Memorandum

TO File

FROM Martin F. Murphy

DATE April 9, 2001

RE Review of Transfer Articles—**Subject to and Protected by the Attorney-Client and Work Product Privileges**

1. **van Oorschot, et al, "HUMTH01 Validation Studies: Effect of Substrate Environment and Mixtures," Journal of Forensic Sciences Vol. 41, No. 1 January 1996 (142-145).**

   For this study, it does not appear that the authors used a 310 or other capillary electrophoresis method. Instead: "Amplification (including the use of bovine cerium albumin) polyacrylamide gel electrophoresis, silver staining and genotyping were performed as described by van Oorschot, et al, 1994. "

   "Sensitivity studies (data not shown) examining serial dilutions from 0.005ng to 50ng DNA using our standard protocols, showed that typings were obtainable from as little as 0.05ng DNA. An extra five cycles (total 35) of amplifications produced typings from 0.01ng. High molecular weight bands and very weak additional bands of 1-2 repeat units smaller than the actual alleles were frequently present when using 10ng or more template DNA (1-5ng when using 35 cycles). No false typings were generated when using an additional five cycles."

   "Table 2 shows that the minor component is still detectable from the following mixtures: 0.1ng DNA: 5ng DNA…" *[In other words, in a mixture containing a ratio containing five thousand picograms of DNA of the major contributor and 100 picograms of DNA the minor component is detectable. 100 picograms DNA equals approximately 15 cells.]*

2. **van Oorschot "DNA fingerprints from fingerprints," Nature, June 19,1997 at 767.**

   "We swabbed specific areas of hands and objects with cotton cloth dampened with sterile water using disposable forceps."

   "Initial tests showed that we can readily obtain correct genetic profiles from swabs taken directly from the palm of a hand (13 of 13). DNA yields varied from 2 to

150ng (average 48.6ng). Dry hands and those that had been washed recently tended to provide the least DNA." "Swabs of objects handled regularly by specific individuals all provided genetic typings that matched the user."

The specific items tested, and results, were as follows:

Regularly Handled Items

ITEM	Number	Mean Quantity
Leather Briefcase Handles	3	75ng
Pens	3	1.6ng
Car Key	1	1.1ng
Personal Locker Handles	1	3.7ng
Telephone Headsets*	5	10.3ng

*One Handset also clearly displayed the genetic profile of a known secondary (minor) user.

Pre-Cleaned Items Held for 15 Minutes

ITEM	Number	Mean Quantity
Plastic Knife Handle	6	17.8ng
Mug	1	6.8ng
New vinyl Gloves**	8	51ng

**[Worn 20-90 min]

"DNA yields from swabs of polypropylene tubes held for varying lengths of time (5 seconds, 30 seconds, 3 minutes, 10 minutes) did not differ significantly indicating that substantial transfer of material occurs during the initial conduct."

"Objects handled by many individuals all produce profiles with multiple alleles of varying intensity. To determine the effect of multiple handlers, we exchanged polypropylene tubes between the individuals (2 or 3, 10 minutes each) with different genotypes. Although the material left by the last holder was usually present on the tube, that of previous holders was also retrieved to varying extents. The strongest profile obtained was not always that of the person who last held the object, but was dependent on the individual. We regularly observed profiles of previous holders of a tube from swabs of hands involved in these exchanges, showing that in some cases material from which DNA can be retrieved is transferred from object to hand (secondary transfer)."

"Also, hands swab before and after a 1-minute handshake revealed the transfer of DNA from one individual to another in one of the four hands tested. Thus genetic profiles from objects handled by several people or from a new blood stains on touched objects may be difficult to interpret.".

3. **Findlay, et al, "DNA fingerprinting from single cells" Nature, Volume 389, October 9, 1997 (555-556).**

"Genetic profiles can be obtained from single cells using an STR profiling system already in routine use in the United Kingdom, which gives a matching probability of roughly 1 in 50 million." The authors "analyzed 226 buccal cells from four different individuals isolating each cell using micro manipulation procedures. DNA was amplified using a routine forensic identification system with modifications (different primer concentrations, Ampli-Taq Gold and 34 cycles)."

"We amplified DNA in 91% cells, containing a full DNA profile in 50% and an acceptable profile (four or more STRs) in (64% of these cells)."

"These results show great promise and could be applied, for example, to smudged fingerprints, single flakes of dandruff, single sperm in multiple rape cases, and small samples left on weapons or vehicles."

"However, they must be considered with some caution. Although we obtained an acceptable profile in almost two-thirds of cells tested, care must be taken with the interpretation of the results. Stochastic effects cause preferential amplification and allele drop out."

4. **Van Hoofstat, et. al. "DNA typing of fingerprints using capillary electrophoresis: effect of dactyloscopic powders," Electrophoresis 1999, 20, 2870-2876.**

The question raised by this article is whether DNA analysis can be done on fingerprints even after fingerprint powder has been applied. The results of the study were mixed because many applications of powders will in fact interfere with DNA

profiling. In one of the experiments, investigators took a sample of ten physical fingerprints collected from a wooden plate. "These results show that the amount of DNA extracted from the consecutive application of ten fingers is at the detection limit, "which is about 0.15 nanograms." (This study used the 310 analyzer.)

"A varying number of physical fingerprints from one individual applied on wood and glass plates were extracted using the chelex extraction method, and subjected to DNA profiling using the above-described STR-capillary electrophoresis technology. Samples of the physical fingerprints, obtained by the application of one, three, five, eight and ten consecutive figures, were analyzed in order to be able to determine the detection limit. Part of the plates without fingerprints was wiped off and used as blank."

"As expected, a decrease in the number of physical fingerprints results in a lower intensity of the peaks on the electropherogram. Good results were obtained when a sample consisting of a consecutive application of five physical fingerprints was analyzed. With three physical fingerprints, the intensity of the peaks was at the limit of detection for wood and was five times lower than the background for glass, which we kept as norm for the lower limit."

"To obtain a better profile, the number of PCR cycles could be increased. Another possibility for increasing the sensitivity is reducing the amount of chelex (normally 200 microliters) so that the extracted DNA would be less diluted. We extracted DNA using 100 microliters at 5% chelex instead of 200 microliters. With this method it is possible to determine a full profile from the traces left by one fingerprint. However, the intensity of the DNA profile is very low and may be difficult to interpret." *[Note: In our case, Cellmark concentrated the samples to 11 microliters].*

"The physical fingerprints were visualized by slightly brushing them using a sterile swab. For each powder, a new sterile swab was used. This swab was also extracted and amplified with the other samples as reference material. Normally a brush is used to apply the powder. We used a swab instead of a brush because it is easy to analyze it because it also gave us the possibility to check if the powder really inhibits DNA typing or if the brush causes a wiping off of the DNA. The experiment was repeated twice."

"Two white powders (the faurot white and a BVKDA white), one magnetic powder (magnetic black) and one black powder (special black) were no problems at all for DNA typing. All four gave good results with good intensity. Analyzing the swab also resulted in a full profile which means that by using the swab and applying the powder, DNA was partly wiped off. Apparently, enough DNA was left on the plates to obtain a profile directly from the traces of the fingerprints."

(Note: This means that there was sufficient DNA for typing left on (a) the original fingerprint and (b) the swab used to apply the fingerprint powder.")

In the concluding remarks section, the authors note: "DNA profiling can be performed on fingerprints left on glass or wood."

5. **Ladd, et al, "A Systematic Analysis of Secondary DNA Transfer," 1999 Journal of Forensic Science 44(6): 1270-12272. "**

This study attempts to cast doubt on van Oorschot, but the reported results of the study are as follows:

Items Handled By One Person

ITEM	Number	Average Quantity DNA
Briefcase Handles	6	15ng
Steering Wheels	6	12ng

Items Handled by More than One Person

ITEM	Number	Avg. Yield	Comments
Lab Door Handles	6	1.6ng	Mixtures Detected. Locus dropout with some amplifications.
Telephone Mouth Pieces	6	7ng	Mixtures Detected. Locus dropout with some amplifications.
Freezer Door Handles	6	12ng	Mixtures Detected. Locus dropout with some amplifications.
Closet Door Handles	6	1ng	Mixtures Detected. Locus dropout with some amplifications.
File Cabinet Handles	6	1ng	Mixtures Detected. Locus dropout with some amplifications.
Computer Key Boards	6	5ng	Mixtures Detected. Locus dropout with some amplifications.
Computer Mouse	6	6ng	Mixtures Detected. Locus dropout with some amplifications.
Coffee Cups	6	6ng	Mixtures Detected. Locus dropout with some amplifications.

Handshakes

Duration	No.	Avg. Yield	Comments
1s	6	nd	Negative
5s	6	1ng	Primary transfer detected with some amplification-- highly dependent on individual.
10s	6	2ng	Primary transfer detected with some amplification--highly dependent on individual.
30s	6	5ng	Primary Transfer detected with some amplification--highly dependent on individual.
60s	6	4ng	Primary Transfer detected with some amplification--highly dependent on individual.

Hand to Hand to Door Handle

Duration	No.	Avg. Yield	Comments
10s/5s	6	1ng	No secondary transfer detected (no peaks <75 RFU) or Amplifications predominately negative, no peaks above background. Some peaks >75 RFU from the second individual present. Allele and locus dropout were observed.
30s/5s	6	1ng	No secondary transfer detected (no peaks <75 RFU) or Amplifications predominately negative, no peaks above background. Some peaks >75 RFU from the second individual present. Allele and locus dropout were observed.

Hand to Object to Hand

Object	No.	Avg. Yield	Comments
Coffee Cup	6	6	No secondary transfer detected (no peaks >75 RFU) or Amplifications predominately negative, no peaks above background. Some peaks <75 RFU from the second individual present. Allele and locus dropout were observed.

Other interesting observations are as follows:

"We have generated interpretable results with as little as 100/200pg of high molecular weight DNA."

"With respect to secondary transfer, peaks above background (15-20 RFU) from the second individual were not detected for most STR amplifications. On occasions minor peaks (below 75 RFU from the second individual were observed. However in these instances, allelic dropout was routine. The complete secondary profile was never detected, even if the data were analyzed in the 50/75 RFU range. It should also be noted that, generally, amplification would not be attempted on many of the experimental sample we tested since the manufacture recommends using a minimum of 250pg (35 cells) of DNA template for the PCR. At 125pg or less, peak heights are close to background; the standard peak height threshold recommended by the manufacture if 150 RFU."

6. **Szibor, et al "Efficiency of Forensic mt DNA analysis: Case examples demonstrating the identification of traces," Forensic Science International 113 (2000) 71-78.**

   In this report, the authors describe a series of tests performed on strangulation tools, fire arms, and traces on folder papers, using Mitochondrial DNA and STRs. The author successful typed DNA from a variety of strangulation tools (including, electrical cable, nylon cord and rope). The author has also successfully typed the identities of police officers based on DNA traces recovered from the trigger cock, butt, hammer, magazine, and cartridges. The author successfully developed a profile from a piece of folder writing paper. The author focused principally on Mitochondrial DNA and STRs using a commercial kit developed by a company called "Serac."

7. **Fregeau, et. al. Fingerprint Enhancement Revisited and the Effects of Blood Enhancement Chemicals on Subsequent Profiler Plus Florescent Short Tandem Repeat DNA Analysis of Fresh and Aged Bloody Fingerprints." Journal of Forensic Science 2000 45(2) 354-380**.

The main point of this article was to determine whether a variety of blood enhancement reagents could successfully help detect bloody fingerprints on surfaces without interfering with the ability to obtain DNA profiles from the relevant stains. The authors used a 377 for the work.

The article does, however, contain the following interesting points:

"Benzedrine and Ortho-Tolidine, routinely used in the early days of fingerprint analysis have been banned by many laboratories because of their potential carcinogenic properties."

The authors used diluted blood to create bloody fingerprints. They diluted blood at 1:10 and 1:50. "Fingerprints prepared using five microliters of undiluted blood provided an abundance of genetic material. The 1:50 dilution yielded sufficient DNA to subject the sample to one or two rounds of typing with a Profiler Plus STR multiplex system…." (359). A significant drop in DNA yields (2.5 to 4 – fold reduction) was noted when the blood was transferred from fingertip to substrate, compared to the situation where the blood was deposited directly on the linoleum as drops. This was anticipated because only a fraction of the blood deposited on the finger will be transferred to the surface. For the same aliquot of undiluted blood (i.e. 20 microliters), 1500ng and 625ng of DNA were recovered from drops and fingerprints, respectively." (359). *[The point here is that the first round of transfer left a deposit of approximately 40% of the DNA originally present.]*

The authors tested some controls, which included "untreated nonbloody fingerprints from individuals A and B produced on glass, wood and clothing: In all instances, incomplete Profiler Plus profiles were observed. The intensity of the signals range from between 40 relative fluorescent units (RFU) to 150 RFU i.e. above the threshold limit of detection of alleles set at 40 RFU during our extensive STR validation studies performed at the RCMP Forensic Laboratory using the Profiler Plus multiplex STR system." (364) "Interestingly in the two situation were excessive pressure was applied by fingers during the preparation of the nonbloody finger prints (e.g., those applied to glass), the Profiler Plus profiles observed were almost complete and were consistent with the profiles of the contributor of the finger print (panels B & C). These results are in agreement with a recent report by van Oorschot and Jones (67) which indicated that STR profiles, under some circumstances, could be obtained epithelial cells left on pens, car keys, telephone receivers and briefcases. These results further reiterate that caution should be exercised when handling materials or samples that could potentially be submitted to a forensic laboratory for DNA typing

analysis with a judicious use of gloves at crime scenes, evidentiary samples will not show contamination."

8. **A.E. Kisilevsky, et al, "DNA PCR STR Profiling of Skin Cells Transferred through Handling." Abstract from the 46th Annual Meeting of the Canadian Society of Forensic Scientists (Edmonton, Alberta, November 16-21) 1999.**

"Human test subjects independently handled four knife handles of varying substrate (plastic, metal, wood/no veneer and wood/worn veneer) for varying lengths of time. DNA recovered from each knife handle was extracted and quantified. DNA yield was compared within and between subjects, at various times, across all substrate." A 377 was used for the testing.

"It was found that the amount of DNA transferred to a substrate handling is:

(a) independent of handling time. Transferred DNA from sloughed epithelial cells, to a substrate during handling, is instantaneous;

(b) dependent on the individual handler. Certain individuals are; 'good' epithelial cell donors ("sloughers") while other individuals are poor epithelial cell donors. ("non-sloughers"); and

(c) dependent on the handled substrate. Porous substances adhere sloughed epithelial cells more readily than non-porous substances."

9. **van Oorschot et al, "Retrieval of Genetic Profiles from Touched Objects, First International Conference on Forensic Human Identification in the Millenium.**

Referring to a 1998 study, apparently published in the proceedings of the 14th International Symposium on the Forensic Sciences of Australia and New Zealand Forensic Science Society, October 12-16, 1998, the authors state that with respect to hand to table to hand experiments, they "identified the presence of a profile secondary to the expected primary profile in a small percentage of occasions. Furthermore, the profiler originating from the hand or known depositor was always present and in nearly all cases was equal or greater in signal intensity (detectability) to that originating from the secondary transfer. Further studies by van Oorschot et al (1998) to illustrate that swabs of the outside of gloves worn while folding an object that had been frequently touched (such as door handles and used gloves that had been turned inside out) revealed a genetic profile that matched that obtained from the swabs of the fondled objects."