable genotyping of samples with very low DNA quantities using PCR
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC146079/
An experimental procedure (including a mathematical model) for PCR of very low amounts of template DNA, taking into account drop-ins and drop-outs.
At least 35 pg mammalian DNA is needed to get 99% confidence in typing with the procedures in the paper.

The application of ultraviolet irradiation to exogenous sources of DNA in plasticware and water for the amplification of low copy number DNA
An optimization of UV-irradiation (with Stratalinker 2400) for cleaning plastics and water used in LCN-work.
Lining the area around the samples with aluminum foil decreased the irradiation time needed. An inch raise in the UV source improved the efficiency of the irradiation for PCR-plates.
Precise guideline presented in paper.

Forensic trace DNA: a review
http://investigativegenetics.biomedcentral.com/articles/10.1186/2041-2223-1-14
History and discussion on STRs and PCR.
Touch/trace DNA should not be confused with LCN.
Touch DNA = collection of minute biological samples. Touched objects does not always result in low amounts of DNA.
LCN = often used to describe increased cycle numbers.
Trace DNA is herein defined as “any sample which may fall below the recommended thresholds at any stage of the process - detection, collection, extraction, amplification and interpretation.” Usually less than 100 pg DNA.
A sample defined as trace DNA in the recovery phase does not necessarily have to be trace DNA in later stages of the process.
The precise location of where to recover the trace DNA is very important.
Double swabbing (not necessarily wet + dry, can be wet+wet if all the moisture is recaptured by the first swab) is recommended for collection of trace DNA.
If the swab is allowed to dry before extraction less DNA will be extracted, if the dried swab is frozen before extraction the DNA recovery will be similar to if it would still have been wet.
LMD can be useful to differentiate relevant cells from other cell types which allow efficient DNA analysis from the minor cell type in a sample. Flow cytometry can be used to separate sperm cells from vaginal wash fluid. Chelex and organic extraction can lead to up to 75 % loss of DNA. However, this loss is often not relevant. Partial or complete profiles have been obtained from samples that did not pass the quantification limit. Trace DNA samples should not always be discarded due to low quantification.

Reviews amplification methods for LCN samples, detection of the amplified product and interpretation. Common problems with trace DNA amplification: 1) allele drop-out, 2) decreased heterozygote balance, 3) allele dop-in (stutter), 4) allele drop-in (contamination).

In analysis it can be useful with detection thresholds such as LOD (limit of detection), T (low template DNA threshold), MIT (match interpretation threshold) and LOQ (limit of quantification). However, the peak intensity should be included in the exclusion calculation and continuous measures should be used instead of definite thresholds.

Replicates should be employed for trace DNA analysis, four replicates with reported alleles detected in at least two of the replicates seems to be the most accurate. Bayesian based likelihood ratios of drop-outs can also be employed. Mixed profiles can be challenging due to e.g. biased drop-outs, complicating drop-ins and over-amplification of some alleles. Contamination of trace-DNA can be a serious problem. The contamination can be the major profile in the sample. Contamination can occur before the crime is committed (background DNA), between the crime is committed and the crime scene is secured, during the crime scene investigation and in the forensic laboratory.

A comparison of the characteristics of profiles produced with the AMPFISTR® SGM Plus™ multiplex system for both standard and low copy number (LCN) STR DNA analysis

Interpretation guide for LCN profiles under AMPFISTR® SGM Plus® conditions (28 cycles, 1 ng DNA compared with 34 cycles, 12 and 25 pg DNA).

Effect of 1,2-indanedione on PCR-STR typing of fingerprints deposited on thermal and carbonless paper

“Indanedione did not adversely affect the DNA profiles obtained from the treated fingerprints. Partial DNA profiles were obtained at all post-development time frames.”
Both Chelex and Qiamp were used and “Both extraction methods produced comparable profiles although more “drop-ins” were observed with the Qiamp method.”

3.13 Penile swabs

Oral intercourse or secondary transfer? A Bayesian approach of salivary amylase and foreign DNA findings

Oral intercourse leads to salivary-α-amylase in penile swabs. However, salivary-α-amylase in penile swabs does not necessarily mean oral intercourse but may be due to secondary transfer.

Setup: This is a study of background salivary-α-amylase in underwear after normal (12 hour) wear (69 male subjects). Masturbation with saliva as lubricant was allowed but oral intercourse was not.

Test method: Phadebas press test for screening and RSID-Saliva for confirmation.

Results: 44 % of the underwear had stains containing amylase on the inside front, 13 % of these stains were found to be salivary-α-amylase (5.7 % of total). 50 % of these produced a mixed DNA profile with a minimum of two donors.
A positive control group with oral intercourse showed larger salivary-α-amylase positive stains in the male underwear than the background-test group. 80% of the underwear in this group gave DNA profiles other than the wearers.

“...the evidence of salivary-α-amylase on male underwear (RSID™-Saliva positive) is 17 times more likely to occur with the allegation of fellatio than the alternative hypothesis of secondary transfer. However the finding of no salivary-α-amylase (RSID™-Saliva negative) upon the male underwear is a 100 times more likely if fellatio did not occur rather than if somebody performed fellatio on Mr B. Likewise, it is evident that if salivary-α-amylase and DNA other than the wearer is present on the inside-front of male underpants that it is 27 times more likely if fellatio occurred rather than fellatio did not occur. In saying that, if salivary-α-amylase is found yet the DNA profile does not contain a profile other than that of the wearer, it is 13 times more likely if fellatio occurred rather than if nobody performed fellatio on an alleged victim”

This paper also contains a section about the sensitivity and specificity of the Phadebas press test and RSID-Saliva; Phadebas press test cannot distinguish between salivary-α-amylase and pancreatic amylase which leads to false positive results, therefore it should only be used for screening.

DNA transfer through nonintimate social contact.

A study on how much female DNA can transfer to male underwear and to the penis through nonintimate social contact.

Setup: After staged nonintimate contact with females (massaging the female face for 2 min and rubbing hands for 3 min) and simulated urination, penile swabs and underwear were collected either 5 min or 6 hrs after the experiment. Samples from the underwear were collected by mini-taping five different areas (waistband, front panels (in- and outside) and back (in- and outside)). Penile swabs and underwear were also collected after unprotected sexual intercourse (without ejaculation). The underwear used was new and worn for 5 min after the intercourse before collected.

Results: After nonintimate contact and sample collection after 5 min matching female (partial) profiles were detected in 5/30 waistbands and 1/30 outside panels (1 matching allele, 56 rfu). The maximum peak height on the waistbands ranged from 180-816 rfu. 4/30 penile swabs resulted in female DNA with a maximum of 5 alleles and 166 rfu. When samples and underwear was collected after 6 hrs female DNA was detected in 1/14 waistbands (maximum peak height 161 rfu) and in no penile swabs. When unprotected sexual intercourse preceded the sample collection female profiles were found in all samples. Maximum peak height ranges were: waistband (all full profiles) 1386-1357 rfu, inside front (all full profiles) 1898-3157 rfu, penile shaft (all full profiles) 958-5835 rfu. Full profiles were also detected on all samples from outside front as well as inside and outside the back of the underwear.

Conclusion: The authors claim that “if DNA matching the female complainant is found on the waistband of a male suspect’s underwear, the data obtained in this study suggest that depending on the time delay before the underpants are seized, a matching female DNA profile below 1000 rfu might be explained by nonintimate social contact with secondary transfer of female DNA from the male’s hands.”

3.14 Persistence

PCR DNA typing of washed stains
http://link.springer.com/chapter/10.1007%2F978-3-642-78782-9_90#page-1

Large amounts of semen, blood and saliva could be recovered after washing.
The yield was inversely proportional to washing temperature (several washes at 95°C did not yield any DNA).
Addition of detergent substantially reduced the amount of recovered DNA.
Cotton and denim yielded the highest amounts of DNA.
E.g. a 10 •L semen stain on cotton could easily be extracted after 95°C wash with detergent.

73 (119)
A study on the effects of immersion in river water and seawater on blood, saliva, and sperm placed on objects mimicking crime scene exhibits

Study of the kinetics of DNA degradation of materials immersed in water for 6 h to 3 months.
Background: Recovery was possible after 3 months on e.g. condoms, cigarette butts and chewing gum.
Bones and teeth were ok for 1mth to 50 yrs in humidity and soil. Skeletons in water were ok for 3 yrs.
Ribs, skin, nails were ok for two months. Soft tissue --> rapid degradation. Bitemark on body yielded DNA after 5.5 hrs submerged in water. Spermatozoa could be found after full laundry (references in paper).

Results: Sperm and saliva was quite resistant to degradation in water, blood was more quickly degraded. Fibre-based (porous) substrates protected against degradation. Smooth surfaces did not preserve DNA efficiently. DNA in chewing gum was protective against degradation. No significant differences regarding dry/wet stains at the point of immersion in water. Saltwater --> much faster degradation.

Persistence of DNA from laundered semen stains: Implications for child sex trafficking cases

Setup: semen stains (one or two (1:1) donors) were placed on clothes (T-shirt, trousers, tights) and stored in a wardrobe for 8 months. Items were washed (together with unstained socks) at 30°C or 60°C and with non-biological or biological detergent.
Results: High quantities of DNA (6-18 µg) were recovered irrespective of washing conditions. The quantity did not decline significantly with repeated washes. T-shirt was better than trousers when there was more than one donor (trousers --> one major DNA profile). DNA could be recovered from the unstained socks washed together with the stained clothes.

Probability of detection of DNA deposited by habitual wearer and/or the second individual who touched the garment

Setup: 63 males wore their own underpants for 12 h, the inside waistband was then touched by one of 11 females for 15 s. The waistband was mini-taped and subjected to DNA profiling.
Results: The wearer was detected in 51 % of the samples and always as the major contributor. The test-female was detected in 11 % of the samples. Reportable background DNA (non-wearer, non-test-female) was found in 14 % of the samples. There was no statistical difference in DNA quantification results depending on the time of collection (0 or 12 weeks after wearing and touching).
If a single DNA profile is found on e.g. a pair of underwear, it is 5 times more likely that the person has worn the garment than just touched it. A high total DNA concentration would support wearing the garment.

The effect of laundering on the detection of acid phosphatase and spermatozoa on cotton T-shirts
http://www.tandfonline.com/doi/abs/10.1080/00085030.2000.10757498#.VeU7APntkXs

Setup: 12 cotton T-shirts were stained with 500 µL seminal fluid from one donor. The T-shirts were taken to different dry-cleaners for “normal treatment” or washed in a domestic washing machine at 18°C or 30°C with normal amount of detergent, two T-shirts were treated with “spot cleaner” before washing.
Results: Results from dry-cleaning: seminal fluid was detected after dry-cleaning if no spot-cleaner was used. Results from washing machine: Only 18°C without detergent gave positive result with the AP-
test (acid phosphatase), however, even if the CP-test was negative there was still spermatozoa and STR DNA profiles could be identified after all different washing procedures.

**Spermatozoa recovered on laundered clothing**

**Setup:** Cotton briefs treated with semen were washed by different programmes. 40°C or 60°C washes with detergent or 60°C with detergent and softener.

**Results:** None of the samples were positive for acid phosphatase (AP) and all 60°C samples were negative for prostate specific antigen (PSA). Spermatozoa was detected in all 40°C washed samples and in ~50 % of the 60°C washed samples (could depend on longer storage before analysis). DNA was recovered from all the randomly selected samples, detection of twice the amount of DNA after 40°C wash compared to 60°C wash.

**The transfer and persistence of DNA under the fingernails following digital penetration of the vagina**

**Setup:** Eight volunteer couples in transfer study, four couples in persistence study.

**Results:** Full female profiles from all male samples collected after 0-6 hours after digital penetration and from ¾ of samples collected after 12 hours. For samples collected 18 hours after penetration the majority showed mixed profiles. “… hand washing had a significant effect on the persistence of the female DNA profiles”.

**Biological and DNA evidence in 1000 sexual assault cases**

**Setup:** Analysis of what proportion of sexual assault cases (1000 cases) provided interpretable DNA profiles from bodily samples.

**Results:** Teenagers and adults: 46-56 % success rate, children: 11-14 % success rate. Vaginal swabs: 3 days persistence (maximum 100 hours). For all other bodily samples (anal swabs, skin swabs) the persistence is shorter (24 hours). The persistence of an oral wash is maximum 15 hours. The Acid phosphatase (AP) semen detection test is not a good predictor for whether DNA evidence will be found or not in the sperm fraction.

**DNA transfer within forensic exhibit packaging: potential for DNA loss and relocation.**

Analysis of DNA persistence and transfer during packaging in forensic investigations.
“DNA can be transferred from the deposit area to other parts of the item or to the bag (package) itself and usually to both”. “The effect of bag size on transfer was limited but loose bags can, in certain situations, permit more transfer”.
“DNA was lost to the inside of the container holding bloodied knives”, also a lot of re-distribution of DNA on the knives. Tighter fitting of the container prevented re-distribution of DNA from the tip to the handle.
Cigarette butts should always be packed separately.
Less re-distribution of DNA on gloves when they were packed in paper compared with plastic.

**Semen detection: a retrospective overview from 2010**

46 % of the studied cases had microscopically verified spermatozoa even though the acid phosphatase test (AP) was negative. Vulva samples seem to produce more negative AP reactions despite the presence of spermatozoa. AP-test, microscopy verification and DNA analysis correlate the best for textiles.
Timing is of importance for the AP-test and could be used as a determinant for time. If AP is positive and spermatozoa is verified it is likely that the assault took place within 24 hours before sampling.

Application of Low Copy Number STR Typing to the identification of aged, degraded skeletal remains
Identification of very old remains (80 years).

The Persistence of seminal constituents on panties after laundering. Significance to investigations of sexual assault
Test-methods: 1) Blue Test for acid phosphatase, 2) microscopic identification of spermatozoa and 3) PSA ANAcard Test for detection of prostate specific antigen (PSA).
Results: Laundered clothing with semen stains could still produce DNA profiles, the efficiency depended on the type of fabric (cotton retained spermatozoa/DNA better than nylon). Negative AP-test was not reliable.

Effect of water immersion on seminal stains on cloth
Intact spermatozoa could be detected on cotton cloth after 120 hours immersion in water. However, the acid phosphatase activity was decreased and the spermatozoa were largely tail-less. At 144 hours (six days) the AP activity was still detectable but the number of intact sperm heads was decreased.

Everything clean? Transfer of DNA traces between textiles in the washtub
Transfer of DNA from worn clothing (without bloodstains) to another garment is highly unlikely both during hand- and machine washing. Blood can easily be transferred to other garments during the washing procedures.

The retention and transfer of spermatozoa in clothing by machine washing
http://www.nlada.org/DMS/Documents/1031178399.08/Laundry-sperm%20transfer.pdf
Washing: 10 minute warm wash, cold rinse and phosphate-free detergent of semen stained clothes rendered negative AP-tests but significant numbers of spermatozoa were retained. Small numbers of spermatozoa were transferred to previously unstained clothes in the washing-procedure.

The recovery and persistence of salivary DNA on human skin
Setup: Saliva from men was deposited on female skin and left there for 96 hours.
Results: Full male profiles were obtained after 96 hours in 8 of 9 cases. Tapelifting was the most efficient method to recover the DNA (compared to wet and dry swabbing). Dried salivary DNA on skin transferred to fabric, cotton was most efficient and leather least efficient for DNA transfer.
Evaluation of methodology for the isolation and analysis of LCN-DNA before and after dactyloscopic enhancement of fingerprints
http://www.isfg.org/files/6834bde1ff72de29232127e2a49d99fa490b26e_05013919_810758811900.pdf
Setup: Cellotape, swab or gauze for recovery of DNA from fingerprints. Persistence of DNA on swabs used for fingerprint sampling.
Results: Swabbing fingerprints for DNA is more efficient (in quantity) than cellotape or gauze. Room temp storage (several weeks) of the swabs did not lead to significant degradation of DNA. The dactyloscopic enhancement of fingerprints was tested on one good shedder and two poor shedders, there was no conclusive data on whether the method influences DNA recovery.

Using hydrophilic adhesive tape for collection of fingerprints for forensic DNA analysis
When collecting human samples (blood, saliva and semen) there is a risk of DNA degradation due to the moisture. A hydrophilic adhesive tape (HAT) was examined for non-invasive collection (taping of ankle, arm, behind ear, behind fingers and back of the neck) of human cells for DNA analysis. Authors claim it is a good method and the samples were stable for a month. Ears resulted in the highest success-rate.

Prevalence and persistence of foreign DNA beneath fingernails
Setup: Casework data, general population and experimental scratching setup.
Results: 33 % of casework fingernail samples contained foreign DNA of which 63 % had 5 or more loci. 19 % of the general population fingernail samples contained foreign DNA of which 35 % had 5 or more loci. 33 % of fingernail samples after deliberate scratching contained DNA from the scratched person but only 7 % retained the donor DNA after 6 hours.
Conclusion: “… more than just casual contact is required for fingernails to acquire and retain foreign DNA and that it generally will not persist for long periods.

The deposition and persistence of indirectly-transferred DNA on regularly-used knives
Setup: "Volunteers each used a set of knives regularly over a period of two days, after which, each of these ‘regular users’ shook hands with another person (‘handshaker’) and then immediately, without touching anything else, repeatedly stabbed one of their own regularly-used knives into foam for 60 s. DNA was recovered from the knife handles using mini-tapes approximately one hour, one day, and one week after the stabbings."
Results: Secondary transfer occurred in three out of four experiments. User: Hand shaker 10:1. Alleles from the handshaker could be found after a week but numbers and peak heights were reduced.

Stability of acid phosphatase activity and spermatozoa in semen stains washed with water
http://ci.nii.ac.jp/naid/110000310895/en
Setup: Morphology and acid phosphatase activity of spermatozoa was studied after 1) washing with distilled water (in solution), 2) storage in water on a piece of cotton cloth 3) washing by sprinkled tap water on a piece of cotton cloth.
Results: 1) Morphology did not change significantly after washing with distilled water. 2) Morphology did not change after storage in water on a piece of cloth (5 days). The Acid phosphatase activity was significantly reduced after two days and abolished after three days. 3) Water sprinkled on semen-stained cloth resulted in negative acid phosphatase activity after three hours. Morphology was not changed after 10 hours of sprinkling.
Changes in yield of contact DNA over time after a physical assault
Setup: study to “determine how touch DNA collected from a victim’s wrist after a simulated sexual assault changed over time” (15 minute intervals after the “assault”). 30 seconds holding with struggle, double (wet/dry) swabbing for recovery.
Results: The DNA quantities were generally low (0-10 pg/µL).

Forensic identification of a murderer by LCN DNA collected from the inside of the victim’s car
http://www.isfg.org/files/1a528d0fcee17e821a369c61d28d759fbaed8d30.03018557_129265714647.pdf
They collected samples with FTA-paper from steering wheel, gear lever and handbrake from a car and successfully matched the profiles from a suspect’s profile. Quantity of DNA from the extractions were less than 100 pg, 16 markers were analyzed.
“…it is possible to type even low copy number (LCN) DNA if a proper and immediate collection of biological evidence is performed.”

Trace DNA and street robbery: a criminalistic approach to DNA evidence
DNA background levels on handbags and wallets was determined as well as the probability of transfer during and after a robbery.
Both owner and non-owner DNA could be recovered from “un-robbed” items. Simulated robberies resulted in majority mixtures or single profiles of the robber in 40 % of the cases.

Trace evidence characteristics of DNA: a preliminary investigation of the persistence of DNA at crime scenes
Casework data gave no clear correlation between the amount of DNA recovered and time of the object spent outdoors.
Experimental setup with human buffy coat showed that two weeks outdoors halved the amount of recovered DNA while the amount was negligible after 6 weeks. Storage at dark and cold locations could preserve the DNA for at least 6 weeks.

Assessing trace DNA evidence from a residential burglary: abundance, transfer and persistence
Both background DNA and deposited DNA at sites of burglary entry points were low. 29 residences were double swabbed (doors, windows) resulting in 150 swabbed locations. 20 different residences (39 different swabblings) produced alleles after amplification.
DNA was deposited by grabbing a pre-cleaned door-frame for 1 minute (without prior handwashing). 40 % of the surfaces yielded 10-200 pg DNA.
DNA (solution) was deposited on wooden window-frames and sampled between 0-6 weeks, after 2 weeks no DNA could be recovered.

A novel approach for genotyping of LCN-DNA recovered from highly degraded samples
http://www.isfg.org/files/91a748919b0f16087a260b2ab392c8c879a21c0f.05017577_537759952848.pdf
“A new kind of STRs profiling system is based on the amplification of shorter fragments compared to the conventional STR multiplexes; because of their length, these MiniQ STR amplicons could be obtained even from extremely degraded DNA and/or from few copies of template DNA.‘……
“MiniSTRs turned out to be more robust and sensitive markers than the traditional ones.”
A further study to investigate the effect of fingerprint enhancement techniques on the DNA analysis of bloodstains

Setup: Bloodstains of varying ages on different surfaces were treated with different fingerprint-enhancement techniques and then DNA was recovered.

Results: “…magnetic powder, multimetal deposition (MMD) and ultraviolet (UV) irradiation is not recommended for use in a sequence of analyses involving DNA typing.” “Strong white light, white and aluminum fingerprint powders, physical developer (PD) after 1,8-diaza-9-fluorenone (DFO), PD after ninhydrin with cadmium (Cd) salt treatment, and cyanoacrylate with gentian violet or Ardrox stains may be used successfully in a sequence of analyses involving DNA typing. Ninhydrin with secondary metal salt treatment, DFO, amido black, diaminobenzidine (DAB), black powder, Stickyside Powder, cyanoacrylate with rhodamine stain, and luminol may be used before DNA analysis but care must be taken to ensure that sufficient DNA is extracted and analyzed.”

An investigation into the transference and survivability of human DNA following simulated manual strangulation with consideration of the problem of third party contamination

Setup: A man “strangled” a woman for 1 minute 29 separate times. Samples were collected by moist swabbing between 1 minute and 10 days after the “assault”. Samples were taken from man’s fingertips and woman’s neck, the neck was not touched or washed after the assault, the man’s fingertips were allowed normal daily routine. The pair worked in the same building but was not allowed direct contact during the experiment.

Results: Neck – 7 of 29 neck-swabs showed full offender profile up to 6 hours after the assault. By LCN offender alleles were present up to 10 days. Fingertips – 7 of 29 showed an offender + victim profile. 6 of 7 were partial victim profiles detected up to 24 hours after the assault. Caution: Control areas of neck and control fingertips also showed profiles from the other person in some cases up to 10 and 5 days after assault resp. Third party profiles were found on both control sites and test sites.

Trace DNA analysis: If your DNA is on the evidence, did you really touch it?
Ryan SR., Posted on LinkedIn 2014; June 26.
https://www.linkedin.com/pulse/20140626163650-13967252-trace-dna-analysis-if-your-dna-is-on-the-evidence-did-you-really-touch-it

“…simply touching an object can leave anywhere from 0-169ng of DNA” (Meakin and Jamieson 2013).
“In 14 of 24 individuals tested, non-self DNA was found on their necks” (Graham and Rutty 2008).
“41 % of fingernail samples tested showed some evidence of a mixed DNA profile” (Dowlman et al. 2010)
“Touch DNA is known to last for up to 2 weeks outside and 6 weeks or longer inside” (Raymond et al. 2009).

Touch DNA. What is it? Where is it? How much can be found? And, how can it impact my case?
A question and answer guide to all things touch DNA
Ryan SR., Ryan Forensic DNA Consulting, January 2012.
http://www.ryanforensicdna.com/yahoo_site_admin/assets/docs/Touch_DNA_article.59101908.pdf
Review on: definition of touch DNA, how much DNA is left behind when an object is touched, is DNA always left on an object via touch? Factors that increase the amount of shed DNA, persistence of touch DNA, secondary transfer, can we tell who handled the item last? Which items can contain touch DNA?
Yield of male touch DNA from fabrics in an assault model
Setup: Grab and struggle for 15 seconds was used to deposit male touch DNA to cotton, polyester or cotton/polyester blend on a female (hands were washed 15 minutes before the test). Sampling was done by cutting the fabric after 12 hours or 7 days.
Results: A maximum of 7 pg/µL DNA was extracted after 12 hours. No significant difference after 7 days. Fabric types could not be compared due to the low yield (Quantifiler Duo was used which has a limit of 23 pg/µL).

The effect of washing on the detection of blood and seminal stains
http://www.tandfonline.com/doi/abs/10.1080/00085030.1971.10757279#.VeRiUfntkXs
Setup: Washing of semen or blood or semen+blood -stains was planted on new underwear (cellulose acetate fabric or cotton fabric). Detergents used were 1) for hot water 2) for cold water 3) for hot or cold water with enzymatic activity. 12 different washing procedures were used combining different lengths of soaking before washing and different detergents.
Results: No visible blood stains on cellulose acetate underwear were detected after washing and most washing procedures resulted in negative detection of blood with benzidine. The blood on cotton underwear was however more resistant and all washing procedures resulted in positive reaction with benzidine even though no visible stain was detected. Seminal stains were visibly not detected after any washing procedure on either cellulose acetate or cotton and the “fast blue” seminal stain was negative for most washing procedures, however, only the detergent containing enzymatic activity washed away the spermatozoa as detected by microscopy (spermatozoa could be found in some samples even after washing with the enzymatic detergent as well).

Analysis of salivary DNA evidence from a bite mark on a body submerged in water
A body was recovered after 5.5 hours in water and the perpetrator was found as the minor profile along with the victims profile from a swabbed bite mark (4 single swabs, 4 different parts of the bite mark). Less than 1.5 ng/µL was recovered from each swab so the extracts were pooled for typing. The minor profile yielded a frequency of 1:220 of the Canadian Caucasian population but together with the characteristics of the bite-mark the perpetrator was identified.

PCR-based DNA typing of saliva stains recovered from human skin
Saliva was deposited on cadavers and collected by double swabbing after 5 min, 24 hrs, and 48 hrs. The DNA concentration in saliva on cadavers seemed to decrease significantly during the first 24 hours but was stable between 24-48 hours.

DNA profiles from fingerprints: A mock case study
Uncleaned substrates were handled for less than 15 s to create a fingerprint and left outdoors (a glass door was not left outdoors but kept in place indoors). Samples were recovered after 24 hours and 8 days.
“Direct PCR” (without extraction) was used.
Results: Glass had the highest rate of DNA recovery, masking tape was second best. Mixed DNA profiles were recovered but the donor profile was always the major one.
Persistence of DNA deposited by the original user on objects after subsequent use by a second person
“Our findings show that the profile, percentage contribution of the 1st user relative to the 2nd user of an object declines in a linear manner, over time.”

Setup:
a) Non-porous, hard, flat surface object (pens and pen lids). Person 1 rubbed 49 new, cleaned pens between his/her hands and the lids was removed and replaced once a day for four days (30s/60s/60s/60s rubbing/day). Five of the rubbed pens were taken as controls. The rest of the 44 pens were given to Person 2 (one person/pen). Person 2 used the pen as normally when writing and documented the duration, the activity and details about e.g. contact with skin and other materials. Sample collection by wet+dry swab.
b) Porous worn object (bracelets of elastic fabric). Person 1 wore rubber-band bracelets for 34 hours (divided in five days) and also rubbed the bracelets with his/her hand for 30s before removing them every day. 4x17 of these bracelets were extra rubbed and given to a person 2. Six were also rubbed again and stored as control. Person 2 used the bracelets and documented when they were temporarily removed, total usage for person 2 was 5, 10, 20, 40 h and 1, 2, 4, 8 days. Sample collection by cutting the bracelets into small pieces.
c) Wide range of every day personal objects used by a second person. Sample collection by wet+dry swab on hard surfaces and tape lifting on soft/porous surfaces.

Results:
a) The 2nd user was the dominant profile on the pen after 30 minutes of use (50/50 after 1 minute) and on the lid after 3 times of removing/replacing the lid (50/50 after 1 time).
b) A large number of “1st wearer derived unknown alleles” in the control bracelets (15 %). The 2nd user was the dominant profile on the bracelet after 29 hours of use, the increase of the 2nd user was linear over time.
c) Many details to consider in this part of the experiment. Well-worn watches retained the 1st user as dominant after 10 days of use by 2nd user. E.g. sunglasses, perfume bottles, lighters etc. were dominated by the 2nd user relatively quickly. A well-worn cap used by 2nd user for 2 weeks (12 hours in total) was dominated by the 2nd users profile but the 1st user was still a full profile.

DNA typing of epithelial cells after strangulation
Experimental study of DNA typing after strangulation. Success rate was > 70 %. Profiles were often a mix of the “suspect” and the “victim”.
Setup: 16 pairs. Upper arm used for strangulation for 1 min including arm movements by the victim. Collection of DNA by 1) glass fibre pieces or 2) moistened cotton swabs. Polyacrylamide gel electrophoresis for visualization of the alleles.
Results: 0.5-1 ng DNA extracted for 14 of the 16 samples. For the other 2 samples ~2 ng was extracted. Case study: Strangled victim found after 48 h. The neck was swabbed and the suspect could be identified.

Recovery of salivary DNA from the skin after showering.
http://link.springer.com/article/10.1007%2Fs12024-014-9635-7
Setup: 50 µl male saliva was deposited on female skin and allowed to dry for 15 min. The volunteers showered and put on clean clothes after 4 hours of normal routine. Samples were collected 5 hours later by double swabbing or M-Vac.
Results: Because of the overwhelming amount of female DNA the AmpFISTER® Y-filer® kit was used for analysis. The maximum amount of collected DNA was 15 ng. Male DNA was quantified in 58 of 96 samples. Full Y-STR profiles were detected in 47 of 96 samples and 15 samples resulted in no loci. There were no differences in DNA recovery between swabbing and the M-Vac.
3.15 Review articles

**Internal quality control in forensic DNA analysis**
Review on the importance of quality controls.

**Validity of low copy number typing and applications to forensic science**
[http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2702736/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2702736/)
“LCN typing simply can be defined as the analysis of any DNA sample where the results are below the stochastic threshold for reliable interpretation.” “Some touch DNA samples do not qualify as LCN samples in that they contain sufficient DNA for routine conventional analyses. Conversely, many crime scene samples do meet the criteria of LCN samples. Such samples should be clearly distinguished and analyzed and interpreted accordingly.” If LCN samples are not handled correctly they can be useless in trial. Difficult (not possible) to use LCN for exculpatory purposes. LCN can basically only be used to identify human remains/missing persons according to the authors.

**Low Copy Number - consideration and caution**
Stochastic amplification may occur --> imbalance of two alleles at a given heterozygous locus, or allelic dropout/dropin.
A stutter allele can have larger height/area that the true allele in LCN.
Contaminations can occur even though the negative control shows no contamination.
Considerations with LCN:
Difficult to exclude an individual after LCN.
Drop-in, contamination
Causal contact between suspect and victim must be considered.
Heterozygous peak imbalance.
Increased stutter.
Difficult to separate contributions in a mixture.

**A review of the science of low template DNA analysis**
Budlowe (2009) is critical to the conclusions of this review: “The reviewers found that LCN typing as practiced specifically in the United Kingdom was “robust” and “fit for purpose” but offered a number of recommendations to improve the methodology. The findings of the commission seem inconsistent with the nature of LCN typing and LCN typing warrants a more in-depth evaluation by the greater scientific community.”
This review address concerns about Low template DNA analyses (LTDNA) for legal purposes, instigated by the Forensic Science Regulator and contains guidelines, recommendations and validation for use of LTDNA.
Transfer of cellular material:
Individuals vary in their propensity to transfer their cellular material to an object.
Time and pressure affects the efficiency of the transfer.
(Person--> object = primary transfer. To next object/person = secondary transfer.)
Forensic DNA evidence is not infallible.
Cale CM., Nature 2015; 526: 611
This is a one page “personal take” on the subject of secondary transfer of DNA. For more detailed reading see “Could secondary DNA transfer falsely place someone at the scene of a crime?” by Cale et al. J Forensic Sci 2015.

DNA transfer: informed judgement or mere guesswork?
Champod C., Front genet 2013; 4: 300.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3872334/
Opinion article
“…the quantity of DNA or the quality of the profile cannot be used “to reliably infer the mode of transfer by which the DNA came to be on the surface of interest.” (Meakin and Jamieson 2013).

Science in court: DNA’s identity crisis
Discussion about the reliability of LCN (by increased number of cycles) in forensic investigations.

Anything you touch may be used against you
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2892330/
How reliable is DNA evidence?
Care must be taken when analyzing DNA from cold cases since the sampling procedures were not the same as now and contaminations are likely.

Experience is the name that everyone gives to their mistakes
Jamieson A, Meakin G., Barrister Mag 2010; 45.
Courts are skeptical to experts and prefer experience.
The habitual wearer tends to be the major source of DNA on a garment. DNA can be deposited onto an object that the donor has never touched through transfer, depending on shedder status the transferred DNA can provide the major profile.

Effects of latent fingerprint development reagents on subsequent forensic DNA typing: A review
Most fingerprint enhancement methods can be used without interfering with DNA extraction. The DNA extraction efficiency can in some cases depend on the time between development and extraction. The type of surface the fingerprint is placed on can be of importance.

Use of low copy number DNA in forensic inference
http://www.isfg.org/files/31f9316afbc584bc0befd4454d6cd38c4f064f3a.02004843_693490260903.pdf
Discussion about shedder status and the likelihood of getting DNA profiles from Touch DNA and used garments. E.g. a good shedder can be the major profile on a garment worn by a poor shedder. Also, a good shedder can be the major profile after a secondary transfer event with a poor shedder.

Understanding DNA results within the case context: importance of the alternative proposition
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3853867/
Examples and probabilities of how a bloodstain from the victim ended up on the suspect and how likely it is that a person’s DNA ends up on a handled item (weapon).
The likelihood is 0.3 that DNA is transferred from shooter to gun and 0.7 that DNA is not transferred. Thus, it is only slightly more likely to transfer DNA to the gun than the chance of finding background DNA on the gun (35% mixed profiles and 24% single background profiles). Conclusively, not finding a suspect’s DNA on a gun does not eliminate the suspect as the shooter.

DNA transfer: Review and implications for casework
Review on factors (shedder status, surfaces, time aspects…) and probabilities of transfer.

Mitochondrial DNA recovery and analysis from spent cartridge casings
Metchikian, M., Michigan State University, 2013 masters thesis.
http://etd.lib.msu.edu/islandora/object/etd%3A2258/datastream/OBJ/view
“…mtDNA analysis is a reliable method to generate genetic profiles recovered from spent cartridge casings.”
The introduction can be read as a review on cartridges in forensic work.

Touch DNA: From the crime scene to the crime laboratory
Minor J., Forensic Magazine 04/12/2013.
A review on touch DNA and contamination, and guidelines on how to avoid the contaminations.

The recovery and analysis of DNA from fired cartridge casings
No association found between DNA yield and profile quality. Cumulative swabbing of several casings resulted in higher quantities of DNA but not better profile quality. However, the author suggests that cumulative swabbing is the best approach.
Introduction and discussion can be read as a review.

Trace DNA: An underutilized resource or Pandora’s box? A review of the use of trace DNA analysis in the investigation of volume crime
Since touch DNA cannot be derived to a specific source the activity level of the evidence might be problematic.
Secondary transfer reviewing.
Discussion of the problems with LCN in court.

The end of the (forensic science) world as we know it? The example of trace evidence
http://rstb.royalsocietypublishing.org/content/370/1674/20140260

The urban myths & conventional wisdom of transfer: DNA as trace evidence
Review of trace DNA and transfer – believers and non-believers of secondary transfer, the occurrence (or not) of good and bad shedders…
Trace DNA analysis: If your DNA is on the evidence, did you really touch it?
Ryan SR., Posted on LinkedIn 2014; June 26.
https://www.linkedin.com/pulse/20140626163650-13967252-trace-dna-analysis-if-your-dna-is-on-the-evidence-did-you-really-touch-it

“…simply touching an object can leave anywhere from 0-169 ng of DNA” (Meakin and Jamieson 2013).
“In 14 of 24 individuals tested, non-self DNA was found on their necks” (Graham and Rutty 2008).
“41 % of fingernail samples tested showed some evidence of a mixed DNA profile” (Dowlman et al. 2010).
“Touch DNA is known to last for up to 2 weeks outside and 6 weeks or longer inside” (Raymond et al. 2009).

Touch DNA. What is it? Where is it? How much can be found? And, how can it impact my case?
A question and answer guide to all things touch DNA
Ryan SR, Ryan Forensic DNA Consulting, January 2012.
http://www.ryanforensicdna.com/yahoo_site_admin/assets/docs/Touch_DNA_article.59101908.pdf

Review on definition of touch DNA, how much DNA is left behind when an object is touched, is DNA always left on an object via touch? Factors that increase the amount of shed DNA, persistence of touch DNA, secondary transfer, can we tell who handled the item last? Which items can contain touch DNA?

Evaluating forensic DNA evidence – essential elements of a competent defense review
Thomson WC, Ford S, Doom T, Raymer M, Krane DE.
http://www.americanbar.org/content/dam/aba/events/legal_aid_indigent_defendants/2015/ls_sclaid_summit_03c_champion2.authcheckdam.pdf

Tertiary transfer in trial. “He and his wife had shared a towel the morning of the murder - perhaps his DNA was transferred from his face to the towel, and from the towel to his wife’s face. His wife was later attacked by a glove-wearing stranger who struck her on the face, strangled her, and stabbed her, in the process transferring husband’s DNA from his wife’s face to the gloves and the knife.” The scenario was tested and found plausible.

Forensic trace DNA: a review
http://investigativegenetics.biomedcentral.com/articles/10.1186/2041-2223-1-14

History and discussion on STRs and PCR.
Touch/trace DNA should not be confused with LCN.
Touch DNA = collection of minute biological samples. Touched objects does not always result in low amounts of DNA.
LCN = often used to describe increased cycle numbers.
Trace DNA is herein defined as “any sample which may fall below the recommended thresholds at any stage of the process - detection, collection, extraction, amplification and interpretation.” Usually less than 100 pg DNA.
A sample defined as trace DNA in the recovery phase does not necessarily have to be trace DNA in later stages of the process.
The precise location of where to recover the trace DNA is very important.
Double swabbing (not necessarily wet + dry, can be wet+wet if all the moisture is recaptured by the first swab) is recommended for collection of trace DNA.
If the swab is allowed to dry before extraction less DNA will be extracted, if the dried swab is frozen before extraction the DNA recovery will be similar to if it would still have been wet.
LMD can be useful to differentiate relevant cells from other cell types which allow efficient DNA analysis from the minor cell type in a sample. Flow cytometry can be used to separate sperm cells from vaginal wash fluid.
Chelex and organic extraction can lead to up to 75 % loss of DNA, however, this loss is often not relevant.
Partial or complete profiles have been obtained from samples that did not pass the quantification limit.
Trace DNA samples should not always be discarded due to low quantification.
Reviews amplification methods for LCN samples, detection of the amplified product and interpretation. Common problems with trace DNA amplification: 1) allele drop-out 2) decreased heterozygote balance 3) allele dop-in (stutter) 4) allele drop-in (contamination).

In analysis it can be useful with detection thresholds such as LOD (limit of detection), T (low template DNA threshold), MIT (match interpretation threshold) and LOQ (limit of quantification). However, the peak intensity should be included in the exclusion calculation and continuous measures should be used instead of definite thresholds.

Replicates should be employed for trace DNA analysis, four replicates with reported alleles detected in at least two of the replicates seems to be the most accurate. Bayesian based likelihood ratios of drop-outs can also be employed.

Mixed profiles can be challenging due to e.g. biased drop-outs, complicating drop-ins and over-amplification of some alleles.

Contamination of trace-DNA can be a serious problem. The contamination can be the major profile in the sample. Contamination can occur before the crime is committed (background DNA), between the crime is committed and the crime scene is secured, during the crime scene investigation and in the forensic laboratory.

**Trace DNA: A review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact**


The use of trace-DNA is possible in forensic applications. Examples and guidelines.

**Touch DNA: Forensic collection and application to investigations**


“…the scraping/tape lift methods are ideal in situations where the scientist can locate areas on the item which are most likely to contain the perpetrator’s skin cells.”

“…an increased chance of obtaining mixed DNA profiles containing DNA from individuals that may have come into contact with the victim/evidence item near the time of the crime. Contributors to these mixtures could include the victim’s spouse or children, and again, elimination samples may need to be collected from these individuals.”

“Some evidence items are also not recommended for the collection of Touch DNA samples. Such items include those that are severely degraded (for example, moldy clothing), have been exposed to extreme environmental conditions (such as weapons left outside for months or years), have been washed, or are heavily soaked in the victim’s body fluids. Also, items that are likely to have been touched by many people, such as a public pay phone or store counter are usually not good sources for probative or interpretable Touch DNA profiles.”

### 3.16 Touch DNA

**Fingerprints as evidence for genetic profile: morphological study on fingerprints and analysis of exogenous and individual factors affecting DNA typing**


http://www.crime-scene-investigator.net/fingerprintDNAextraction.pdf

Setup: 347 fingerprints, 11 persons, glass/wood/metal, 30s, with/without handwashing.

Results: Recovery 0,04-0,2 ng.

Handwashing reduced the amount of recovered DNA. “Shedder status” of donor is very important regarding how much DNA can be recovered from a fingerprint.

“… LCN method enhances the amplification of spurious alleles present in fingerprints, but laboratory-based contamination cannot be excluded, since standard negative controls of amplification cannot reveal lower levels of contamination”
“When fingerprints were made after subjects had touched objects of routine use, including computer keyboards used by many people, mixed alleles from multiple donors were observed. In many cases, the peaks of accessory alleles reached those of true alleles, hindering definite allele typing.”

**Fingerprints from fingerprints**
http://www.isfg.org/files/8113b53efe142f5fac93aab44ea8e1cfdec0456.02002303_961657393440.pdf
Latent fingerprints usually contain a small number of epithelial cells. The cells may also be corneocytes only (no nuclei).
Tested on white paper.
Minimal incidence of nucleated cells (microscopic inspection), but enough for analysis.

**STR genotyping and mtDNA sequencing of latent fingerprint on paper**
Setup: Fingerprints were deposited on ordinary white paper for 1-60 s.
Results: Most cells deposited on the paper were nuclei-free corneocytes but enough nucleated cells for DNA extraction were found.

**The use of adhesive tape for recovery of DNA from crime scene items**
Adhesive tape has shown to be superior in recovering shed epithelial cells (over swabbing/cutting) for e.g. shoe insoles, baseball caps, jackets and cadavers (refs in paper).
Tape inside and outside of gloves separately to avoid contamination.
Taping large areas might require several tapes. However, the need to pool and concentrate the samples increased the risk for both DNA loss and contamination.

**LCN DNA typing from touched objects**
http://www.isfg.org/files/91a748919b016087a266b2ab392c8c8f79a21c0f.0501420_311586137609.pdf
Marking pen and comb swabbing (1x PBS) --> less than 100 pg DNA --> full DNA profile with LCN.

**Evaluation of samples comprising minute amounts of DNA**
Setup: Single donor and two- and three- person samples, 3-12 pg DNA/contributor. Standard and enhanced CE-settings.
Conclusion: “… an assessment of the criminalistic relevance of a sample carrying minute amounts of DNA is essential prior to applying enhanced interrogation techniques and/or calculating a weight of evidence statistic.”

**Validity of low copy number typing and applications to forensic science**
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2702736/
“LCN typing simply can be defined as the analysis of any DNA sample where the results are below the stochastic threshold for reliable interpretation.” “Some touch DNA samples do not qualify as LCN samples in that they contain sufficient DNA for routine conventional analyses. Conversely, many crime scene samples do meet the criteria of LCN samples. Such samples should be clearly distinguished and analyzed and interpreted accordingly.” If LCN samples are not handled correctly they can be useless in trial. Difficult (not possible) to use LCN for exculpatory purposes. LCN can basically only be used to identify human remains/missing persons according to the authors.
Analysis of cellular and extracellular DNA in fingerprints
Cellular DNA – from skin cells.
Extracellular DNA – from body fluids (s.a. sweat)
Setup: Glass slides and plastic pens were wet swabbed (TE buffer).
Preparation methods: centrifugation, acoustofluidics.
Analysis method: qPCR, 45 cycles.
Results: Extracellular fractions contain more DNA than cellular fractions. Some people are better “shedders” than others.
Some contaminations through carryover in blank runs.

DNA profiling success and relevance of 1739 contact stains from casework
Statistics on 1739 real cases with contact stains.
More DNA was recovered when stains were collected with the double swab (wet swab + dry swab) technique (both for contact stains and saliva stains).
Clothes has the highest success-rate (61 %) (the proportion of DNA profiles that were suitable, 1 person > 5 loci, 2 persons > 7 loci), car items second best (37 %), plugs and cables third (29 %). Stones were most difficult (7 %).
Apparently the relevance of car-contact-stains was high suggesting that the “borrowing” persons DNA covers the car owners DNA.

The transfer of touch DNA from hands to glass, fabric and wood
Setup: Glass/fabric/wood, 100 volunteers per type of material, 60 s contact, objects were mini taped.
Results: Wood gave the best yield of DNA (36 % gave handler profile) followed by fabric (23 %) and then glass (9 %).
10 % of the total number of samples gave mixed profiles indicating secondary transfer.

Comparison of stubbing and the double swab method for collecting offender epithelial material from a victim’s skin
Setup: Manual strangulation by a man on a woman was simulated. DNA was secured by double swab or by a tape-lifting method called stubbing.
Results: Fewer alleles from the victim were detected after double swabbing compared with stubbing. There was no difference in the number of alleles from the offender between swabbing and stubbing.

The complexities of DNA transfer during a social setting
A study on “effects of unstructured social interactions on the transfer of touch DNA”.
Setup: Three individuals were video-recorded while having a drink (one central jug and individual glasses) together around a table for 20 minutes. All relevant surfaces were sampled for DNA and the video was used to identify how the DNA was transferred.
Results: Transfer occurred during everyday interactions and was found to be bi-directional. The last person to touch an object was often but not always the major or the only DNA contributor. The participants often introduced DNA from persons not included in the experiment setup.
DNA transfer during social interactions
Very similar to Goray 2015.
Setup: Three individuals having a drink (one central jug and individual glasses) together around a table for 20 minutes. All relevant surfaces were sampled for DNA. Same results as Goray 2015.

Investigation of secondary DNA transfer of skin cells under controlled test conditions
"The transfer rates showed that both the primary and secondary type of substrate and the manner of contact are important factors influencing transfer of skin cells… the freshness of the deposit in most instances is not." "Skin cells deposited on a non-porous primary substrate transferred more readily to subsequent substrates than those deposited on a porous substrate. Porous secondary substrates, however, facilitated transfer more readily than non-porous secondary substrates, from both porous and non-porous surfaces. Friction as the manner of contact significantly increased the rate of transfer.”

Success rate of a forensic tape-lift method for DNA recovery
http://www.forensicscience.pl/component/option,com_jbook/task,view/Itemid,2/catid,67/id,628/lang,en/
For trace DNA recovery mini taping provided a lower degree of inhibited DNA extracts and a higher proportion of usable DNA results than conventional techniques. However, the proportion of mixed profiles found was also increased.

“Getting blood from a stone”: Ultrasensitive forensic DNA profiling of microscopic bio-particles recovered from “Touch DNA” evidence
Hanson E, Ballantyne J., Methods Mol Biol 2013; 1039: 3-17.
Description of “…an optimized and efficient removal strategy for the collection of cellular microparticles present in “touch DNA” samples, as well as enhanced amplification strategies to permit the recovery of short tandem repeat profiles of the donor(s) of the recovered microparticles” in order to avoid “blind-swabbing” which increases the risk of mixed profiles and contamination of DNA between different areas of a sample.
This approach includes: collection of bio-particles on a WF Gel-Film, cell staining, removal of bio-particles from the film, nuclear isolation, and STR amplification and detection of amplified products.

Specific and sensitive mRNA biomarkers for the identification of skin in 'touch DNA’evidence
When analyzing touch DNA it is difficult to determine the tissue of origin. The paper presents five potential mRNA biomarker (especially promising was LCE1C) for the identification of skin. At time of publication the use of these biomarkers in actual forensic work was not an option due to e.g. variability.

Trace DNA collection - performance of minitape and three different swabs
Comparison of the recovery of DNA using three different swabs (two synthetic and one cotton) or mini tape. There was no major difference between the three kinds of swabs but the mini tape was the best option for recovery of DNA from absorbent materials (like textiles). (No statistics calculated).
Zip lock poly bags in drug cases - a valuable source for obtaining identifiable DNA results?
http://www.fsigeneticssup.com/article/S1875-1768(08)00050-4/pdf
Retrospective study of 183 swabs on zip lock poly bags from drug related casework. 14 % of the swabs contained enough DNA for identification.

Low Copy Number DNA: Reality vs Jury Expectations
Hoffman Wulff P., Silent Witness Newsletter 2006; 10(3).
Discussion about the limitations and the future of LCN.

Experience is the name that everyone gives to their mistakes
Jamieson A, Meakin G., Barrister Mag 2010; 45.
Courts are skeptical to experts and prefer experience.
The habitual wearer tends to be the major source of DNA on a garment. DNA can be deposited onto an object that the donor has never touched through transfer, depending on shedder status the transferred DNA can provide the major profile.

Touch DNA – The prospect of DNA profiles from cables.
This study was performed with metal theft in mind. Cable sheaths are often removed and left behind when the metal inside is stolen. The material is often made of black plastic polymers and both DNA and fingerprint investigations are of interest. For fingerprint visualization on this type of material cyanoacrylate (CAN) fuming or wet powder suspensions (WPS) are often used.
Setup: Sweat, extracted DNA or touch DNA was deposited on smooth non-porous black cable (0.02 m diameter). Sweat and extracted DNA were from stock solutions collected from volunteers and touch DNA was deposited with unwashed hands by rolling the top-half of each finger on the cable. DNA was recovered by either double swabbing or mini-taping, before or after fingerprint visualization by CAN fuming or WPS treatment.
Results: There was no difference in DNA yield between double swabbing and mini-taping. Treatment with CAN fuming before swabbing or tapping resulted in a significant increase in amount of recovered DNA (~8 times more). However, treatment with WPS before collection of DNA decreased the recovered yield when samples were collected by swabbing and increased the yield when mini-tape was used. Pre-treatment by CAN fuming resulted in significantly higher amounts of DNA than WPS treatment.
Conclusions: Full profiles could be recovered from touch DNA on the cables. CAN fuming significantly increased the amount of recovered DNA, possibly because the collection area was clear and thus limited to only the stained parts.

Good shedder or bad shedder—the influence of skin diseases on forensic DNA analysis from epithelial abrasions
http://link.springer.com/article/10.1007%2Fs00414-011-0579-0#page-1
Certain skin diseases (e.g. atopic dermatitis, ulcer and psoriasis) lead to high proliferation rate of the keratinocytes. This could mean that the individuals are “good shedders” and render very good DNA profiles from touch DNA. However, the shedder status can be affected by disease-treatment. Moreover, shed cells can contain partially degraded DNA.
Use of low copy number DNA in forensic inference
http://www.isfg.org/files/31f9316aafbc584bc0befd4454d6cd38c4f064f3a.02004843_693490260903.pdf
Discussion about shedder status and the likelihood of getting DNA profiles from Touch DNA and used garments. E.g. a good shedder can be the major profile on a garment worn by a poor shedder. Also, a good shedder can be the major profile after a secondary transfer event with a poor shedder.

Analysis of DNA from fired cartridge casings
http://waset.org/publications/10002182/analysis-of-dna-from-fired-cartridge-casings
Even though cartridges are subjected to high temperatures at firing the weapon full DNA profiles can be obtained.

Mitochondrial DNA recovery and analysis from spent cartridge casings
Metchikian, M., Michigan State University, 2013 masters thesis.
http://etd.lib.msu.edu/islandora/object/etd%3A2258/datastream/OBJ/view
“…mtDNA analysis is a reliable method to generate genetic profiles recovered from spent cartridge casings.”
The introduction can be read as a review on cartridges in forensic work.

Touch DNA: From the crime scene to the crime laboratory
Minor J., Forensic Magazine 04/12/2013.
A review on Touch DNA and contamination, and guidelines on how to avoid the contaminations.

Optimization of recovery and analysis of touch DNA from spent cartridge casings
Mottar AM., Michigan State University, 2014 masters thesis, publication number 1563498.
http://gradworks.umi.com/15/63/1563498.html
Optimization of DNA extraction from fired casings. Best method: “…double swabbing with organic extraction and amplification with Fusion…”

Touch DNA collection versus firearm fingerprinting: comparing evidence production and identification outcomes
Touch DNA produced a larger volume of evidence than fingerprints from firearms in an Indianapolis police district, however, the identification outcome was equal.

Double swab technique for collecting touched evidence
Evaluation of wet+dry swabbing of touched items. The two swabs were extracted separately and some dry-swabs contained enough DNA for a DNA profile. Double swabbing is recommended.
Setup: Skin cells sloughed off during contact. Wet = water.

DNA analysis and document examination: the impact of each technique in respective analyses
DNA can transfer from the hand and arm when a letter is handwritten. Areas of a document subjected to most touch were fold lines, center top face and middle face. DNA could be successfully recovered from a document by dry/dry-swabbing only, a method that allows other types of document analyses to be performed on the document afterwards. Most fold lines contained detectable DNA probably due to the applied pressure when folding.
Changes in yield of contact DNA over time after a physical assault
Setup: study to “determine how touch DNA collected from a victim’s wrist after a simulated sexual assault changed over time” (15 minute intervals after the “assault”). 30 seconds holding with struggle, double (wet/dry) swabbing for recovery.
Results: The DNA quantities were generally low (0-10 pg/uL).

Influence of an individual's age on the amount and interpretability of DNA left on touched items
Setup: Handprints on plastic syringes from 213 persons, 1-89 years of age, were analyzed for amount of DNA and for STR profiles.
Results: Very old persons (>80 yrs) did not leave full profiles on the syringes. Very young persons (<10 yrs) left the most full profiles.

Maximization of STR DNA typing success for touched objects
Evaluation of sample recovery, extraction, amplification and separation of DNA from fingerprints on different substrates (credit cards, keys and pens). Development of a “specialized swab” that produced at least 2.5 times more DNA than ordinary swabs. Single tube digestion with SDS followed by purification and concentration with Micron column was more efficient than more complicated extraction methods. Most of the samples contained less than 100 pg DNA. ~60 % of the samples contained less than 40 pg DNA. Suggests that samples containing at least 20 pg of DNA may generate database-acceptable DNA profiles. 10-20 pg DNA could be enough for direct comparison of profiles.

Experiments on the DNA contamination risk via latent fingerprint brushes
Setup 1: Analysis of 51 used fingerprint brushes.
Results 1: 86 % of the brushes gave full or partial profiles.
Setup 2: A selection of brushes and some artificially contaminated brushes to test secondary transfer
Results 2: “The larger the brushed area, the greater is the risk for contamination; single fingerprints are less affected.” “Contact of the brush with body fluids such as blood or saliva make secondary transfer highly likely.”

Cell free DNA as a component of forensic evidence recovered from touched surfaces
Keratinocytes have no nuclei but possibly residual amounts of DNA. Cell free nucleic acids from sweat may contribute to the DNA recovered from touched objects.
Cell free sweat samples gave full DNA profiles in 40 % of the cases, which is to be compared with the real casework success-rate of ~5 % for touch-DNA (time delay between deposition and recovery could be a factor). High inter- and intra-individual variety in the amount of DNA in sweat.

Trace DNA and street robbery: a criminalistic approach to DNA evidence
DNA background levels on handbags and wallets was determined as well as the probability of transfer during and after a robbery.
Both owner and non-owner DNA could be recovered from “un-robbed” items. Simulated robberies resulted in majority mixtures or single profiles of the robber in 40 % of the cases.
Trace evidence characteristics of DNA: a preliminary investigation of the persistence of DNA at crime scenes
Casework data gave no clear correlation between the amount of DNA recovered and time of the object spent outdoors.
Experimental setup with human buffy coat showed that two weeks outdoors halved the amount of recovered DNA while the amount was negligible after 6 weeks. Storage at dark and cold locations

Trace DNA success rates relating to volume crime offences
Compilation of trace DNA (252 samples) collected in burglaries. Full or major profiles (12 alleles or more) were recovered from 14 % of the samples. 8 % gave a full single profile. 21 % gave mixed profiles. No DNA was recovered from 16 % of the samples. Least efficient was recovery from firearms and points of entry. Average amount of DNA (in the 252 samples) was 1.7 ng.

Assessing trace DNA evidence from a residential burglary: abundance, transfer and persistence
Both background DNA and deposited DNA at sites of burglary entry points was low. 29 residences were double swabbed (doors, windows) resulting in 150 swabbed locations. 20 different residences (39 different swabblings) produced alleles after amplification.
DNA was deposited by grabbing a pre-cleaned door-frame for 1 minute (without prior handwashing). 40 % of the surfaces yielded 10-200 pg DNA.
DNA (solution) was deposited on wooden window-frames and sampled between 0-6 weeks, after 2 weeks no DNA could be recovered.

Trace DNA: An underutilized resource or Pandora's box? A review of the use of trace DNA analysis in the investigation of volume crime
Since touch DNA cannot be derived to a specific source the activity level of the evidence might be problematic.
Secondary transfer reviewing.
Discussion of the problems with LCN in court.

The effect of common fingerprint detection techniques on the DNA typing of fingerprints deposited on different surfaces
The recovery of DNA from a fingerprint was determined to be more dependent on the surface type than the method used to develop the fingerprint. Fingerprints on paper and aluminum foil did not give any DNA profiles. Fingerprints on plastic bags, glass slides and adhesive tape resulted in DNA profiles.

Comparison of collection methods from touch samples on metal and wearer samples from simulated mixtures on clothing
Puritan sterile cotton swabs can contain up to 23 pg of human DNA.
Touch DNA on metal: comparison between DNA sterile cotton swabs, DNA free cotton swabs and foam tipped DNA free swabs (organic extraction, qPCR, PCR STR multiplex amplifications and capillary electrophoresis). The foam tipped DNA free swabs worked poorly on both stainless steel and
brass. DNA sterile cotton swabs worked the best for stainless steel and DNA free cotton swabs worked best for brass.

Touch DNA on clothes (different types): Comparison between swabbing, scraping and adhesive taping (Gel-Pak `0`). All three methods resulted in mixed profiles. Swabbing and Gel-Pak `0` were comparable in that the last wearers profile was the most distinct one. Gel Pak `0` gave generally less DNA. Scraping recovered more DNA from the habitual wearers.

The end of the (forensic science) world as we know it? The example of trace evidence
http://rstb.royalsocietypublishing.org/content/370/1674/20140260

The urban myths & conventional wisdom of transfer: DNA as trace evidence
Review of trace DNA and transfer – believers and non-believers of secondary transfer, the occurrence (or not) of good and bad shedders…

Trace DNA analysis: If your DNA is on the evidence, did you really touch it?
Ryan SR., Posted on LinkedIn 2014; June 26.
https://www.linkedin.com/pulse/20140626163650-13967252-trace-dna-analysis-if-your-dna-is-on-the-evidence-did-you-really-touch-it
 “…simply touching an object can leave anywhere from 0-169ng of DNA” (Meakin and Jamieson 2013).
“In 14 of 24 individuals tested, non-self DNA was found on their necks” (Graham and Rutty 2008).
“41 % of fingernail samples tested showed some evidence of a mixed DNA profile” (Dowlman et al. 2010).
“Touch DNA is known to last for up to 2 weeks outside and 6 weeks or longer inside” (Raymond et al. 2009).

Touch DNA. What is it? Where is it? How much can be found? And, how can it impact my case?
A question and answer guide to all things touch DNA
Ryan SR, Ryan Forensic DNA Consulting, January 2012.
http://www.ryanforensicdna.com/yahoo_site_admin/assets/docs/Touch_DNA_article.59101908.pdf
Review on: definition of touch DNA, how much DNA is left behind when an object is touched, is DNA always left on an object via touch? Factors that increase the amount of shed DNA, persistence of touch DNA, secondary transfer, can we tell who handled the item last? Which items can contain touch DNA?

Yield of male contact DNA evidence in an assault simulation model
Grab setup: Man with washed hands grabbed the washed wrist and washed upper arm of a female for 5 seconds (females did not resist or struggle). Wrists were double swabbed within 30 minutes.
Struggle setup: Man with washed hands grabbed the washed wrist and washed upper arm of a female for 10 seconds while she was struggling.

Results: “The maximum concentration of total DNA extracted was 40 pg/µL in “grab” situations and 90 pg/µL in “struggle” situations, whereas estimates of male DNA from the female’s skin were up to 20 pg/µL in the “grab” situations and 40 pg/µL in the “struggle” Situations”. “... the difference in DNA transferred during grab and struggle situations were not statistically significant in this study.” “Our results showed that no full or usable profiles were attainable with the AmpFISTR® Identifiler® PCR Amplification kit. Even the Yfiler™, which was designed to detect trace male DNA in a predominantly female background and has been shown to generate complete profiles with less than 60 pg of DNA, did not yield usable profiles.”
The hand on the shoulder. A case report where low copy number (LCN) DNA analysis was vital for solving a robbery
DNA from suspect was successfully recovered from victims clothing using adhesive tape even though the time of physical contact was short.

Yield of male touch DNA from fabrics in an assault model
Setup: Grab and struggle for 15 seconds was used to deposit male touch DNA to cotton, polyester or cotton/polyester blend on a female (hands were washed 15 minutes before the test). Sampling was done by cutting the fabric after 12 hours or 7 days.
Results: A maximum of 7 pg/µL DNA was extracted after 12 hours. No significant difference after 7 days. Fabric types could not be compared due to the low yield (Quantifiler Duo was used which has a limit of 23 pg/µL).

Trace evidence scrapings: a valuable source of DNA?
“One method of trace evidence collection in the (FBI) Laboratory involves scraping items to remove hairs, fibers, and other debris adhering to the item.”
They collected scrapings from clothes (T-shirt and hosiery) worn a day. Scraping = hanging the clothing on a metal rack over a table covered in clean paper, scrapings were then collected in a pillbox, the scrapings were then collected from the pillbox on swabs. Samples were also collected by “friction swabs” = moistened swabbing around e.g. neckline of a T-shirt.
“The average amount of DNA recovered was approximately 4 ng from the friction swabs and 21 ng from the pillboxes. In this study, on average, more DNA was recovered from T-shirts than from hosiery, with knee-highs yielding the least quantity of DNA.”

DNA transfer by examination tools – a risk for forensic casework?
High risk vectors: scissors, forceps, gloves.
Setup: Substrates: glass and cotton. “Dried blood or touch DNA, deposited on the primary substrate, was transferred via the vector to the secondary substrate, which was either DNA-free or contained a target sample (dried blood or touch DNA).”
Results: Touch DNA transferred less than blood. Transfer did not interfere with the target profile on the second substrate but could complicate the analysis when no suspect is known.

DNA profiles from fingermarks: A mock case study
Uncleaned substrates were handled for less than 15 s to create a fingerprint and left outdoors (a glass door was not left outdoors but kept in place indoors).
Samples were recovered after 24 hours and 8 days.
“Direct PCR” (without extraction) was used.
Results: Glass had the highest rate of DNA recovery, masking tape was second best. Mixed DNA profiles were recovered but the donor profile was always the major one.
Evaluating forensic DNA evidence – essential elements of a competent defense review
Thomson WC, Ford S, Doom T, Raymer M, Krane DE.
http://www.americanbar.org/content/dam/aba/events/legal_aid_indigent_defendants/2015/ls_sclaid_summit_03c_champion2.authcheckdam.pdf
Tertiary transfer in trial. “He and his wife had shared a towel the morning of the murder - perhaps his DNA was transferred from his face to the towel, and from the towel to his wife’s face. His wife was later attacked by a glove-wearing stranger who struck her on the face, strangled her, and stabbed her, in the process transferring husband’s DNA from his wife’s face to the gloves and the knife.” The scenario was tested and found plausible.

Forensic trace DNA: a review
http://investigativegenetics.biomedcentral.com/articles/10.1186/2041-2223-1-14
History and discussion on STRs and PCR.
Touch/trace DNA should not be confused with LCN.
Touch DNA = collection of minute biological samples. Touched objects does not always result in low amounts of DNA.
LCN = often used to describe increased cycle numbers.
Trace DNA is herein defined as “any sample which may fall below the recommended thresholds at any stage of the process - detection, collection, extraction, amplification and interpretation.” Usually less than 100 pg DNA.
A sample defined as trace DNA in the recovery phase does not necessarily have to be trace DNA in later stages of the process.
The precise location of where to recover the trace DNA is very important.
Double swabbing (not necessarily wet + dry, can be wet+wet if all the moisture is recaptured by the first swab) is recommended for collection of trace DNA.
If the swab is allowed to dry before extraction less DNA will be extracted, if the dried swab is frozen before extraction the DNA recovery will be similar to if it would still have been wet.
LMD can be useful to differentiate relevant cells from other cell types which allow efficient DNA analysis from the minor cell type in a sample. Flow cytometry can be used to separate sperm cells from vaginal wash fluid.
Chelex and organic extraction can lead to up to 75 % loss of DNA. However, this loss is often not relevant.
Partial or complete profiles have been obtained from samples that did not pass the quantification limit.
Trace DNA samples should not always be discarded due to low quantification.
Reviews amplification methods for LCN samples, detection of the amplified product and interpretation.
Common problems with trace DNA amplification: 1) allele drop-out, 2) decreased heterozygote balance, 3) allele dop-in (stutter), 4) allele drop-in (contamination).
In analysis it can be useful with detection thresholds such as LOD (limit of detection), T (low template DNA threshold), MIT (match interpretation threshold) and LOQ (limit of quantification). However, the peak intensity should be included in the exclusion calculation and continuous measures should be used instead of definite thresholds.
Replicates should be employed for trace DNA analysis, four replicates with reported alleles detected in at least two of the replicates seems to be the most accurate. Bayesian based likelihood ratios of drop-outs can also be employed.
Mixed profiles can be challenging due to e.g. biased drop-outs, complicating drop-ins and over-amplification of some alleles.
Contamination of trace-DNA can be a serious problem. The contamination can be the major profile in the sample. Contamination can occur before the crime is committed (background DNA), between the crime is committed and the crime scene is secured, during the crime scene investigation and in the forensic laboratory.
Are you collecting all the available DNA from touched objects?
http://www.isfg.org/files/31f9316afbc584bc0befd4454d6cd38c4f064f3a.02004983_748131740352.pdf
Single swabbing might not be enough to retrieve all DNA. Extraction methods may not be as efficient as needed.
Fingerprint powder affected the DNA recovery by 25 %. Moreover, the powder can inhibit the DNA amplification.

DNA fingerprints from fingerprints
http://www.nature.com/scitable/content/DNA-fingerprints-from-fingerprints-11782
DNA can easily be extracted from the palm of a hand with a water-moistened cotton cloth. Dry hands or recently washed hands yielded less DNA.
Swabbing of regularly used objects all provided user-matched profiles. Washed objects used for a limited time also produced user profiles.
Indications of secondary transfer were observed.
The strongest profile on an object handled by several users was not always from the last user.

Preliminary investigation of differential tapelifting for sampling forensically relevant layered deposits
Evaluation of differential tape-lifting to separate different layers of DNA.
Setup touch/touch: Donor A rubbed a hand over a piece of polyester-cotton for 60 s three times. Donor B rubbed the same way on plastic. The fabric and the plastic were put together after 24 hours, either touchside against touchside or touchside of plastic against backside of fabric.
Setup saliva/touch: Saliva from donor C was applied to fabric. Donor A rubbed a hand on plastic. The fabric and the plastic were put together after 24 hours, either touchside against touchside or touchside of plastic against backside of fabric.
Setup: touch/saliva: Donor A rubbed a hand on fabric. Saliva from donor C was applied on the touchside or the backside of the fabric and allowed to dry for 24 hours.
Setup sample collection: Light tapping = one taping /area on the substrate. Maximum collection = 16 tapings/substrate. Tapes used: Scotch® Magic™, Scenesafe FAST™. Taped substrate was also cut for extraction of DNA.
Results: “This study has demonstrated that there is no clear preference of sampling method when attempting to differentially sample deposits of touch DNA layered over a pre-existing DNA background.” “…the selective collection of saliva from a background of touch DNA is not enhanced by tapelifting; rather, it seems that the resulting profile reflects the ratio of the respective deposits.” “…DNA is easily transferred through the polyester–cotton fabric to the other side.”

Evaluation of tapelifting as a collection method for touch DNA
Comparison between Scotch® Magic™, Scenesafe FAST™ and wet/dry-swabbing for sampling touch DNA on different fabric types.
“Significantly more DNA was extracted, and a higher proportion of alleles detected, from Scenesafe FAST tape than from Scotch Magic tape.” Additionally, profiles were of higher quality with Scenesafe compared with Scotch. Tapelifting was more efficient than swabbing for all fabric types examined except flannelette where swabbing was as efficient.
Trace DNA: A review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact
The use of trace-DNA is possible in forensic applications. Examples and guidelines.

Touch DNA: Forensic collection and application to investigations
“… the scraping/tape lift methods are ideal in situations where the scientist can locate areas on the item which are most likely to contain the perpetrator’s skin cells.”
“…an increased chance of obtaining mixed DNA profiles containing DNA from individuals that may have come into contact with the victim/evidence item near the time of the crime. Contributors to these mixtures could include the victim’s spouse or children, and again, elimination samples may need to be collected from these individuals.”
“Some evidence items are also not recommended for the collection of Touch DNA samples. Such items include those that are severely degraded (for example, moldy clothing), have been exposed to extreme environmental conditions (such as weapons left outside for months or years), have been washed, or are heavily soaked in the victim’s body fluids. Also, items that are likely to have been touched by many people, such as a public pay phone or store counter are usually not good sources for probative or interpretable Touch DNA profiles.”

DNA typing of shed epithelial cells
Winkler L-M., 2011 research project.
http://dare.uva.nl/cgi/arno/show.cgi?fid=342000
Recommends the collection of skin flakes with the aid of microscopy instead of swabbing/taping in order to avoid mixed profiles.

DNA fingerprinting secondary transfer from different skin areas: morphological and genetic studies
Touch DNA is traditionally considered to come from shed keratinocytes. This study shows that sebaceous fluid represents an important vector responsible for DNA transfer.
“…we confirmed that in DNA secondary transfer, under the ‘ideal’ conditions of clean objects (glass slides) and washed hands, the full profile of an individual can be recovered from an item that he/she had not touched while the profile of the person (vector) having contact with that item was not observed. Thus the ‘single full profile’ may be misleading in terms of determining who actually had contact with an item.”
“The results obtained indicate that ‘touch DNA’ secondary transfer is indeed an important phenomenon but we should consider the specific touched cutaneous area in the evaluation of the genetic results.” …” we show that secondary transfer of DNA traces originates from sebum rather than from keratinocytes, following contact with different skin areas.”

3.17 Transfer
Fingerprints as evidence for genetic profile: morphological study on fingerprints and analysis of exogenous and individual factors affecting DNA typing
http://www.crime-scene-investigator.net/fingerprintDNAextraction.pdf
Setup: 347 fingerprints, 11 persons, glass/wood/metal, 30s, with/without handwashing.
Results: Recovery 0,04-0,2 ng.
Handwashing reduced the amount of recovered DNA. “Shedder status” of donor is very important regarding how much DNA can be recovered from a fingerprint.

“… LCN method enhances the amplification of spurious alleles present in fingerprints, but laboratory-based contamination cannot be excluded, since standard negative controls of amplification cannot reveal lower levels of contamination.”

“When fingerprints were made after subjects had touched objects of routine use, including computer keyboards used by many people, mixed alleles from multiple donors were observed. In many cases, the peaks of accessory alleles reached those of true alleles, hindering definite allele typing.”

**Persistence of DNA from laundered semen stains: Implications for child sex trafficking cases**


**Setup:** semen stains (one or two (1:1) donors) were placed on clothes (T-shirt, trousers, tights) and stored in a wardrobe for 8 months. Items were washed (together with unstained socks) at 30°C or 60°C and with non-biological or biological detergent.  

**Results:** High quantities of DNA (6-18 µg) were recovered irrespective of washing conditions. The quantity did not decline significantly with repeated washes.  

T-shirt was better than trousers when there was more than one donor (trousers --> one major DNA profile).  

DNA could be recovered from the unstained socks washed together with the stained clothes.

**Probability of detection of DNA deposited by habitual wearer and/or the second individual who touched the garment**


**Setup:** 63 males wore their own underpants for 12 h, the inside waistband was then touched by one of 11 females for 15 s. The waistband was mini-taped and subjected to DNA profiling.  

**Results:** The wearer was detected in 51 % of the samples and always as the major contributor. The test-female was detected in 11 % of the samples.  

Reportable background DNA (non-wearer, non-test-female) was found in 14 % of the samples. There was no statistical difference in DNA quantification results depending on the time of collection (0 or 12 weeks after wearing and touching).  

If a single DNA profile is found on e.g. a pair of underwear, it is 5 times more likely that the person has worn the garment than just touched it. A high total DNA concentration would support wearing the garment.

**Oral intercourse or secondary transfer? A Bayesian approach of salivary amylase and foreign DNA findings**


Oral intercourse leads to salivary-α-amylase in penile swabs. However, salivary-α-amylase in penile swabs does not necessarily mean oral intercourse but may be due to secondary transfer.  

**Setup:** This is a study of background salivary-α-amylase in underwear after normal (12 hour) wear (69 male subjects). Masturbation with saliva as lubricant was allowed but oral intercourse was not.  

**Test method:** Phadebas press test for screening and RSID-Saliva for confirmation.  

**Results:** 44 % of the underwear had stains containing amylase on the inside front, 13 % of these stains were found to be salivary-α-amylase (5,7 % of total). 50 % of these produced a mixed DNA profile with a minimum of two donors.  

A positive control group with oral intercourse showed larger salivary-α-amylase positive stains in the male underwear than the background-test group. 80 % of the underwear in this group gave DNA profiles other than the wearers.  

“…the evidence of salivary-α-amylase on male underwear (RSID™-Saliva positive) is 17 times more likely to occur with the allegation of fellatio than the alternative hypothesis of secondary transfer. However the finding of no salivary-α-amylase (RSID™-Saliva negative) upon the male underwear is a 100 times more likely if fellatio did not occur rather than if somebody performed fellatio on Mr B.
Likewise, it is evident that if salivary-α-amylase and DNA other than the wearer is present on the inside-front of male underpants that it is 27 times more likely if fellatio occurred rather than fellatio did not occur. In saying that, if salivary-α-amylase is found yet the DNA profile does not contain a profile other than that of the wearer, it is 13 times more likely if fellatio occurred rather than if nobody performed fellatio on an alleged victim.”

This paper also contains a section about the sensitivity and specificity of the Phaebas press test and RSID-Saliva; Phadebas press test cannot distinguish between salivary-α-amylase and pancreatic amylase which leads to false positive results, therefore it should only be used for screening.

A review of the science of low template DNA analysis

Budlowe (2009) is critical to the conclusions of this review: “The reviewers found that LCN typing as practiced specifically in the United Kingdom was “robust” and “fit for purpose” but offered a number of recommendations to improve the methodology. The findings of the commission seem inconsistent with the nature of LCN typing and LCN typing warrants a more in-depth evaluation by the greater scientific community.”

This review address concerns about Low template DNA analyses (LTDNA) for legal purposes, instigated by the Forensic Science Regulator and contains guidelines, recommendations and validation for use of LTDNA.

Transfer of cellular material:
Individuals vary in their propensity to transfer their cellular material to an object.
Time and pressure affects the efficiency of the transfer.
(Person→ object = primary transfer. To next object/person = secondary transfer.)

Could secondary DNA transfer falsely place someone at the scene of a crime?

Setup: Hand to hand contact for two minutes, then handling a knife (smooth or rough handled knives). Knives were immediately swabbed for DNA.
Results: Secondary transfer was detected in 85 % of the samples. In some cases the secondary contributor was the major or the only identified DNA profile.
There was no significant difference in concentration of DNA between smooth and rough handled knives.
For one smooth-handled knife the major contributor was not one of the test-subjects nor one of the personnel.

Forensic DNA evidence is not infallible.
Cale CM., Nature 2015; 526: 611
This is a one page “personal take” on the subject of secondary transfer of DNA. For more detailed reading see “Could secondary DNA transfer falsely place someone at the scene of a crime?” by Cale et al. J Forensic Sci 2015.

DNA transfer: informed judgement or mere guesswork?
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3872334/
Opinion article.
“…the quantity of DNA or the quality of the profile cannot be used “to reliably infer the mode of transfer by which the DNA came to be on the surface of interest.” (Meakin and Jamieson 2013).
The transfer of touch DNA from hands to glass, fabric and wood
Setup: Glass/fabric/wood, 100 volunteers per type of material, 60 s contact, objects were mini taped.
Results: Wood gave the best yield of DNA (36 % gave handler profile) followed by fabric (23 %) and then glass (9 %). 10 % of the total number of samples gave mixed profiles indicating secondary transfer.

Assessing primary, secondary and tertiary DNA transfer using the Promega ESI-17 Fast PCR chemistry
Setup: Volunteers washed thoroughly, spent an hour doing every day activities, shook hands with one or two other persons in the experiment for 30 s and then gripped a plastic tube. The tube was double swabbed for DNA.
Results: In some samples there was more DNA from secondary transfer than from primary. In some samples there was DNA from a donor not in the study.

Assessment of individual shedder status and implication for secondary DNA transfer
The shedder status of an individual is very important for recovery of DNA from handled items. If the primary user is a poor shedder and a secondary transfer person is a good shedder the dominating (or only) profile could be from the secondary person.

Contamination during criminal investigation: Detecting police contamination and secondary DNA transfer from evidence bags
More sensitive analyses require more attention regarding contamination.
Setup: 1) Environmental DNA monitoring of commonly touched surfaces in examination rooms at two large police units.
3) Study of whether DNA from the outside of evidence bags can transfer to the material (swabs and fabric).
Results: 1) High risk- low risk- and medium risk surfaces (rulers, chairs, glove boxes, sides of roles with bench paper…) contained significant amounts of DNA from the police officers working in that room. Only 12 of the analyzed 45 areas had no or few peaks above threshold (200 rfu).
2) 16 incidents of police officer contamination were found in the retrospective matching of DNA and case work. In 6 of these cases the detected police officer was not directly involved in the case work. 12 of the contaminations were from swabs collected by the police officers and 4 were from exhibits sent to the laboratory for DNA collection. However, the number of contaminated samples related to the total amount of samples is < 1 %. The laboratory caused significantly less contamination incidents than police officers.
3) Evidence bags were handled without gloves on the outer surface and then handled as usual. 60 swabs were analyzed and one contamination event was detected (full profile). 20 pieces of fabric were analyzed and one full profile and 11 partial profiles (0-6 alleles >200 rfu, 10-29 alleles >30 rfu) were recovered. There was a correlation between the amount of DNA on the bags and the contamination events.
Conclusion: Secondary transfer can occur during examination. Shaking a contaminated evidence bag over the material can lead to contamination of the material. Contaminated opening edges of the evidence bags can also contaminate the material. The authors present guidelines on how to avoid contamination.
Secondary and subsequent DNA transfer during criminal investigation
Detection of DNA transfer from object to plastic gloves worn by investigator (disposable nitrile-gloves) to new object. The amount of DNA deposited on the first object and the object material-type influenced the transfer.

Assessment of the possibility of DNA accumulation and transfer in a superglue chamber
DNA can be transferred from one object to another when treated in a superglue chamber (fingerprint technique) where multiple objects are often treated together or one after the other.

Analysis and implications of the miscarriages of justice of Amanda Knox and Raffaele Sollecito
LCN-levels of victim-DNA (no detection of blood) on a knife, analyzed without replication and with only a few alleles, were used to convict a suspect. Moreover, the location of the suspect-DNA on the handle was interpreted (without scientific grounds) as a result from stabbing rather than cutting food. The suspect and the victim shared an apartment. Evidence was collected using dirty gloves and in non-sterile containers. Mixed profiles of the two roommates were interpreted as one’s involvement in the murder of the other but was most probably just normal background DNA from two people living together.
“Selective cleaning” was believed to have erased the DNA from two suspects but not the third at the murder-scene.
Negative controls during the DNA analysis was interpreted as that the evidence could not have been contaminated in any way, either before the murder, at collection of the evidence or during handling of the evidence by the police officers.

The complexities of DNA transfer during a social setting
A study on “effects of unstructured social interactions on the transfer of touch DNA”.
Setup: Three individuals were video-recorded while having a drink (one central jug and individual glasses) together around a table for 20 minutes. All relevant surfaces were sampled for DNA and the video was used to identify how the DNA was transferred.
Results: Transfer occurred during everyday interactions and was found to be bi-directional.
The last person to touch an object was often but not always the major or the only DNA contributor. The participants often introduced DNA from persons not included in the experiment setup.

DNA transfer during social interactions
Very similar to Goray 2015.
Setup: Three individuals having a drink (one central jug and individual glasses) together around a table for 20 minutes. All relevant surfaces were sampled for DNA. Same results as Goray 2015.

DNA transfer within forensic exhibit packaging: potential for DNA loss and relocation
Analysis of DNA persistence and transfer during packaging in forensic investigations.
“DNA can be transferred from the deposit area to other parts of the item or to the bag (package) itself and usually to both”. “The effect of bag size on transfer was limited but loose bags can, in certain situations, permit more transfer”.

Nationellt forensiskt centrum, NFC – Biologisektionen
“DNA was lost to the inside of the container holding bloodied knives”, also a lot of re-distribution of DNA on the knives. Tighter fitting of the container prevented re-distribution of DNA from the tip to the handle.
Cigarette butts should always be packed separately.
Less re-distribution of DNA on gloves when they were packed in paper compared with plastic.

**Evaluation of multiple transfer of DNA using mock case scenarios**
Different experimental case scenarios illustrate the likelihood and expected proportion of DNA transfer. Further work is needed to draw conclusions according to the authors.

**Investigation of secondary DNA transfer of skin cells under controlled test conditions**
“The transfer rates showed that both the primary and secondary type of substrate and the manner of contact are important factors influencing transfer of skin cells… the freshness of the deposit in most instances is not.” “Skin cells deposited on a non-porous primary substrate transferred more readily to subsequent substrates than those deposited on a porous substrate. Porous secondary substrates, however, facilitated transfer more readily than non-porous secondary substrates, from both porous and non-porous surfaces. Friction as the manner of contact significantly increased the rate of transfer.”

**Secondary DNA transfer of biological substances under varying test conditions**
“Porous substrates and/or dry samples diminished transfer… whereas non-porous substrates and/or wet samples facilitated the transfer events”. At secondary transfer porous substrates absorbed transferred samples better than non-porous ones. Friction increased the transfer compared to passive or pressure contact.

**Investigation into “normal” background DNA on adults necks: Implications for DNA profiling of manual strangulation victims**
Setup: Optimization of method for collection, extraction and amplification of DNA from skin.
Measurements of background DNA on skin (neck) and simulation of an assault to study the transfer of DNA between offender and victim.
Results: The necks of 24 volunteers were swabbed and 23 % showed non-donor alleles. After simulated strangulation primary, secondary and zero transfer of victim and/or offender DNA could be detected. The background DNA was abundant enough to interfere with DNA profile interpretation after an assault.

**DNA transfer – a never ending story. A study on scenarios involving a second person as carrier**
Setup: The possibility of tertiary transfer onto textile and plastic was investigated. Donor’s neck --> cotton cloth --> carriers hand (with or without glove) --> plastic bag or cotton cloth.
Results: “… a transfer of donor DNA from epithelial cells through a carrier to a second item is possible, even if the carrier does not wear gloves.” In 11 % of the samples full donor profiles were detected and in 8 of these 21 samples the donor was the major profile. In ~50 % of the samples a carrier profile was detected. Full donor profiles were more frequently found when textile was the final object compared with plastic.
Experience is the name that everyone gives to their mistakes.
Jamieson A, Meakin G., Barrister Mag 2010; 45.  
Courts are skeptical to experts and prefer experience.  
The habitual wearer tends to be the major source of DNA on a garment. DNA can be deposited onto an object that the donor has never touched through transfer, depending on shedder status the transferred DNA can provide the major profile.

DNA transfer through nonintimate social contact.
A study on how much female DNA can transfer to male underwear and to the penis through nonintimate social contact.  
Setup: After staged nonintimate contact with females (massaging the female face for 2 min and rubbing hands for 3 min) and simulated urination, penile swabs and underwear were collected either 5 min or 6 hrs after the experiment. Samples from the underwear were collected by mini-taping five different areas (waistband, front panels (in- and outside) and back (in- and outside)). Penile swabs and underwear were also collected after unprotected sexual intercourse (without ejaculation). The underwear used was new and worn for 5 min after the intercourse before collected.  
Results: After nonintimate contact and sample collection after 5 min matching female (partial) profiles were detected in 5/30 waistbands and 1/30 outside panels (1 matching allele, 56 rfu). The maximum peak height on the waistbands ranged from 180-816 rfu. 4/30 penile swabs resulted in female DNA with a maximum of 5 alleles and 166 rfu. When samples and underwear was collected after 6 hrs female DNA was detected in 1/14 waistbands (maximum peak height 161 rfu) and in no penile swabs. When unprotected sexual intercourse preceded the sample collection female profiles were found in all samples. Maximum peak height ranges were: waistband (all full profiles) 1386-1357 rfu, inside front (all full profiles) 1898-3157 rfu, penile shaft (all full profiles) 958-5835 rfu. Full profiles were also detected on all samples from outside front as well as inside and outside the back of the underwear.  
Conclusion: The authors claim that “if DNA matching the female complainant is found on the waistband of a male suspect’s underwear, the data obtained in this study suggest that depending on the time delay before the underpants are seized, a matching female DNA profile below 1000 rfu might be explained by nonintimate social contact with secondary transfer of female DNA from the male’s hands.”

Everything clean? Transfer of DNA traces between textiles in the washtub  
Transfer of DNA from worn clothing (without bloodstains) to another garment is highly unlikely both during hand- and machine washing. Blood can easily be transferred to other garments during the washing procedures.

Good shedder or bad shedder - the influence of skin diseases on forensic DNA analysis from epithelial abrasions  
http://link.springer.com/article/10.1007%2Fs00414-011-0579-0#page-1
Certain skin diseases (e.g. atopic dermatitis, ulcer and psoriasis) lead to high proliferation rate of the keratinocytes. This could mean that the individuals are “good shedders” and render very good DNA profiles from touch DNA. However, the shedder status can be affected by disease-treatment. Moreover, shed cells can contain partially degraded DNA.
The retention and transfer of spermatozoa in clothing by machine washing
http://www.nlada.org/DMS/Documents/1031178399.08/Laundry-sperm%20transfer.pdf
Washing: 10 minute warm wash, cold rinse and phosphate-free detergent of semen stained clothes rendered negative AP-tests but significant numbers of spermatozoa were retained. Small numbers of spermatozoa were transferred to previously unstained clothes in the washing-procedure.

Assessment of DNA transfer involving routine human behavior
Kelley, S., Fort Worth, Tx: University of North Texas Health Science Center; thesis 2010.
http://digitalcommons.hsc.unt.edu/cgi/viewcontent.cgi?article=1098&context=theses
A study on secondary transfer in a setup that mimics normal day behaviour regarding saliva and sweat.
Setup: Volunteers were instructed to e.g. lick their thumb (as when turning a page) and after 5-30 minutes shake hands with a person B who would then grip a plastic tube, samples were collected from the tube. Experiments involving pen-licking and simulated sweat were also included in different combinations.
Results: 28 amplification cycles on 208 samples resulted in 10 samples with one allele or more. Five of these 10 were setup as follows: person A bites a pen for 2 minutes, after 30 minutes the the palm of person B is sprayed with water to stimulate sweat, then person B holds the pen and after different time intervals person B grips a plastic tube which is swabbed for analysis. 12 samples were reanalysed with 34 amplification cycles and the profiles were interpreted, all but one of the profiles were partial. The profiles were dominantly from person A and in two samples only from person A.

The recovery and persistence of salivary DNA on human skin
Setup: Saliva from men was deposited on female skin and left there for 96 hours.
Results: Full male profiles were obtained after 96 hours in 8 of 9 cases. Tapelifting was the most efficient method to recover the DNA (compared to wet and dry swabbing). Dried salivary DNA on skin transferred to fabric, cotton was most efficient and leather least efficient for DNA transfer.

IPV - Bridging the juridical gap between scratches and DNA detection under fingernails of cohabitating partners
(IPV = Intimate partner violence)
The respective partners DNA will be under the fingernails in 17 % of couples that live together.
Setup: Female vigorously scratched male back.
Results: There was a highly significant difference in absolute and relative male DNA under scratching and non-scratching fingers. The mean difference was 16-22-fold depending on finger. However, a negative result for male DNA did not mean the nail was not involved in the scratching.

A systematic analysis of secondary DNA transfer
http://projects.nfstc.org/workshops/resources/literature/A%20Systematic%20Analysis%20of%20SecondaryDNA.pdf
The data in this paper does not suggest that secondary transfer can interfere with DNA typing results under typical forensic conditions.
Setup: 1) Handshaking and holding of object (rubbing in both instances), 2) Coffee mugs were handled for 2 hours and then handled by another user. Palms and objects were swabbed. 3) Commonly handled objects were swabbed for primary transfer.
Results: Secondary transfer was not detected. Primary transfer could yield interpretable results.
Following the transfer of DNA: How does the presence of background DNA affect the transfer and detection of a target source of DNA?
Setup: wet blood, dried blood or touch DNA in five transfer steps on glass or on cotton fabric. Background biological material was deposited on the substrates, either from the same individual on all substrates or from different individuals on different substrates in the transfer series.
Results: In most cases the deposited background DNA affected the DNA profiling of the target sample. Background blood (wet and dry) affected more than touch DNA and if the substrate was cotton background DNA from blood made DNA profiling impossible. If the background DNA was touch DNA full target DNA profiles could mostly be obtained, even after multiple transfer steps. It seemed like the transfer of the target sample was inhibited by the presence of background DNA on the substrates. Moreover, detection of the target DNA decreased with an increased number of background donors.
See paper for specific results regarding different combinations of samples and substrates.

Following the transfer of DNA: How far can it go?
Setup: Wet blood, dry blood or Touch DNA was transferred (15 s, 1,4 kg) on glass or cotton substrates.
Results: Wet blood could transfer full profiles to the fourth cotton and sixth glass substrate. Dry blood and Touch DNA gave full profiles on the first cotton substrate only. Dry blood on glass could transfer full profiles to the sixth substrate while Touch DNA on glass gave partial profiles from the second to the fifth substrate.

Use of low copy number DNA in forensic inference
http://www.isfg.org/files/31f9316afbc584bc0befd4454d6cd38c4f064f3a_02004843_693490260903.pdf
Discussion about shedder status and the likelihood of getting DNA profiles from Touch DNA and used garments. E.g. a good shedder can be the major profile on a garment worn by a poor shedder. Also, a good shedder can be the major profile after a secondary transfer event with a poor shedder.

The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces
Individuals differ in their tendency to deposit DNA. Hand washing seemed to be of importance. A good shedder could deposit DNA close after hand washing while a poor shedder required longer time after washing. If the time period after hand washing is between 2-6 hours the shedder status may not be of big importance. Secondary transfer is a real possibility in forensic case work.

The prevalence of mixed DNA profiles in fingernail samples taken from couples who co-habit using autosomal and Y-STRs
Setup: Fingernail swabs from 12 cohabiting couples.
Results: Mixed profiles in 17 % of the samples. The majority on non-donor alleles belonged to the partner. Mixture ratios ranged from 20:1 to 1:2. Female swabs were analyzed with Y-filer and in 63 % of the samples gave full or partial Y-chromosome profiles even though no foreign profile was detected during regular analysis.
Sex-specific age association with primary DNA transfer
Addresses the question why DNA can sometimes be found on objects handled without gloves and sometimes not.

Setup: 83 males and 45 females, aged 18-75 years of age, held a plastic tube for 5 minutes in each of their unwashed hands (768 samples).

Results: Men were found to be better shedders than women. Younger men were better shedders (under age of 45) than older men, no statistical significance for female age. Mean shedding was found highest from the non-dominant hand in men (not sign for women). Shedding status changed between the repetitions of the experiment for most test-subjects.

Primary transfer: “… 11 % of the attempts resulted in no detectable genetic profiles, 72 % produced partial genetic profiles, and 17 % revealed the presence of full genetic profiles.” “…in 51 %, no secondary transfer was observed and in 34 % secondary transfer was low (one to three alleles).” “… secondary transfer of six or more alleles was observed in 9 % of the samples.”
Secondary transfer: 85 % resulted in 0-3 non-donor alleles. 9 % resulted in 6-34 non-donor alleles. The secondary transfer could always be distinguished from the primary transfer due to lower peak-heights.

The deposition and persistence of indirectly-transferred DNA on regularly-used knives
Setup: “Volunteers each used a set of knives regularly over a period of two days, after which, each of these ‘regular users’ shook hands with another person (‘handshaker’) and then immediately, without touching anything else, repeatedly stabbed one of their own regularly-used knives into foam for 60 s. DNA was recovered from the knife handles using mini-tapes approximately one hour, one day, and one week after the stabbings.”

Results: Secondary transfer occurred in three out of four experiments. User:Handshaker 10:1. Alleles from the handshaker could be found after a week but numbers and peak heights were reduced.

DNA transfer: Review and implications for casework
Review on factors (shedder status, surfaces, time aspects…) and probabilities of transfer.

Exploring the relative DNA contribution of first and second object’s users on mock touch DNA mixtures
Setup: First user handled an object (plastic, metal, fabric or nitrile) for 8-10 days. Second user handled the object for 5 min, 30 min or 120 min. Hands were washed at least two hours before handling the object. The % contribution of the second user increased in proportion to the time the object was handled.

Results: A plastic bracelet gave 40 % second user even after 5 minutes handling. The bracelet also showed 10 % profile that did not belong to either the first or second user. In one third of the evaluated samples the second user was eventually the major contributor.

DNA profiling of trace DNA recovered from bedding
Setup: Five volunteers slept one night (in their own, freshly laundered night wear) on a new lower bed sheet (the rest of the sheets were unwashed) in their own bed and then one night in a foreign bed. Samples were collected from upper shoulder, mid body and foot areas on the sheets.

Results: Own bed, new lower sheet – “DNA from a second individual was obtained from at least one of the sheet samples collected from three of the five volunteers” (from member of household).
Foreign bed, new lower sheet, same nightwear as in own bed – “At least one of the three samples collected from each volunteer’s sheet provided the DNA profile of the volunteer sleeper after one night in a bed foreign to them. In addition, DNA profiles from at least one sample collected from sheets of four of the volunteers showed the presence of additional DNA resulting in mixed DNA profiles.”

**The tendency of individuals to transfer DNA to handled items**

**Setup:** 60 volunteers held a sterile plastic tube for 10 s with one washed or unwashed hand. (washed hands were used normally for 15 minutes before touching the tube).

**Definition:** Good shedder – leaves a median result of a full profile after washing their hands, Bad shedder – leaves partial or no profiles (Lowe et al.).

**Results:** A persons shedding-status changes over time (4 days) and a person can vary in himself as much as compared with other persons. No good shedders were identified in the study. The longer the persons went without washing their hands, the more DNA they seemed to shed. People seemed to shed more from their dominant hand when hands were unwashed and more from the non-dominant hand when washed. The level of secondary transfer was very low. Compares with Lowe et al. where e.g. 18 of 30 volunteers were good shedders.

**Influence of an individual's age on the amount and interpretability of DNA left on touched items**

**Setup:** Handprints on plastic syringes from 213 persons, 1-89 years of age, were analyzed for amount of DNA and for STR profiles.

**Results:** Very old persons (>80 yrs) did not leave full profiles on the syringes. Very young persons (<10 yrs) left the most full profiles.

**Beware; gloves and equipment used during the examination of exhibits are potential vectors for transfer of DNA-containing material**
Poy A, van Oorschot RAH., Int Congr Ser 2006; 1288: 556-558. [Link](http://www.isfg.org/files/91a748919b016087a260b2ab392c8c8f79a21c0f.05014093_845812755086.pdf)

A paper very similar to the other paper by Poy 2006.

**Conclusions:** Regularly change gloves during examinations. Avoid contact with areas of the exhibit that are likely to be sampled for DNA analysis. Regularly clean tools and objects that may come in contact with an exhibit.

**Trace DNA presence, origin and transfer within a forensic biology laboratory and its potential effect on casework**

Gloves were at high risk of acting as vectors for DNA transfer.

Two other high contamination risk objects were a plastic ruler and a drying line for clothes.

DNA could be found on most investigated vectors in the whole forensic laboratory chain but the risk of transferring a profile that would interfere with analysis were medium to low.

**Trace DNA and street robbery: a criminalistic approach to DNA evidence**

DNA background levels on handbags and wallets was determined as well as the probability of transfer during and after a robbery.

Both owner and non-owner DNA could be recovered from “un-robbed” items. Simulated robberies resulted in majority mixtures or single profiles of the robber in 40 % of the cases.
Trace DNA: An underutilized resource or Pandora's box? A review of the use of trace DNA analysis in the investigation of volume crime
Since touch DNA cannot be derived to a specific source the activity level of the evidence might be problematic.
Secondary transfer reviewing.
Discussion of the problems with LCN in court.

The urban myths & conventional wisdom of transfer: DNA as trace evidence
Review of trace DNA and transfer – believers and non-believers of secondary transfer, the occurrence (or not) of good and bad shedders…

An investigation into the transference and survivability of human DNA following simulated manual strangulation with consideration of the problem of third party contamination
Setup: A man “strangled” a woman for 1 minute 29 separate times. Samples were collected by moist swabbing between 1 minute and 10 days after the “assault”. Samples were taken from man’s fingertips and woman’s neck, the neck was not touched or washed after the assault, the man’s fingertips were allowed normal daily routine. The pair worked in the same building but was not allowed direct contact during the experiment.
Results: Neck – 7 of 29 neck-swabs showed full offender profile up to 6 hours after the assault. By LCN offender alleles were present up to 10 days. Fingertips – 7 of 29 showed an offender + victim profile. 6 of 7 were partial victim profiles detected up to 24 hours after the assault. Caution: Control areas of neck and control fingertips also showed profiles from the other person in some cases up to 10 and 5 days after assault resp. Third party profiles were found on both control sites and test sites.

Trace DNA analysis: If your DNA is on the evidence, did you really touch it?
“…simply touching an object can leave anywhere from 0-169ng of DNA” (Meakin and Jamieson 2013).
“In 14 of 24 individuals tested, non-self DNA was found on their necks” (Graham and Rutty 2008).
“41 % of fingernail samples tested showed some evidence of a mixed DNA profile” (Dowlman et al. 2010).
“Touch DNA is known to last for up to 2 weeks outside and 6 weeks or longer inside” (Raymond et al. 2009).

Touch DNA. What is it? Where is it? How much can be found? And, how can it impact my case?
A question and answer guide to all things touch DNA
Review on: definition of touch DNA, how much DNA is left behind when an object is touched, is DNA always left on an object via touch? Factors that increase the amount of shed DNA, persistence of touch DNA, secondary transfer, can we tell who handled the item last? Which items can contain touch DNA?
Stabbing simulations and DNA transfer
Setup: 64 stabbing experiments by 4 persons. The same person participated both at 11 am and 4 pm for 8 days. Samples were collected by wet+dry swabbing. Knives were cleaned before the experiments.
Results: High intra and inter-person variability in DNA quantity (1-97 pg/µL). The stabber was the major profile in 83 % of the cases and the single profile in 3 % of the cases, the stabber was never the minor profile. In 50 % of the cases DNA was transferred from an unknown source and interpreted as a minor profile.

http://www.astm.org/DIGITAL_LIBRARY/JOURNALS/FORENSIC/PAGES/JFS2002315.htm
Caution must be taken regarding contamination from fingerprint powder and brushes when extracting DNA from fingerprints.

Residual DNA on examination tools following use
High risk vectors: scissors, forceps, gloves.
Setup: Dried blood or touch DNA, deposited on the primary substrate of cotton or glass, was transferred via the vector to the secondary DNA-free substrate. The vector was sampled directly after contact with the secondary substrate.
Results: The vectors contained DNA after touching the second substrate which means that further contamination can occur.

DNA transfer by examination tools – a risk for forensic casework?
High risk vectors: scissors, forceps, gloves.
Setup: Substrates: glass and cotton. “Dried blood or touch DNA, deposited on the primary substrate, was transferred via the vector to the secondary substrate, which was either DNA-free or contained a target sample (dried blood or touch DNA).”
Results: Touch DNA transferred less than blood. Transfer did not interfere with the target profile on the second substrate but could complicate the analysis when no suspect is known.

The potential transfer of trace DNA via high risk vectors during exhibit examination
High risk vectors: scissors, forceps, gloves.
Light contamination: single touch/cut for 2-3 seconds with vector.
Heavy contamination: multiple cuts/touches.
Results: “DNA was transferred for all vectors in both heavy and light contamination scenarios”. Acceptable profiles were observed for all scenarios except forceps light contamination.

Evaluating forensic DNA evidence – essential elements of a competent defense review
Thomson WC, Ford S, Doom T, Raymer M, Krane DE.
http://www.americanbar.org/content/dam/aba/events/legal_aid_indigent_defendants/2015/ls_sclaid_summit_03e_champion2.authcheckdam.pdf
Tertiary transfer in trial. “He and his wife had shared a towel the morning of the murder - perhaps his DNA was transferred from his face to the towel, and from the towel to his wife’s face. His wife was
later attacked by a glove-wearing stranger who struck her on the face, strangled her, and stabbed her, in
the process transferring husband’s DNA from his wife’s face to the gloves and the knife.” The scenario
was tested and found plausible.

Activities between activities of focus – Relevant when assessing DNA transfer probabilities
Genet Suppl Ser 2015; 5: e75-77.
A study of how long every-day objects are touched by video-monitoring.
On average, a person performs ~15 touches/60 seconds.
“As more items are contacted by the originally deposited biological sample, the greater the likelihood
of it no longer being detected on the surface it was originally deposited on, or on the surface it was
considered ultimately to have been transferred.”

DNA transfer: The role of temperature and drying time
“The speed at which blood dries is dependent on the temperature, with the drying process complete
within 15–60 min.” “The percentage of deposited DNA transferred upon contact follows an exponential
pattern of decline from soon after deposition, decreasing until the sample is dry.” “When considering
the likelihood of a proposed scenario that incorporates one or more contact situations it is important to
consider the timing of the potential transfer event(s) relative to when the biological sample in question
was initially deposited.”

Persistence of DNA deposited by the original user on objects after subsequent use by a second
person
“Our findings show that the profile, percentage contribution of the 1st user relative to the 2nd user of an
object declines in a linear manner, over time.”
Setup:
a) Non-porous, hard, flat surface object (pens and pen lids). Person 1 rubbed 49 new, cleaned pens
between his/her hands and the lids was removed and replaced once a day for four days (30s/60s/60s/60s
rubbing/day). Five of the rubbed pens were taken as controls. The rest of the 44 pens were given to
Person 2 (one person/pen). Person 2 used the pen as normally when writing and documented the
duration, the activity and details about e.g. contact with skin and other materials. Sample collection by
wet+dry swab.
b) Porous worn object (bracelets of elastic fabric). Person 1 wore rubber-band bracelets for 34 hours
(divided in five days) and also rubbed the bracelets with his/her hand for 30s before removing them
every day. 4x17 of these bracelets were extra rubbed and given to a person 2. Six were also rubbed
again and stored as control. Person 2 used the bracelets and documented when they were temporarily
removed, total usage for person 2 was 5, 10, 20, 40 h and 1, 2, 4, 8 days. Sample collection by cutting
the bracelets into small pieces.
c) Wide range of every day personal objects used by a second person. Sample collection by wet+dry
swab on hard surfaces and tape lifting on soft/porous surfaces.
Results:
a) The 2nd user was the dominant profile on the pen after 30 minutes of use (50/50 after 1 minute) and
on the lid after 3 times of removing/replacing the lid (50/50 after 1 time).
b) A large number of “1st wearer derived unknown alleles” in the control bracelets (15 %). The 2nd user
was the dominant profile on the bracelet after 29 hours of use, the increase of the 2nd user was linear
over time.
c) Many details to consider in this part of the experiment. Well-worn watches retained the 1st user as
dominant after 10 days of use by 2nd user. E.g. sunglasses, perfume bottles, lighters etc. were dominated
by the 2nd user relatively quickly. A well-worn cap used by 2nd user for 2 weeks (12 hours in total) was
dominated by the 2nd users profile but the 1st user was still a full profile.
Forensic trace DNA: a review
http://investigativegenetics.biomedcentral.com/articles/10.1186/2041-2223-1-14
History and discussion on STRs and PCR.
Touch/trace DNA should not be confused with LCN.
Touch DNA = collection of minute biological samples. Touched objects does not always result in low amounts of DNA.
LCN = often used to describe increased cycle numbers.
Trace DNA is herein defined as “any sample which may fall below the recommended thresholds at any stage of the process - detection, collection, extraction, amplification and interpretation.” Usually less than 100 pg DNA.
A sample defined as trace DNA in the recovery phase does not necessarily have to be trace DNA in later stages of the process.
The precise location of where to recover the trace DNA is very important.
Double swabbing (not necessarily wet + dry, can be wet+wet if all the moisture is recaptured by the first swab) is recommended for collection of trace DNA.
If the swab is allowed to dry before extraction less DNA will be extracted, if the dried swab is frozen before extraction the DNA recovery will be similar to if it would still have been wet.
LMD can be useful to differentiate relevant cells from other cell types which allow efficient DNA analysis from the minor cell type in a sample. Flow cytometry can be used to separate sperm cells from vaginal wash fluid.
Chelex and organic extraction can lead to up to 75 % loss of DNA. However, this loss is often not relevant.
Partial or complete profiles have been obtained from samples that did not pass the quantification limit.
Trace DNA samples should not always be discarded due to low quantification.
Reviews amplification methods for LCN samples, detection of the amplified product and interpretation.
Common problems with trace DNA amplification: 1) allele drop-out, 2) decreased heterozygote balance, 3) allele dop-in (stutter), 4) allele drop-in (contamination).
In analysis it can be useful with detection thresholds such as LOD (limit of detection), T (low template DNA threshold), MIT (match interpretation threshold) and LOQ (limit of quantification). However, the peak intensity should be included in the exclusion calculation and continuous measures should be used instead of definite thresholds.
Replicates should be employed for trace DNA analysis, four replicates with reported alleles detected in at least two of the replicates seems to be the most accurate. Bayesian based likelihood ratios of drop-outs can also be employed.
Mixed profiles can be challenging due to e.g. biased drop-outs, complicating drop-ins and over-amplification of some alleles.
Contamination of trace-DNA can be a serious problem. The contamination can be the major profile in the sample. Contamination can occur before the crime is committed (background DNA), between the crime is committed and the crime scene is secured, during the crime scene investigation and in the forensic laboratory.

Impact of relevant variables on the transfer of biological substances
Setup: Calculations based on data from Goray et.al (2009) on how much biological material would have had to be deposited at the original source to yield enough DNA (1 ng) after up to 5 transfer steps.
Results: “We demonstrate that, in many scenarios incorporating multiple transfer steps, unrealistically large amounts of biological material would need to be present at source to generate a detectable level of DNA from the targeted crime scene surface.”
DNA fingerprints from fingerprints
http://www.nature.com/scitable/content/DNA-fingerprints-from-fingerprints-11782
DNA can easily be extracted from the palm of a hand with a water-moistened cotton cloth. Dry hands or recently washed hands yielded less DNA. Swabbing of regularly used objects all provided user-matched profiles. Washed objects used for a limited time also produced user profiles. Indications of secondary transfer were observed. The strongest profile on an object handled by several users was not always from the last user.

The influence of substrate on DNA transfer and extraction efficiency
Setup: Nine types of substrates were examined regarding their influence on DNA transfer from blood.
Results: Less DNA was transferred if the blood was dry. More DNA was transferred if friction was applied. Porous substrates retained more DNA and transferred less. Fabrics transferred less DNA than porous substrates. Natural fibers are considered more porous than artificial fibers. Blood on polyester formed droplets on top of the fabric --> transfer can occur easier. The extraction efficiency differed between the different substrates. Extraction from plastic provided the least DNA (modified Chelex protocol). Suggestion: Correction factors should be applied to reduce errors associated with loss of DNA during processing.

An evaluation of the transfer of saliva-derived DNA
Moisture and a smooth surface were shown to increase transfer. Moisture in the first event of transfer was more significant than moisture in the following events. When saliva is the source of DNA the primary depositor was found to be the major profile. In each transfer event substantial losses of DNA was observed.

Trace DNA: A review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact
The use of trace-DNA is possible in forensic applications. Examples and guidelines.

Transfer of biological stains from different surfaces
An experimental contamination study on blood and saliva.
Setup: 50 µL saliva or blood was placed on paper/cotton/plastic and allowed to dry.
   a) 2 s pressing a thumb on the stain with or without gloves.
   b) 10 s rubbing a thumb on the stain with or without gloves.
   c) 10 s rubbing a thumb on the stain with or without gloves and then the thumb was placed on a piece of paper.
Sample collection by swabbing.
Results: DNA from saliva was transferred from paper and cotton in low but detectable amounts with both setup a) and b). < 10pg/µL. Setup c) resulted in very low amounts of DNA (<0.1 pg/µL) in the 50 % of the cases where DNA was detected at all. The transfer from plastic resulted in much higher DNA yields in all experimental setups (primary transfer up to 100 pg/µL and secondary transfer 0-10 pg/µL). DNA from blood was transferred poorly from fabric (~0.1 pg/µL) but higher yields were detected from paper and plastic (in some cases more than 100 pg/µL, even in secondary transfer).
DNA typing of epithelial cells after strangulation


Experimental study of DNA typing after strangulation. Success rate was > 70 %. Profiles were often a mix of the “suspect” and the “victim”.

Setup: 16 pairs. Upper arm used for strangulation for 1 min including arm movements by the victim.

Collection of DNA by 1) glass fibre pieces or 2) moistened cotton swabs. Polyacrylamide gel electrophoresis for visualization of the alleles.

Results: 0.5-1 ng DNA extracted for 14 of the 16 samples. For the other 2 samples ~2 ng was extracted.

Case study: Strangled victim found after 48 h. The neck was swabbed and the suspect could be identified.

DNA fingerprinting secondary transfer from different skin areas: morphological and genetic studies


Touch DNA is traditionally considered to come from shed keratinocytes. This study shows that sebaceous fluid represents an important vector responsible for DNA transfer.

“…we confirmed that in DNA secondary transfer, under the “ideal” conditions of clean objects (glass slides) and washed hands, the full profile of an individual can be recovered from an item that he/she had not touched while the profile of the person (vector) having contact with that item was not observed. Thus the “single full profile” may be misleading in terms of determining who actually had contact with an item.”

“The results obtained indicate that “touch DNA” secondary transfer is indeed an important phenomenon but we should consider the specific touched cutaneous area in the evaluation of the genetic results.” …” we show that secondary transfer of DNA traces originates from sebum rather than from keratinocytes, following contact with different skin areas.”

3.18 Washed stains/clothes

PCR DNA typing of washed stains


http://link.springer.com/chapter/10.1007%2F978-3-642-78782-9_90#page-1

Large amounts of semen, blood and saliva could be recovered after washing. The yield was inversely proportional to washing temperature (several washes at 95°C did not yield any DNA).

Addition of detergent substantially reduced the amount of recovered DNA.

Cotton and denim yielded the highest amounts of DNA.

E.g. a 10 µL semen stain on cotton could easily be extracted after 95°C wash with detergent.

A study on the effects of immersion in river water and seawater on blood, saliva, and sperm placed on objects mimicking crime scene exhibits


Study of the kinetics of DNA degradation of materials immersed in water for 6 h to 3 months.

Background: Recovery was possible after 3 months on e.g. condoms, cigarette butts and chewing gum. Bones and teeth were ok for 1mth to 50 yrs in humidity and soil. Skeletons in water were ok for 3 yrs. Ribs, skin, nails were ok for two months. Soft tissue --> rapid degradation. Bitemark on body yielded DNA after 5.5 hrs submerged in water. Spermatozoa could be found after full laundry (references in paper).

Results: Sperm and saliva was quite resistant to degradation in water, blood was more quickly degraded. Fibre-based (porous) substrates protected against degradation. Smooth surfaces did not preserve DNA efficiently. DNA in chewing gum was protective against degradation. No significant differences regarding dry/wet stains at the point of immersion in water. Saltwater --> much faster degradation.

Persistence of DNA from laundered semen stains: Implications for child sex trafficking cases
Setup: semen stains (one or two (1:1) donors) were placed on clothes (T-shirt, trousers, tights) and stored in a wardrobe for 8 months. Items were washed (together with unstained socks) at 30°C or 60°C and with non-biological or biological detergent.
Results: High quantities of DNA (6-18 µg) were recovered irrespective of washing conditions. The quantity did not decline significantly with repeated washes.
T-shirt was better than trousers when there was more than one donor (trousers --> one major DNA profile).
DNA could be recovered from the unstained socks washed together with the stained clothes.

The effect of laundering on the detection of acid phosphatase and spermatozoa on cotton T-shirts
http://www.tandfonline.com/doi/abs/10.1080/00085030.2000.10757498#.VeU7APntkXs
Setup: 12 cotton T-shirts were stained with 500 µL seminal fluid from one donor. The T-shirts were taken to different dry-cleaners for “normal treatment” or washed in a domestic washing machine at 18°C or 30°C with normal amount of detergent, two T-shirts were treated with “spot cleaner” before washing.
Results: Results from dry-cleaning: seminal fluid was detected after dry-cleaning if no spot-cleaner was used. Results from washing machine: Only 18°C without detergent gave positive result with the AP-test (acid phosphatase), however, even if the AP-test was negative there was still spermatozoa and STR DNA profiles could be identified after all different washing procedures.

Spermatozoa recovered on laundered clothing
Setup: Cotton briefs treated with semen were washed by different programmes. 40°C or 60°C washes with detergent or 60°C with detergent and softener.
Results: None of the samples were positive for acid phosphatase (AP) and all 60°C samples were negative for prostate specific antigen (PSA). Spermatozoa was detected in all 40°C washed samples and in ~50 % of the 60°C washed samples (could depend on longer storage before analysis). DNA was recovered from all the randomly selected samples, detection of twice the amount of DNA after 40°C wash compared to 60°C wash.

The Persistence of seminal constituents on panties after laundering. Significance to investigations of sexual assault
http://www.tandfonline.com/doi/abs/10.1080/00085030.2003.10757551#.VeU6VfnTkXs
Test-methods: 1) Blue Test for acid phosphatase, 2) microscopic identification of spermatozoa and 3) PSA ANAcard Test for detection of prostate specific antigen (PSA).
Results: Laundered clothing with semen stains could still produce DNA profiles, the efficiency depended on the type of fabric (cotton retained spermatozoa/DNA better than nylon). Negative AP-test was not reliable.
Effect of water immersion on seminal stains on cloth
Intact spermatozoa could be detected on cotton cloth after 120 hours immersion in water. However, the acid phosphatase activity was decreased and the spermatozoa were largely tail-less. At 144 hours (six days) the AP activity was still detectable but the number of intact sperm heads was decreased.

Everything clean? Transfer of DNA traces between textiles in the washtub
Transfer of DNA from worn clothing (without bloodstains) to another garment is highly unlikely both during hand- or machine washing. Blood can easily be transferred to other garments during the washing procedures.

The retention and transfer of spermatozoa in clothing by machine washing
http://www.nlada.org/DMS/Documents/1031178399.08/Laundry-sperm%20transfer.pdf
Washing: 10 minute warm wash, cold rinse and phosphate-free detergent of semen stained clothes rendered negative AP-tests but significant numbers of spermatozoa were retained. Small numbers of spermatozoa were transferred to previously unstained clothes in the washing-procedure.

Stability of acid phosphatase activity and spermatozoa in semen stains washed with water
http://ci.nii.ac.jp/naid/110000310895/en
Setup: Morphology and acid phosphatase activity of spermatozoa was studied after 1) washing with distilled water (in solution), 2) storage in water on a piece of cotton cloth 3) washing by sprinkled tap water on a piece of cotton cloth.
Results: 1) Morphology did not change significantly after washing with distilled water. 2) Morphology did not change after storage in water on a piece of cloth (5 days). The Acid phosphatase activity was significantly reduced after two days and abolished after three days. 3) Water sprinkled on semen-stained cloth resulted in negative acid phosphatase activity after three hours. Morphology was not changed after 10 hours of sprinkling.

The effect of washing on the detection of blood and seminal stains
http://www.tandfonline.com/doi/abs/10.1080/00085030.1971.10757279#.VeRiUFntkXs
Setup: Washing of semen or blood or semen+blood -stains was planted on new underwear (cellulose acetate fabric or cotton fabric). Detergents used were 1) for hot water 2) for cold water 3) for hot or cold water with enzymatic activity. 12 different washing procedures were used combining different lengths of soaking before washing and different detergents.
Results: No visible blood stains on cellulose acetate underwear were detected after washing and most washing procedures resulted in negative detection of blood with benzidine. The blood on cotton underwear was however more resistant and all washing procedures resulted in positive reaction with benzidine even though no visible stain was detected. Seminal stains were visibly not detected after any washing procedure on either cellulose acetate or cotton and the “fast blue” seminal stain was negative for most washing procedures, however, only the detergent containing enzymatic activity washed away the spermatozoa as detected by microscopy (spermatozoa could be found in some samples even after washing with the enzymatic detergent as well).
3.19 Weapons & arms

Persistence of biological traces at inside parts of a firearm from a case of multiple familial homicide
Backspatter on firearms. Profilable DNA could be found in both the barrel and in other places inside the firearm.

Persistence of biological traces in gun barrels - an approach to an experimental model
A study of backspatter (blood) in gun barrels: “…recovery of typable victim DNA from bloodstains within gun barrels is possible and that fully typable DNA in a gun barrel may endure even the physical strain produced by subsequent shots.”

DNA transfer within forensic exhibit packaging: potential for DNA loss and relocation
Analysis of DNA persistence and transfer during packaging in forensic investigations. “DNA can be transferred from the deposit area to other parts of the item or to the bag (package) itself and usually to both”. “The effect of bag size on transfer was limited but loose bags can, in certain situations, permit more transfer”. “DNA was lost to the inside of the container holding bloodied knives”, also a lot of re-distribution of DNA on the knives. Tighter fitting of the container prevented re-distribution of DNA from the tip to the handle. Cigarette butts should always be packed separately. Less re-distribution of DNA on gloves when they were packed in paper compared with plastic.

A single approach to the recovery of DNA and firearm discharge residue evidence
http://www.scienceandjusticejournal.com/article/S1355-0306(04)71680-9/abstract
The mini-tape used for recovery of firearm discharge residue (FDR) in this paper was successfully used for extraction of DNA after the FDR identification was done.

Development of STR profiles from firearms and fired cartridge cases
Test of different kits for DNA recovery from firearms and fired cartridge cases and where it is useful or not to swab for DNA (hull vs. head of shotshell cases and firearm surface areas).

Understanding DNA results within the case context: importance of the alternative proposition
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3853867/
Examples and probabilities of how a bloodstain from the victim ended up on the suspect and how likely it is that a person’s DNA ends up on a handled item (weapon). The likelihood is 0.3 that DNA is transferred from shooter to gun and 0.7 that DNA is not transferred. Thus, it is only slightly more likely to transfer DNA to the gun than the chance of finding background DNA on the gun (35 % mixed profiles and 24 % single background profiles). Conclusively, not finding a suspects DNA on a gun does not eliminate the suspect as the shooter.
Touch DNA collection versus firearm fingerprinting: comparing evidence production and identification outcomes

Touch DNA produced a larger volume of evidence than fingerprints from firearms in an Indianapolis police district, however, the identification outcome was equal.

An investigation of DNA recovery from firearms and cartridge cases

Weapons setup: Different types of weapons were cleaned and then fired in environments similar to a crime scene. Each weapon was shot by two persons without cleaning in between. 128 samples (32 from each firearm).

Cartridge setup: Samples were collected from both fired and unfired cartridges. Also a test made with swabbed-on saliva on cartridges.

Results weapons: The most successful DNA recovery was from the grips of the firearms (80 % of the samples had enough quantity of DNA) and slide serrations of the pistol (87.5 %). 30 of 47 samples gave mixed DNA profiles. 24 of these 30 had clear major profiles, however, the major profile was not always the designated shooter. 8 of 19 samples resulted in mixed profiles after shooter one only, some mixtures were investigated and could be attributed to boyfriend and office partner.

Results cartridges: results indicate DNA from fired/unfired cartridges or the magazine holding cartridges. There seemed to be a considerable loss of DNA by the movement through the weapon, even when saliva was swabbed-on.

Conclusion: A DNA profile from a weapon does not mean it was the last person to touch/use the weapon. Furthermore, it does not indicate that direct contact has occurred between the weapon and the individual.

Trace DNA success rates relating to volume crime offences

Compilation of trace DNA (252 samples) collected in burglaries. Full or major (12 alleles or more) were recovered from 14 % of the samples. 8 % gave a full single profile. 21 % gave mixed profiles. No DNA was recovered from 16 % of the samples. Least efficient was recovery from firearms and points of entry. Average amount of DNA (in the 252 samples) was 1.7 ng.

Comparison of collection methods from touch samples on metal and wearer samples from simulated mixtures on clothing

Puritan sterile cotton swabs can contain up to 23 pg of human DNA.

Touch DNA on metal: comparison between DNA sterile cotton swabs, DNA free cotton swabs and foam tipped DNA free swabs (organic extraction, qPCR, PCR STR multiplex amplifications and capillary electrophoresis). The foam tipped DNA free swabs worked poorly on both stainless steel and brass. DNA sterile cotton swabs worked the best for stainless steel and DNA free cotton swabs worked best for brass.

Touch DNA on clothes (different types): Comparison between swabbing, scraping and adhesive taping (Gel-Pak ‘0’). All three methods resulted in mixed profiles. Swabbing and Gel-Pak ‘0’ were comparable in that the last wearers profile was the most distinct one. Gel Pak ‘0’ gave generally less DNA. Scraping recovered more DNA from the habitual wearers.
Persistence of biological traces in gun barrels after fatal contact shots
“For samples taken after the first shot DNA-analysis yielded STR profiles eligible for reliable individualization in 17 of 20 cases. After a second shot had been fired 8 or more STR systems were amplified successfully in 14 of 20 barrels.”
Conclusion: DNA could be recovered from the inside of the barrels after close contact shots, even after subsequent firing.