Article

# Multiple DNA Transfer Events in a Social Setting Complicates Interpretation of DNA Evidence

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**Abstract**: Advances in forensic DNA typing and increases in the sensitivity of STR kits have allowed for the analysis of DNA transferred both directly and indirectly between individuals and objects. The present study was adapted from previous research and illustrates the transfer of genetic material between individuals and objects in a simulated social setting. Presterilized objects were handled by four participants sitting at a table. The order and timing of handling were predetermined and controlled by researchers to test for evidence of primary, secondary, and tertiary DNA transfer. Participant behavior and the actions of nonparticipants were not controlled by the researchers thereby mimicking a social situation. The handled objects and participants' hands were swabbed throughout the experiment. The DNA was purified, quantified, and amplified for DNA profiling. DNA was detected in 92% of the samples; however, only 50% produced profiles that met casework requirements for interpretation. Eighty percent of the interpretable DNA profiles were characterized as mixtures of DNA from two or more individuals, with 60% of those mixtures having identifiable major and minor contributors. Extraneous DNA was observed in 75% of the DNA profiles. Profiles obtained from the objects displayed no pattern regarding which participant most recently touched the object, and many of the samples were inconclusive because of the complexity of the mixtures. The open-air setting of this experiment and the extraneous DNA detected in the samples complicated reconstructing the order of transfer events. This study highlights the complexity of DNA transfer between individuals and objects when multiple transfer events occur.

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## Introduction

Recent advances increasing the sensitivity of STR kits have allowed for the detection of trace amounts of DNA that have been transferred to various everyday objects that an individual may or may not have directly contacted [1–5]. As a result, extraneous DNA that is not associated with a criminal event is often detected and amplified [6]. The ability of next generation kits to detect DNA that has been transferred by secondary or even tertiary routes often results in the generation of complex, mixed DNA profiles from evidentiary items. In an attempt to better understand the relationship between DNA evidence and a criminal act, there has been an increase in the number of studies testing transfer DNA in various real-life situations [2–4, 7–13]. Understanding the active and passive processes that can lead to DNA transfer, the multiple variables that can impact DNA transfer, and the limits of DNA transfer can assist in determining the probative nature of DNA found on evidentiary items. A situation in which multiple individuals come into contact with the same object over a short period of time would be common in most social settings; therefore, the evaluation of a multihandling scenario with respect to DNA transfer would be of interest to both scientific and judicial communities.

This study builds upon research conducted and published by Goray and van Oorschot [8], where the effects on DNA transfer were explored when multiple people touched the same objects. Specifically, they looked at the DNA profiles obtained from samples collected after a simulated situation involving three individuals drinking from glasses filled from a communal jug of juice. The researchers placed no restrictions on talking, timing, order, or duration of item handling. The 20-minute interaction was filmed using two video cameras. Swabbing for DNA took place only at the end of this 20 minutes.

The present study mimicked the social setting established by Goray and van Oorschot (2013); however, the experimental design took a more systematic approach. Although no restrictions were placed on talking, handling order and timing were strictly controlled by the researchers. Additionally, swabbing took place throughout the duration of the experiment to test for evidence of DNA transfer. This method of experimentation allowed for a comparison between the behaviors in the reenacted social event and the DNA profiles generated from the objects and individuals. This research project investigated DNA transfer between a group of four people handling a communal jug and four plastic cups over a short period of time. This study aimed to address the following null hypotheses: (1) DNA transfer will not occur and will not be detected on dominant hands, (2) DNA transfer will not occur and will not be detected on the cups, (3) a mixed DNA profile from all participants will not be found on the jug handle, and (4) the order of object-handling will not be discerned from the DNA data.



## Materials and Methods

Prior to experimentation, a plastic jug and four plastic cups were obtained and prepared for the study (Figure 1). The jug handle was divided into four equal quadrants 3 cm in size and labeled with numbers 1 through 4 using a felt-tip permanent marker. The quadrants were positioned so that Quadrant 1 was the most inferiorly placed. This order was chosen to prevent the buffer used during the collection procedure from dripping onto subsequent quadrants and potentially compromising the DNA evidence. Each cup was labeled with a letter (A, B, C, or D) corresponding to the participant who would be handling it: A would be handled by Participant 1, B would be handled by Participant 2, C would be handled by Participant 3, and D would be handled by Participant 4.

*Figure 1*
*Jug handle quadrant positioning.*

In an effort to remove extraneous DNA from the objects' surfaces, each item was cleaned with a 10% bleach solution and exposed to UV light for 15 minutes under a laminar flow hood. The table surface was also wiped with a 10% bleach solution prior to experimentation. To verify the effectiveness of the decontamination procedures, the entire jug handle and the circumference of Cup A beneath the rim were swabbed using a wet swab technique. Both the jug handle and Cup A were allowed to dry completely before proceeding with the experiment. These samples were designated as "control" samples.

The four participants[1] were directed to lightly wash their hands with soap and water and air dry for 1 minute in order to remove any extraneous DNA from the skin surface. Afterwards, they put on nitrile gloves for a period of 15 minutes prior to the start of the experiment. During this 15-minute period, the researchers explained the scenario and sequence of events to the four participants. The participants were instructed to handle the entirety of the jug handle, sufficiently contacting each of the four labeled quadrants. The participants were also instructed to carry on conversations throughout the entire experimental process, as would take place in a real-world social situation. Other individuals were present in the room when the experiment took place. These nonparticipants were allowed to freely move around, leave the room, and talk at their leisure, simulating the surrounding environment that might occur at a restaurant or bar.

Once the participants removed their gloves, they sat facing each other at a square table. Each participant had a plastic cup in front of him or her, and the plastic jug was placed in the center of the table. Participants were instructed to handle objects with their dominant hands. Participant 1 handled the jug handle for 60 seconds and then placed it back in the center of the table. Following Participant 1's handling of the jug, Quadrant 1 of the jug handle was swabbed and allowed to dry completely. Participant 2 then handled the jug for 60 seconds. Using the same hand, Participant 2 then handled Cup B for 60 seconds. The researchers then swabbed Quadrant 2 of the jug handle, Cup B, and Participant 2's dominant hand. This handling and swabbing procedure was replicated three additional times: one for Quadrant 3 of the jug handle, Cup C, and Participant 3's dominant hand; another for Quadrant 4 of the jug handle, Cup D, and Participant 4's dominant hand; and finally, Cup A and Participant 1's dominant hand. The individuals collecting the DNA samples throughout the experiment were not any of the participants handling the objects.

Because the participants were directly handling the jug, the handle was swabbed to detect primary DNA transfer. Participants' hands were swabbed to detect secondary DNA transfer from the jug handle. The cups were swabbed to test for tertiary DNA transfer from the participants' hands.

Samples were extracted using the QIAamp DNA Mini Kit (Qiagen, Venlo, Netherlands) as per manufacturer's instructions. In addition, a negative extraction control was included to ensure that the reagents that were used for sampling and extraction in this study were devoid of human DNA. All samples were quantified using Quantifiler Trio (Life Technologies Corporation, Carlsbad, CA) on an Applied Biosystems (Foster City, CA) 7500 Real-Time PCR System, and amplified using the Globalfiler Amplification Kit (Life Technologies Corporation) on a GeneAmp (Life Technologies Corporation) PCR System 9700. In addition, positive and negative amplification controls were included to ensure that amplification was successful and that the reagents were devoid of human DNA. Capillary electrophoresis was performed on a 3130xl platform using protocol J6_3kv_05sec. Data was collected using Data Collection Software 4 (Life Technologies Corporation) and analyzed using GMID-X version 1.5 (Life Technologies Corporation). Data was interpreted following established guidelines with the aid of the GMID-X Mixture Analysis Tool. DNA profiles were analyzed using analytical thresholds and stochastic thresholds outlined in Table 1. Application of thresholds was based on the overall peak heights observed in the profile. Peak height ratios were used to aid in the deconvolution of mixtures as shown in Table 2.

[1] The research design was evaluated by the University of Indianapolis Human Research Protections Program and determined that activities do not meet the federal definition of Human Subject Research, as set forth in 45CFR 46.102, because data was deidentified in such a way that specific profiles were not being connected to specific identified persons.

| Analysis Method | Minimum Analytical Threshold | | 50 RFU | 100 RFU |
|---|---|---|---|---|
| Analytical Method | B: 20 RFU<br>G: 35 RFU<br>Y: 20 RFU | R: 25 RFU<br>P: 30 RFU<br>O: 50 RFU | 50 RFU | 100 RFU |
| Stochastic Threshold | 125 RFU | | 170 RFU | 220 RFU |

*Table 1*

*Analysis and interpretation thresholds.*

| Allele Peak Height (RFU) | Expected Peak Height Ratio |
|---|---|
| 20–300 | >50% |
| 301–600 | 50% |
| 601–1000 | 60% |
| >1000 | 70% |

*Table 2*

*Peak height ratios.*

## Results

The quantification results are illustrated in Table 3. Human DNA was detected in 92% of the samples collected from the jug handle, participant hands, and cups. Cup A was the only item that was swabbed in which human DNA was not detected. The amount of human DNA that was detected in the remaining samples that were collected from the jug handle, the cups, and the participants' hands ranged from 10 to 240 picograms.

No human DNA was detected, and no DNA profiles were obtained from the control samples or the negative extract control. The positive amplification control produced the expected DNA profile, and the negative amplification control was devoid of human DNA. Data was obtained from all samples including Cup A, which had no quantifiable DNA. Despite this, only 50% of the DNA profiles that were obtained met casework requirements for interpretation. Of the interpretable DNA profiles, 80% were characterized as mixtures of DNA from at least two individuals, with 60% of the mixtures having identifiable major and minor contributors. The profiles that were obtained from the samples ranged from partial to complete. For example, in the Cup C sample, data was obtained at only two of 24 genetic loci. Although no more than four alleles were identified at any one locus in any one sample, there were some indications that there might be more than two contributors, but there was insufficient data to support the affirmative conclusion of more than two contributors. For example, in the hand 4 sample, four alleles were identified at 11 of 24 genetic loci, but no more than four alleles were observed. However, after comparing the DNA profile to the participants' DNA profiles, it was determined that more than two individuals could be contributing to the profiles. In fact, after comparisons were completed, extraneous DNA was observed in 75% of the DNA profiles. For the purposes of the study, extraneous DNA includes any alleles not consistent with any of the four participants in this study. These results can be viewed in Table 4. Observed participant profiles listed in parentheses were not expected as contributors based on the order of handling.

| Sample | Volume (µl) | DNA (pg/µl) | Calc. Target (pg) |
|---|---|---|---|
| Control Jug | 150 | 0.0 | 0.0 |
| Control Cup | 150 | 0.0 | 0.0 |
| Negative Extraction | 150 | 0.0 | 0.0 |
| Jug Quadrant 1 | 150 | 0.2 | 20 |
| Jug Quadrant 2 | 150 | 0.2 | 30 |
| Jug Quadrant 3 | 150 | 0.6 | 90 |
| Jug Quadrant 4 | 150 | 0.2 | 30 |
| Hand 1 | 150 | 1.7 | 240 |
| Hand 2 | 150 | 0.5 | 80 |
| Hand 3 | 150 | 0.2 | 30 |
| Hand 4 | 150 | 1.1 | 160 |
| Cup A | 150 | 0.0 | 0.0 |
| Cup B | 150 | 0.1 | 10 |
| Cup C | 150 | 0.1 | 10 |
| Cup D | 150 | 0.2 | 30 |

*Table 3*

*DNA quantification results.*

| Item | Handler | Expected Participant Profiles | Observed Participant Profiles |
|---|---|---|---|
| Jug Quadrant 1 | 1 | 1 | 1, (2), (3), extraneous |
| Jug Quadrant 2 | 1,2 | 2,1 | 2, 1, extraneous |
| Jug Quadrant 3 | 1,2,3 | 3,2,1 | 3, 2, 1, (4), extraneous |
| Jug Quadrant 4 | 1,2,3,4 | 4,3,2,1 | 4, 3, extraneous |
| Hand 1 | 1 | 1,4,3,2 | 1, 4 |
| Hand 2 | 2 | 2,1 | 2, 1, extraneous |
| Hand 3 | 3 | 3,2,1 | 3, 2, (4) |
| Hand 4 | 4 | 4,3,2,1 | 4, 3, 2, 1, extraneous |
| Cup A | 1 | 1,4,3,2 | 1, 4, 3, 2, extraneous |
| Cup B | 2 | 2,1 | 2, 1, (3 or 4), extraneous |
| Cup C | 3 | 3,2,1 | 3 |
| Cup D | 4 | 4,3,2,1 | 4, 3, extraneous |

*Table 4*

*Expected versus observed participant profiles.*

## Discussion

Identification of major and minor contributors was limited to the samples taken directly from the participants' hands and the major profile belonged to that particular individual. For the samples collected from the jug handle and cups, there was no discernable pattern regarding whose DNA was detected and the timing or length of contact with the object. In other words, the last participant to touch an object was not consistently the major contributor to the profile that was obtained, and the amount of DNA that was detected was not dependent on the length of time the participant handled the item.

The uncontrolled social nature of this experiment, specifically the lack of restrictions on talking and the actions of nonparticipants in the room, made reconstructing the order of object-handling difficult. Potential aerosolization of DNA through sneezing, coughing, or talking, all of which were observed, could have been the source of extraneous alleles not consistent with the DNA profiles of the four participants. Additionally, the extraneous alleles could have been introduced to the participants' hands while drying, applying gloves, or by surviving the hand-washing process. For example, the detection of DNA on the cups that did not belong to the direct handler of the cup could be evidence for tertiary DNA transfer or could be the result of DNA transfer through aerosolization. From an academic standpoint, researchers were able to assign alleles identified in the samples to the individual participants and document how genetic material potentially moved to the objects involved in the social interaction. This unique vantage point made possible by the controlled nature of certain aspects of the research design allows for additional discussions regarding DNA transfer that are not restricted by a forensic casework prospective.

As can be viewed in Table 2, there were discrepancies between the profiles expected to be on an object versus the profiles that were actually observed. For certain samples, the observed profiles were the same as the expected profiles but with extraneous DNA detected. For others, data was missing from the observed participant profiles that were expected to be present. One-half of the DNA profiles that were obtained were considered inconclusive because of the incomplete nature of the profile or the complexity of the mixture that was obtained.



Regarding the null hypotheses in this study:

- The first null hypothesis (Hn1) can be rejected because of the presence of alleles that did not belong to the individual from which the dominant hand swab was taken.
- The second null hypothesis (Hn2) can be rejected because there were participant alleles present on the cups that did not belong to the direct handler of the cup.
- The third null hypothesis (Hn3) can be rejected because DNA from each of the participants was found on the jug handle, resulting in a mixed profile.
- The fourth null hypothesis (Hn4) failed to be rejected. Although major and minor contributors could be identified in approximately 20% of the samples, this was restricted to the hand swabs, and this hypothesis specifically regards the handled objects. The last person to touch an object was not consistently the major contributor to the sample, which did not allow the order of handling to be determined.

## Conclusions

This study contributes to a large body of research demonstrating the challenges and complexity of interpreting DNA transfer events in a social setting where multiple individuals come into contact with the same object. In this study, participants handled presterilized objects, the handlers' DNA profiles were known to allow for better mixture interpretation, and the order of the handling and length of contact with the objects were known.

However, the introduction of extraneous DNA via different transfer pathways (direct, aerosol, and indirect) complicated the ability to reconstruct the DNA transfer events. In a real-world situation, the objects encountered will not be presterilized, and all contact events and contributors will not be known. The presence of extraneous alleles on jug Quadrant 1, an item that was handled by only one participant, is clear evidence of the ease of DNA transfer in situations where multiple people are in the same general area. The detection of extra alleles has the potential to impact the interpretation of the DNA results.

The results of this study are in agreement with others that demonstrate the ease of DNA transfer between people and objects. Additionally, this study illustrates that predictions of mode of DNA transfer, whether direct or indirect, cannot be

made with certainty from DNA typing results, and the interpretation of DNA mixtures becomes increasingly challenging when multiple transfer events have occurred.

Two major points are emphasized in this study regarding situations where DNA transfer occurs as multiple individuals make contact with shared objects: (1) multiple transfer events may impact understanding the relationship between DNA evidence and the objects associated with the criminal act, and (2) one cannot predict the handling order, length of contact, or mode of DNA transfer from DNA quantity or the DNA profile obtained from an object.


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