Original article

Detection of Y-STR Touch DNA for Personal Identification on a Wooden Knife Handle Used as a Stabbing Weapon

Boonpim M, Kanchai C and Bhoopat T

Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University

Objectives The purpose of this study was to examine the effectiveness of Y-STR profiling of touch DNA from a wooden-handle knife used as a stabbing weapon.

Methods A group of 60 male volunteers were asked to stab a foam box with a wooden-handle knife held firmly in their hand. DNA from the surface of the knife handles was extracted using a Qiagen® QIAamp DNA Investigator kit and amplified using a modified Investigator® Argus Y-12 QS protocol.

Results The touch DNA profiling found that 6 of 60 of full profile samples (10%) gave a full profile and 21 samples (35%) showed useful partial profiles. No significant difference was observed between right- and left-hand dominant volunteers (p = 0.5615 95% CI = -24.6237 to 44.1160, Chi-squared test of proportion).

Conclusion Y-STR profiling of touch DNA is suitable for use in conjunction with other personal identification methods.
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Keywords: Touch DNA, Y-STR, transfer, knife

Introduction

Analysis of deoxyribonucleic acid (DNA) profiles from touched objects has been conducted for many years (1). The mechanism of touch DNA transference from the skin to touched objects is complicated by the epithelial morphology of skin cells which lack nuclei and are keratinized (2). Wickenheiser explained that DNA from the skin can be transferred via the sloughing process, and that DNA can come from rich DNA cell sources, e.g., the hands or fingers, which can act as vectors (3). DNA providers can be classified into either good shedders or bad shedders on the basis of the number of alleles shed or the success rate in getting a DNA profile from the surface of a touched object (4). The success rate of a full profile from touch DNA depends on the characteristics of the skin of individual who has touched the object as well as their activities prior to touching the object (5). Different object surfaces also show a different likelihood of success in obtaining a profile that can be used to identify the individual. The likelihood of success for different object surfaces has been shown to be approximately 9% for glass, 23% for fabrics and 36% for wood samples (6).

Short tandem repeats on the Y chromosome (Y-STR) DNA can be used in conjunction with standard autosomal DNA to obtain additional information, since Y-STR DNA can be used to identifies an individual as a male. This information can be useful in cases where both male and female traces have been mixed in the same specimen, e.g., in sexual assault cases (7,8). The Y-STR DNA can also be used in crime scene investigations, especially in a search for male traces in forensic evidence with high amounts of female DNA and

Correspondence: Matee Boonpim, MD, Department of Forensic Medicine, Faculty of Medicine Chiang Mai University, Chiang Mai 50200, Thailand
E-mail: boonpim.matee@gmail.com

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can also give information about a number of male perpetrators involved in the crime.

However, the effectiveness and reliability of Y-STR as touch DNA in personal identification has not yet been studied. To help fill this gap, we conducted a study on Y-STR DNA by analyzing traces from a wooden-handle knife used as a stabbing weapon.

Methods

In this study, 21 wooden-handled knives were used: 20 test objects and 1 negative control (Figure 1). The knives were prepared and cleaned using 10% sodium hypochlorite and 70% ethanol, then were sealed in individual autoclave bags and sterilized by autoclaving at 121 °C for 20 minutes to degrade any remaining pre-existing extraneous DNA on the knives. The objects were kept in the autoclave bags until used in the stabbing. Sixty male volunteers of unknown shedder status were divided into three groups of 20 for the experiment (9). To mimic a stabbing, each group was asked to stab a foam box with a wooden knife while holding the knife firmly for 60 seconds. Before touching the knife, all the volunteers' hands were washed with soap and tap-water for 30 seconds then air dried. They held the knife with their dominant hand and firmly stabbed the foam box until the knife hilt reached the box surface. The knife was then put into a paper bag which was tightly sealed and stored at room temperature for not more than two weeks before being sent to the laboratory where each knife handle was swabbed two times, once with a wet swab then with a dry one (10). A total of 60 individual samples, two swabs from each volunteer, were collected for further DNA testing. Information on each volunteer's dominant hand, age and order of participation in the stabbing were recorded on the bag. A buccal swab from each volunteer was also obtained for standard Y-STR profiling for comparison with the DNA results from the knife handle.

Figure 1. The type of wooden-handled knife used in the experiment

Extraction of DNA

Small samples of each wet and dry swab were placed in 1.5 mL micro-centrifuge tubes and DNA was extracted using a method of the Qiagen® QIAamp DNA Investigator protocol (11,12). ATL buffer (400 µL) was added, followed by 20 µL of Proteinase K (supplied with the QIAamp kit), vortexed and incubated at 56°C with shaking at 900 rpm for a minimum of one hour in a thermomixer. After that, 400 µL of prewarmed AL buffer was added and the swabs were vortexed and incubated for ten minutes at 70 °C and shaken at 900 rpm. Next 300 µL of ethanol was added and the mixture was vortexed. Samples were carefully added to columns in collection tubes (QIAamp kit) and centrifuged at 8,000 rpm for one minute after which 500 µL of AW1 buffer was added and the mixture again centrifuged at 8,000 rpm for one minute. Following that, 700 µL of AW2 buffer was added and the mixture centrifuged at 8,000 rpm for seven minutes. Next, 700 µL of ethanol was added, centrifuged at 8,000 rpm for one minute then at 14,000 rpm for three minutes. The columns were removed and incubated at 56°C for three minutes and 15 µL of ATE buffer was added. The mixture was incubated at room temperature for five minutes before centrifugation at 14,000 rpm for one minute. The extract was then stored at -20 °C pending further analysis. A buccal swab from a known source was used as a positive extraction control in each extraction batch.

Amplification and profiling

Samples were amplified for genetic profiling using a modified method of the Investigator®

Argus Y-12 QS protocol (13). Template DNA 8.45 µL was added to the PCR tube, followed by 4.05 µL Master mix (reaction mix 2.5 µL, primer mix 1.25 µL, Taq DNA polymerase 0.3 µL) to a total solution of 12.5 µL. Then the solution was amplified using the GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems) under the conditions shown in Table 1. Profiles were generated using an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). The sample solution of 9 µL included 8.7 µL Hi-Di Formamide (Applied Biosystems) + 0.3 µL DNA Size Standard 550 (BTO) and 1 µL amplified DNA. The analysis was conducted using Genescan® analysis and GeneMapper™ ID SoftwareV3.2 (minimum peak height of 75 rfu for Y-STR testing) (14).

Table 1. Thermal cycling protocol

Temperature	Duration	Number of cycles
94 °C*	4 minutes	-
94 °C	30 seconds	5 cycle
63 °C	120 seconds	
72 °C	75 seconds	
94 °C	30 seconds	25 cycle
61 °C	120 seconds	
72 °C	75 seconds	
68 °C	60 minutes	-

* Hot-started to activate DNA polymerase

Results

Of the 60 volunteers, 50 were right-handed. Ages ranged from 18 to 60 years. The resulting profiles were classified by the total number of alleles obtained, ranging from no alleles to a full DNA profile of all 11 loci (12 alleles). We compared the profiles with buccal cell profiles and categorized the results as no profile, partial profile, full profile and mixed profile. Of the 60 touch DNA samples amplified, 15 samples resulted in no profile, 35 had partial profiles, 6 provided full single profiles and 4 resulted in mixed partial profiles (Tables 2 and 3).

Discussion

By simulating a stabbing, this project studied the transfer of DNA from an individual's hand to a knife handle. We were interested in both the direct contact or primary transfer and the indirect contact or secondary transfer. In the study, the acceptance criteria of useful profiles was set at four or more alleles (15). No useful profiles were obtained in 55% of the cases while 45% yielded useful profiles. Of the useful profiles, 10% provided full profiles. No significant difference was found between right and left hand dominance (p = 0.5615 95% CI = -24.6237 to 44.1160, Chi-squared test of proportion). The results are comparable to D.J. Daly's study in which approximately 36% of 100 wood samples gave useful profiles using standard STR with no significant

Table 2. Number of amplified samples and DNA profiles

Dominant hand	Number of samples generating no profile	Number of samples generating partial profiles	Number of samples generating full profiles (12 Y-STR Alleles)	Number of samples generating mixed profiles
Right	13	30	3	4
Left	2	5	3	0
Total	15	35	6	4

Table 3. Number of amplified samples and DNA profiles

Dominant hand	1-3 Y-STR alleles	4-6 Y-STR alleles	7-9 Y-STR alleles	10-11 Y-STR alleles
Right	11	13	0	6
Left	3	1	0	1
Total	14	14	0	7

differences between standard STR and Y-STR (p = 0.2608 95% CI = -7.4356 to 25.3351, Chi-squared test of proportion) (6). Interpretation of touch DNA profiles may be difficult in cases of high secondary transfer. In this study, only one volunteer was in contact with the object. This, coupled with the fact that the negative controls were free of extraneous DNA, means the incidence of secondary transfer (mixed DNA profiles) of 6.67% makes it reasonable to assume that any DNA transfer occurred via the volunteer. All of mixed profiles had partial profiles with additional minor elements present. The interpretation of mixed profiles using this method was challenging.

Conclusions

This study demonstrated touch DNA from Y-STR. More Y-STR touch DNA can be obtained from primary transfer than from secondary sources. There are no significant differences between right and left hand dominant, standard STR and Y-STR. No significant differences were found in STR and Y-STR between right- and left-hand dominant individuals; however, studies with a larger population are needed.

Declarations

Ethics approval and consent to participate

Ethics

This Research was approval form Research Ethics Committee 4, Faculty of Medicine, Chiang Mai University, Number 238/2017.

Consent for publication

Not applicable

Availability of data and material

Data sharing not applicable to this article as no datasets were generated or analysed during the current study. Please contact author for data requests.

Competing interests

The authors declare that they have no competing interests

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การตรวจดีเอ็นเอสัมผัสชนิด Y-STR ที่ติดกับด้ามมีดไม้จากการแทงเพื่อพิสูจน์บุคคล

เมธี บุญพิมพ์, จาตุรงค์ กันชัย และ ธานินทร์ ภู่พัฒน์
ภาควิชานิติเวชศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

วัตถุประสงค์ เพื่อประเมินประสิทธิภาพและความสำเร็จของการตรวจดีเอ็นเอสัมผัสจากด้ามมีดไม้

วิธีการ ผู้วิจัยได้ศึกษาความเป็นไปได้ของการตรวจดีเอ็นเอสัมผัสจากด้ามมีดไม้โดยให้อาสาสมัครเพศชาย 60 คน ทำการกำที่ด้ามมีดด้วยมือข้างที่ถนัดและแทงส่วนของคมมีดเข้าไปในกล่องโฟม ดีเอ็นเอจากผิวด้ามมีดถูกเก็บและนำไปสกัดโดยใช้ชุดน้ำยาสำเร็จรูป Qiagen® QIAamp DNA Investigator kit และเพิ่มปริมาณดีเอ็นเอโดยใช้ชุดน้ำยาสำเร็จรูป Investigator® Argus Y-12 QS ครึ่งหนึ่งของสูตรปกติ ก่อนจะนำไปเปรียบเทียบกับลายพิมพ์ดีเอ็นเอที่ได้จากกระพุ้งแก้มของอาสาสมัคร

ผลการศึกษา พบว่าสามารถตรวจพบดีเอ็นเอซึ่งเป็นประโยชน์ในการระบุบุคคลจำนวน 27 ตัวอย่างคิดเป็น ร้อยละ 45 จากตัวอย่างทั้งหมดซึ่งสามารถแบ่งเป็นดีเอ็นเอบางส่วน ร้อยละ 35 และดีเอ็นเอ็นครบถ้วนสมบูรณ์ร้อยละ 10 เมื่อเปรียบเทียบผลดีเอ็นเอครบถ้วนสมบูรณ์จากอาสาสมัครซึ่งถนัดมือซ้ายและมือขวาพบว่าไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ (p = 0.5615 95% CI = -24.6237 to 44.1160,Chi-squared test of proportion)

สรุป ผลการศึกษานี้แสดงให้เห็นว่า การตรวจและวิเคราะห์ดีเอ็นเอสัมผัสโดย Y-STR สามารถนำไปใช้ควบคู่กับวิธีการมาตรฐานอื่นเพื่อช่วยเพิ่มโอกาสในการระบุบุคคล **เชียงใหม่เวชสาร 2563;59(3):121-5.**

คำสำคัญ: ดีเอ็นเอสัมผัส Y-STR ส่งต่อ มีด