บทความวิจัย

ผลกระทบของน้ำชนิดต่างๆ ต่อการเก็บคราบเลือด เพื่อวิเคราะห์สารพันธุกรรม

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บทคัดย่อ

การวิเคราะห์วัตถุพยานทางนิติวิทยาศาสตร์โดยเฉพาะประเภทชีววัตถุมีข้อจำกัดเพราะมักพบวัตถุพยานในลักษณะที่เสื่อมสภาพ และมีปริมาณน้อย ดังนั้นการเก็บและการส่งมอบวัตถุพยานต้องกระทำอย่างถูกต้องและเหมาะสม เพื่อรักษาสภาพวัตถุพยานให้คงเหมือนเมื่อพบในสถานที่เกิดเหตุ ในการศึกษานี้ได้ศึกษาผลกระทบของน้ำชนิดต่างๆ ที่หาได้ง่าย 5 ชนิดต่อการเก็บคราบเลือดเพื่อวิเคราะห์สารพันธุกรรมด้วยวิธีปฏิกิริยาลูกโซ่โพลีเมอเรส ได้แก่ น้ำบริสุทธิ์ปราศจากเชื้อ น้ำกลั่นสำหรับเติมแบตเตอรี่รถยนต์ น้ำดื่ม น้ำประปา และน้ำเกลือสำหรับคอนแทคเลนส์ รวม 9 ตัวอย่าง จากการวัดค่าการนำไฟฟ้าพบว่าปริมาณไอออนของน้ำกลั่นเติมแบตเตอรี่รถยนต์และน้ำดื่มมีค่าใกล้เคียงกับน้ำบริสุทธิ์ปราศจากเชื้อมากที่สุด จากการทดลองพบว่าสามารถเพิ่มปริมาณชิ้นสารพันธุกรรมที่มีขนาดใหญ่ถึง 366 คู่เบส จากสารพันธุกรรมที่สกัดจากคราบเลือดที่เก็บด้วยก้านสำลีชุบน้ำทั้ง 9 ตัวอย่าง แต่แถบพันธุกรรมที่ได้มีความเข้มแตกต่างกัน และเมื่อมีการเพิ่มปริมาตรต่างๆ จนสารสกัดก้านสำลีชุบน้ำเกลือสำหรับคอนแทคเลนส์ น้ำดื่มหมายเลข 2 น้ำกลั่นแบตเตอรี่รถยนต์หมายเลข 1 และน้ำประปาหมายเลข 1 ในปฏิกิริยาลูกโซ่โพลีเมอเรส พบว่ามีการยับยั้งการสร้างชิ้นสารพันธุกรรมขนาด 211 คู่เบส การวิเคราะห์สารพันธุกรรมที่เก็บไว้นาน 12 เดือน พบว่าสามารถทำปฏิกิริยาลูกโซ่โพลีเมอเรสเพิ่มชิ้น สารพันธุกรรมทั้ง 3 ขนาด คือ 366, 289 และ 211 คู่เบส ได้จากตัวอย่างสารพันธุกรรมซึ่งสกัดจากคราบเลือดที่เก็บด้วยก้านสำลีชุบน้ำบริสุทธิ์ปราศจากเชื้อ และได้ชิ้นสารพันธุกรรมขนาด 289 และ 211 คู่เบส จากการวิเคราะห์สารพันธุกรรมที่สกัดจากคราบเลือดที่เก็บด้วยก้านสำลีชุบน้ำเกลือสำหรับคอนแทคเลนส์ ดังนั้นหากไม่ใช้น้ำบริสุทธิ์ปราศจากเชื้อในการเก็บคราบเลือดเพื่อวิเคราะห์ลายพิมพ์แล้วนั้น มีโอกาสที่จะได้ลายพิมพ์พันธุกรรมที่ไม่สมบูรณ์หรือไม่ได้ลายพิมพ์พันธุกรรม

คำสำคัญ: การวิเคราะห์สารพันธุกรรมทางนิติวิทยาศาสตร์ การเก็บวัตถุพยาน คราบเลือด น้ำ

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The Effect of Water Types Used for Bloodstain Collection on DNA analysis

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ABSTRACT

Analysis of forensic evidence, especially biological sources, is often limited because its nature is often poor in quality and small in quantity. Therefore, collection and handling of forensic evidence must be properly done to preserve the conditions as when the evidence was found. In this study, 9 water samples of 5 different types, which were easy to obtain, i.e., sterile purified water, water for car battery, drinking water, tap waters, and normal saline for contact lens, were investigated to study the effect of water types used in bloodstain collection on PCR-based DNA analysis. Conductivity measurement suggested that ion contents of water for car battery no. 1 & 2, and bottled drinking water were similar to that of sterile distilled water. The study suggested that DNA fragments up to 366 bp can be PCR amplified from the DNA samples extracted using all 9 water swabs; however, the band intensities were different. Amplification of the 211-bp DNA fragment was partially or fully inhibited when increased volumes of NSS for contact lens, drinking water no. 2, water for car battery no. 1 and tap water no. 1 swabs were added. Analyses of 366, 289 and 211-bp DNA fragments of 12 month-old DNA samples were successful in only one sample in which the bloodstain was collected with sterile purified water. In addition, the 289 and 211-bp DNA fragments were amplified from DNA extracted from NSS swab. Therefore, if water type other than sterile purified water was used for evidence collection, partial or no DNA profile may be obtained from the analysis.

Keywords: Forensic DNA analysis, evidence collection, bloodstain, water

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Introduction

Forensic evidence, especially biological evidence, is prone to degradation. It is essential that evidence must be properly collected and handled to preserve its best condition, as when originally found, for further downstream analytical process. Blood is a type of biological evidence often found in criminal cases. Pattern analysis of blood and bloodstain can provide detail information of the incident occurred. Contributor of the blood or bloodstain can be determined by DNA identity testing. Linkage of the victim, perpetrator, and crime scene of an incident can then be further established [1, 2]. There are different ways to collect blood evidence according to the condition of evidence present in the scene. If wet blood is present, a volume of liquid can be collected in a sterile disposable tube, or using dry sterile cotton swabs. If dried stain is present, cotton swabs moisten with sterile purified water is then used for evidence collection. The use of sterile purified water is suggested because of its sterility and low ionic content which would not interfere with the downstream PCR-based DNA analysis [3, 4].

In various regions of Thailand, it is difficult for regionally forensic offices, set in the rural areas and short of staff members, to obtain and maintain purified water facility for analytical work. Therefore, various water types, locally available such as bottled drinking water, distilled water for car battery, tap water, etc., are sometimes used for forensic evidence collection. The use of these water types may affect the stability of DNA extracted and the result of downstream analytical process.

In this study, we collected dried bloodstains using various types of water swabs and conducted a PCR-based DNA analysis in order to investigate the effect of water types used for bloodstain collection on DNA analysis. The water types which can be used as substitutions of sterile purified water for bloodstain evidence collection was discussed.

Materials and Methods

Water samples

Nine samples of 100 mL with 5 water types filled in an air-tight laboratory glass bottle (Pyrex), were directly transferred to the laboratory. These samples include one sample of each laboratory sterile-purified water and normal saline solution (NSS) for contact lens purchased from a local pharmacy, 2 samples of distilled water for car battery from local petrol stations, 2 samples of drinking water, 3 samples of tap water; one of the samples was ground-tap water collected from Lumlukka district, and the other 2 samples were collected from Mahidol University laboratory (SCFS) and regional police forensic science laboratory sub-division 11 (RTPOFS-11). Conductivity, temperature, and pH of the water samples were measured using

the conductivity meter (Digicon CD-430) and pH meter (Cyberscan 510, Eutech, USA), respectively.

Bloodstains were prepared on glass slides by spotting 200 μL of pig's blood in duplicates, and allowed to dry overnight at room temperature in a dark cabinet. Each bloodstain was then collected by using cotton swabs moisten with each water sample. In addition, 9 swabs of different water samples (no bloodstain) were also collected as a control. The bloodstained and water swabs were left to air dry.

DNA analysis

DNA was extracted from swabs using QIAamp DNA mini kit® (QIAGEN, Germany), and subsequently quantified by UV-spectrophotometry (Nanodrop™ Spectrophotometer, Thermo Scientific, USA).

The nuclear *β*-actin gene locus of *Sus scrofa* was analyzed by PCR using 3 primer pairs to generate 211, 289 and 366 bp DNA fragments (Figure 1). Primer sequences are shown in Table 1.

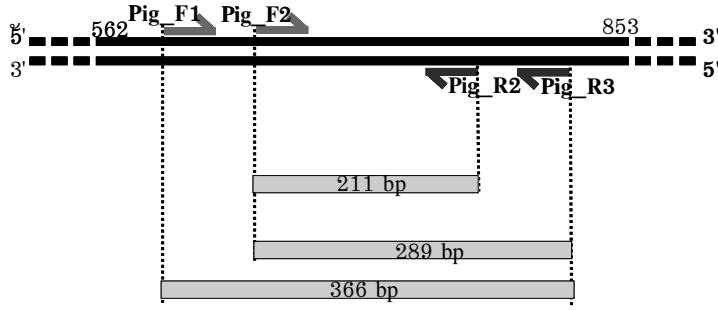


Figure 1 Map showing the primer positions in the *β*-actin gene.

The PCR reaction contained 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 10 pmol of each primer, 1 unit of *Taq* DNA polymerase, and DNA template. Thermo-cycling condition consisted of initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 sec, annealing at 65°C for 30 sec and extension at 72°C for 1 min and final extension at 72°C for 7 min. PCR products were separated by agarose gel electrophoresis and documented. The concentration of PCR products was then estimated by using GeneTool software (SYNGENE, USA).

Table 1 Sequences of primers

Primer sequences	Amplicon size (bp)
(Pig_F2 primer) 5'-GTCACCCACACGGTGCCCAT-3' (Pig_R2 primer) 5'-TGGCCATCTCCTGCTCGAAGTC-3'	211
(Pig_F2 primer) 5'-GTCACCCACACGGTGCCCAT-3' (Pig_R3 primer) 5'-AGCGCTCGTTGCCGATGGTG-3'	289
(Pig_F1 primer) 5'-ACGTGGCCATCCAGGCCGTG-3' (Pig_R3 primer) 5'-AGCGCTCGTTGCCGATGGTG-3'	366

Results

I. pH and conductivity of water samples

Measurements of pH and conductivity of water samples were carried out at 26 ± 0.5 ˚C. As shown in Table 2, the pH of water samples ranged from 5.5 - 8.5. The pH of sterile-purified water was 6.53. The pH of drinking water no. 1 and 2; tap water no. 1 and 2; and water for car battery no. 2 were 6.5-7.5. NSS for contact lens and water for car battery no. 1 had slightly acidic pH of 5.6 and 5.8, respectively, whereas tap water no. 3 (ground water) had an alkaline of pH 8.4. Sterile-purified water had the lowest conductivity of 1.5 µS while that of NSS was highest (15.5 mS). The conductivity of water for car battery no. 1 was 1.7 µS, which was close to that of the sterile-purified water used in the laboratory. Conductivity of drinking water no. 1 and water for car battery no. 2 was 16.9 and 8.6 µS, respectively. The drinking water no. 2 and the tap waters no. 3 had the conductivity ranged from 0.8 - 0.2 mS.

Table 2 pH, temperature, and conductivity of the water samples

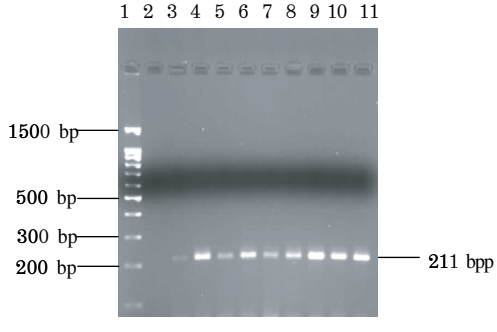
Water types	Sources	pH	Temperature (˚C)	Conductivity (µS)	Resistivity (MΩ)
Laboratory sterile purified water	SCFS Laboratory	6.53	26.4	1.5×10^{0}	6.7×10^{-1}
Normal saline (NSS) for contact lens	Local Pharmacy	5.58	26.0	1.55×10^{4}	6.5×10^{-5}
Water for car battery, no. 1	Local petrol station	5.72	25.7	1.7×10^{0}	5.9×10^{-1}
Water for car battery, no. 2	RTPOFS-11	7.29	26.6	8.6×10^{0}	1.2×10^{-1}
Tap water, no. 1	SCFS Laboratory	7.23	26.5	2.0×10^{2}	5.1×10^{-3}
Tap water, no. 2	RTPOFS-11	7.41	26.3	2.7×10^{2}	3.7×10^{-3}
Tap water, no. 3 (ground water)	Lumlukka distict, Pathumtani	8.43	26.8	8.2×10^{2}	1.2×10^{-3}
Drinking water, no. 1	Local petrol station	6.41	25.8	1.7×10^{1}	5.9×10^{-2}
Drinking water, no. 2	RTPOFS-11	7.46	26.0	2.0×10^{2}	5.0×10^{-3}

II. Analysis of nuclear DNA from bloodstain collected by 9 different water samples

As shown in figure 2, the Pig_F2 - Pig_R2, Pig_F2 - Pig_R3, and Pig_F1 - Pig_R3 primer pairs amplified 211, 289, 366 bp DNA fragments of the *Sus scrofa's* β-actin gene, respectively.

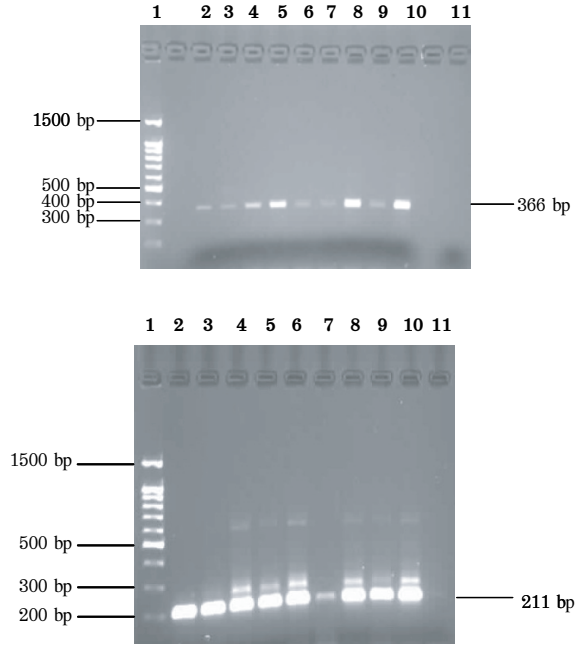
The Pig_F2 and Pig_R2 primer pair was used to amplify DNA extracted from bloodstains collected with 9 different water swabs. Amplification of 10 ng DNA template showed distinct 211-bp DNA bands with slightly different intensities (Fig. 2). Quantity of the amplified 211-bp PCR products ranged from 5.4 to 6.4 ng/μl. Bloodstains collected with sterile-purified water, water for car battery no. 1 and no. 2, and tap water no. 3 (ground water) swabs gave amplification yield of more than 6.0 ng/μl. Amplification yield of drinking water no. 1 and no. 2, tap water no. 1 and no. 2, and NSS swabs were 5.9 and 5.6, 6.0 and 5.6 and 5.4 ng/μl, respectively.

In Figure 3A, the Pig_F1 and Pig_R3 primer pair amplified the 366-bp β-actin gene fragment from all samples, but the intensity of the DNA bands were markedly different. The presence of 211 bp PCR products after re-amplification of all PCR 366-bp reactions using the Pig_F2 and Pig_R2 primers confirmed the validity of 366-bp PCR products (Fig. 3B). No DNA band was obtained in the negative control reaction. In addition, to determine the sensitivity of the PCR reaction using Pig_F2 and Pig_R2 primer pair, the amount of DNA template was diluted to 50, 25, 10, 5 and 1 ng. As shown in figure 4, the sensitivity of detection was equal to 1 ng of DNA template.



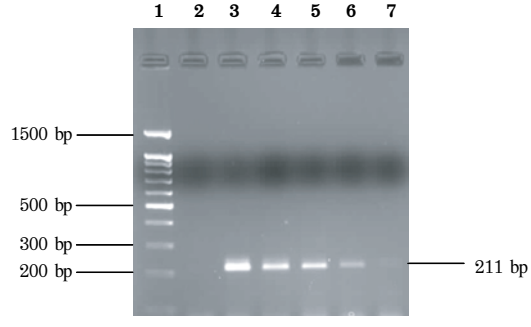
Lane	Description	Concentration of PCR product (ng/μL)
1	100 bp ladder	N/A
2	Negative control	0.000
3	NSS for contact lens swab	5.417
4	Tap water, no. 3 (ground water) swab	6.212
5	Tap water, no. 2 swab	5.558
6	Tap water, no. 1 swab	5.995
7	Drinking water, no. 2 swab	5.581
8	Drinking water, no. 1 swab	5.919
9	Water for car battery, no. 2 swab	6.397
10	Water for car battery, no. 1 swab	6.127
11	Sterile purified water swab	6.231

Figure 2 Ethidium bromide-stained gel showing the 211-bp β-actin PCR products using Pig-F2 and Pig-R2 primers and, DNA templates extracted from bloodstains collected by 9 different water swabs.



Lane	Description
1	100 bp ladder
2	sterile purified water swab
3	NSS for contact lens swab
4	Water for car battery, no. 1 swab
5	Drinking water no. 1 swab
6	Tap water no. 1 swab
7	Tap water no. 2 swab
8	Tap water no. 3 swab
9	Drinking water no. 2 swab
10	Water for car battery no. 2 swab
11	Nagative control

Figure 3 Ethidium bromide-stained gel depicting the 366-bp PCR-amplified DNA fragment using Pig-F1 and Pig-R3 primers (A), the 211-bp DNA fragments were then successfully re-amplified from these PCR products using the internal primer pair, Pig-F2 and Pig-R2 primers (B)



Lane	Description
1	100 bp ladder
2	Nagative control
3	DNA template 50 ng
4	DNA template 25 ng
5	DNA template 10 ng
6	DNA template 5 ng
7	DNA template 1 ng

Figure 4 The sensitivity of PCR reaction using Pig-F2 and Pig-R2 primers using 50, 25, 10, 5 and 1 ng of DNA template extracted from bloodstained sterile purified water swab (lane 3-7).

III. The effect of carry-over PCR inhibitors in water samples after DNA extraction and purification

The effect of carry-over PCR inhibitors in the water samples after DNA purification were demonstrated by adding 3 different volumes (5, 10 and 14.8 µl) of water-swab (or 'no bloodstain' swab) extracts into the PCR reaction to amplify 1 ng of DNA template using Pig_F -Pig_R2 primer pair. The ethidium bromide-stained agarose gels depicting the absence or presence of the 211-bp PCR-amplified DNA fragment are shown in figure 5 and the results are summarized in table 3. The 211-bp DNA fragment was not PCR-amplified from all samples. Results suggested that water used for bloodstain collection can be divided into 3 groups according to 3 different levels of PCR inhibition. The first group composed of NSS and drinking water no. 2 swab extracts. No PCR product was obtained when 5, 10 and 14.8 µl of water-swab extracts were added to the PCR reaction. The second group composed of water for car battery no.1 and tap water no. 1, in which faint 211-bp DNA bands were present when 5 and 10 µl of the extracts were added to the PCR reaction, but absent when the volume was 14.8 µl. The third group composed of sterile-purified water, distilled water for car battery no. 2, drinking water no. 1, tap water no. 2 and 3, in which distinct 211-bp DNA bands were present in all PCR conducted.

Table 3 A summary of tested result of the effect of carry-over PCR inhibitors in water samples after DNA extraction and purification. (✓ = PCR product was present, X = PCR product was absent)

Group	Description	Volume of swab extract added (µL)			
		0	5	10	14.8
1	Normal saline (NSS) for contact lens	✓	X	X	X
	Drinking water no. 2	✓	X	X	X
2	Water for car battery no. 1	✓	✓	✓	X
	Tap water no. 1	✓	✓	✓	X
3	Sterile distilled water	✓	✓	✓	✓
	Water for car battery no. 2	✓	✓	✓	✓
	Drinking water no. 1	✓	✓	✓	✓
	Tap water no. 2	✓	✓	✓	✓
	Tap water no. 3 (ground water)	✓	✓	✓	✓

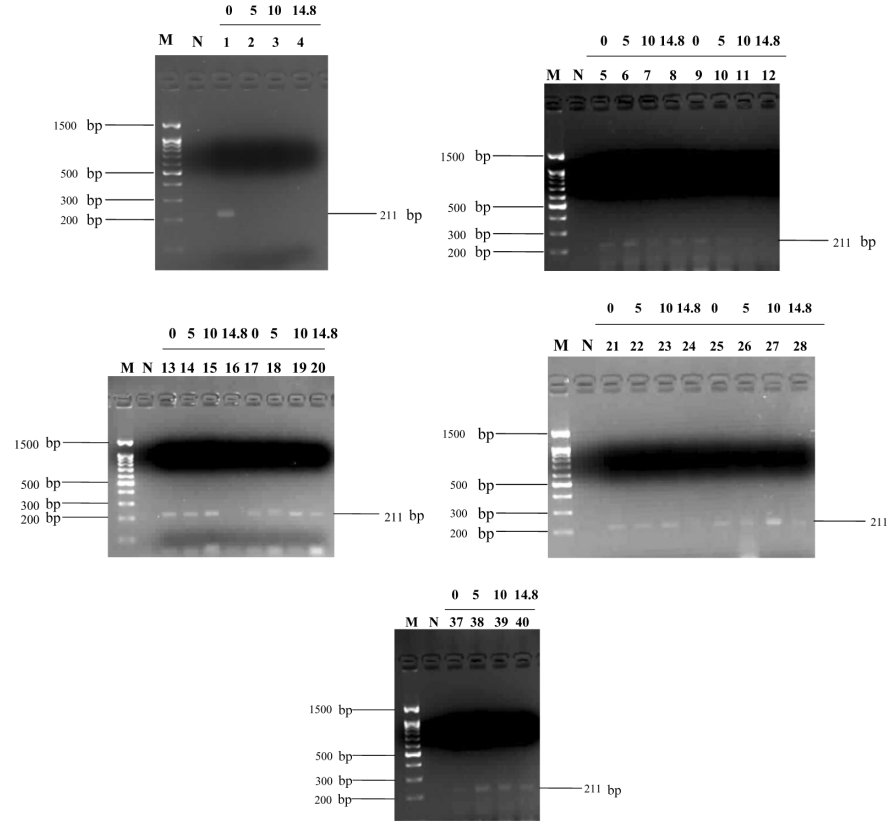


Figure 5 The effect of carry-over PCR inhibitors in the water samples after DNA extraction and purification. The 211-bp β-actin PCR products were amplified from 1 ng DNA template with 0, 5, 10 and 14.8 µL of water-swabs (no bloodstain) extracts as indicated. Lane 1-4; normal saline for contact lens, Lane 5-8; tap water no. 3 (ground water), Lane 9-12; drinking water no. 2, Lane 13-16; water for car battery no. 2, Lane 17-20; drinking water no. 1, Lane 21-24; tap water no. 1, Lane 25-28; tap water no. 2, Lane 37-40; water for car battery no. 2. Lane M; 100 bp DNA ladder, Lane N; negative control.

IV Amplification of 12-month-old DNA extracted sample

After 12 months of storage, all samples were amplified by 3 primer pairs; Pig_F2 - Pig_R2, Pig_F2 - Pig_R3, and Pig_F1 - Pig_R3. No PCR products were detected when 10 ng of DNA template was added to the reaction; therefore, the DNA template was then increased to 20 ng. As summarized in table 4, all three DNA fragments, i.e. 211, 289, 366 bp, were amplified from only one sample, the DNA template extracted from the bloodstain collected with laboratory sterile-purified water swab. However, only 2 fragments, 211 and 289 bp, were PCR-amplified from bloodstained collected with NSS swab.

Table 4. A summary of the amplification of 211, 289, 366-bp β-actin fragments of 12-month-old DNA extracted samples. (✓ = PCR product was present, X = PCR product was absent)

No.	β-actin fragment / Description	211 bp	289 bp	366 bp
1	Sterile distilled water	✓	✓	✓
2	Normal saline (NSS) for contact lens	✓	✓	X
3	Water for car battery no. 1	X	X	X
4	Water for car battery no. 2	X	X	X
5	Drinking water no. 1	X	X	X
6	Drinking water no. 2	X	X	X
7	Tap water no. 1	X	X	X
8	Tap water no. 2	X	X	X
9	Tap water no. 3 (ground water)	X	X	X

Discussions

In this study, 9 water samples of 5 different types were investigated for the effect on DNA analysis of bloodstain collection. All of these water types were easy to obtain when they were urgently needed. They were 2 samples of water for car battery in which the bottles were opened, 2 samples of drinking water (one was bottled, and the other was from a drinking water tank), 3 samples of tap water (from 3 different places and one was ground water), and 1 sample of normal saline for contact lens. Experiments were conducted in parallel with the sterile purified water routinely used in the laboratory.

Measurements of pH and conductivity could be used to divide the level of purity of water samples tested. The pH range of these samples was 5.5 - 8.5. Based on the conductivity, the purity of water types could be estimated in the following order; sterile distilled water, water for car battery, bottled drinking water, tap water no. 1 and drinking water no. 2, tap water no. 2, tap water no. 3, NSS for contact lens, respectively.

DNA was extracted from swabs using the solid-phase column extraction method (QIAamp DNA mini kit, QIAGEN®, Germany). This method was easy and could efficiently recover and purify DNA from the biological sample [5, 6]. DNA samples were analyzed using combinations of the 3 nuclear β-actin gene locus primers which can generate 211, 289 and 366-bp DNA fragments, according to Phengon *et al.* [7,8]. These fragments were designed to cover

the size range of STRs used in forensic human DNA typing. Large DNA fragments ranging between 300-500 bp would be poorly amplified from forensic biological samples [9]. Results showed that the 211-bp DNA fragment could be PCR-amplified from all DNA samples. However, inconsistency of the 366-bp DNA bands was observed when water swabs other than sterile purified water were used for bloodstain collection. Since large amount of DNA template (10 ng) was used for PCR amplification, subtle difference of the amplification products was observed. The 366-bp PCR product was confirmed by nested-PCR approach. The 211-bp DNA fragment was re-amplified from all 366-bp reactions. It confirmed that the amplified 366-bp DNA fragments were part of the β-actin gene. Results from figure 4 suggested that the 211-bp PCR product amplified from DNA template as low as 1 ng was sufficient to be analyzed with the ethidium-bromide stained gel.

The different intensities of the amplified 211 and 366-bp DNA fragments observed in figure 2 and 3 suggested that there may be PCR inhibitors present in the water used for moistening the swabs. Hence, the amount of 0, 5, 10 and 14.8 µL of the 'no bloodstain' or 'water-only' swab extracts, were added to the PCR reaction to test for the inhibitory effect of the carry-over PCR inhibitors. Amplification of the 211-bp DNA fragment from 1 ng of DNA template demonstrated 3 levels of inhibition; complete inhibition, no PCR product was detected in the presence of water swab extract in the amplification reaction; partial inhibition, PCR product was absent when the volume of water swab extract was increased; no inhibition, the PCR product was all detected. As summarized in Table 3, PCR inhibition was observed in drinking water no. 2 but not no. 1. Drinking water no. 1 was a 500-ml bottled drinking water and no. 2 was from a 20-l drinking water cooler. The differences between these two drinking water samples were manufactures, type and size of the two plastic containers. Drinking water in the larger container was prone to be contaminated from various sources such as the reservoir, pipes and outlet of the cooler tower, whereas bottled drinking water was stored in its original sealed-container prior use. Tap water was collected from 3 different sites. PCR inhibition was observed in only one sample when 14.8 µl of the tap water swab extract was added to the amplification reaction, while no inhibition was observed in the others. In this case, factors such as the water source, water reservoirs, pipe material, and the frequency of tap water use would affect the quality of water from the outlet. PCR inhibition was also observed when 14.8 µl of water for car battery no. 1 was added to the amplification reaction, while sample no. 2 showed no inhibition. The two samples of water for car battery were obtained from different manufacturer. Sample no. 1 was left opened over a period of time, while sample no. 2 was freshly opened. These variations suggested that the level of PCR inhibitors vary between sources. The various level of PCR

inhibitors can possibly be contributed by factors such as the nature of water at its origin, manufacturing process, period of time from manufacture date, container type, storage condition, and freshness or frequent of use from the storage container or tap. Conductivity of the water samples also supported the amount of total ions presented in each water sample. In PCR buffers, either NaCl or KCl is present as one of the major component to maintain the optimal ionic strength of the PCR [9]. Results indicated that NSS had the highest conductivity among all water samples and the increased of NSS-swab extract volume in PCR showed complete PCR inhibition. This result suggested the possibility of sodium and chloride ions being carried-over from the swab through DNA extraction and purification, thus destroying the buffering capacity of PCR buffer. As a result no PCR product was obtained from the amplification. Measurement of pH reflected the water impurity caused by dissolved gas and organic matters in the water, for example, CO_2 (g) dissolved in water would decrease pH. In addition, as no PCR products were obtained from amplification of the 12-month-old DNA extracts when non-sterile waters were used for bloodstain collection, this may be because of the presence of DNase in the water used for swabbing. As a practice, it is suggested that water used for sample collection for DNA analysis must be heat-sterilized to destroy DNase activity.

Amount of PCR inhibitors presented in the PCR may depend upon the type of inhibitor, the DNA extraction, and purification protocol used. For example, column-based extraction methods would be appropriate for removing insoluble contaminants in biological samples; and Chelex buffer would be appropriate for removing metal ion contaminants [10, 11]. Moreover, concentrations of DNA and the PCR inhibitor in the DNA extract, and their ratio would be crucial for successful amplification. If concentration of DNA extract is high and the PCR inhibitor is low, this would lead to successful amplification. On the opposite side, it would be more difficult to obtain results from amplification of DNA extracts having high level of PCR inhibitors and low amount of DNA.

Conclusions

This study demonstrated the type of water used for bloodstain collection would affect the downstream PCR-based DNA analysis, especially when only trace amount of material is present. However, results of DNA analysis can be obtained if excess or sufficient amount of biological material is available. DNA fragments less than 289 bp are likely to be successfully PCR-amplified than larger fragments when water other than sterile purified water was used for bloodstain collection.

Conclusions of this study could imply that if quality of water lower than that of sterile purified water was used for sample collection, the downstream PCR-based analysis could be affected. The chance of success would depend on the amount and condition of biological material available for collection, DNA extraction and purification method, the quantity of carry-over PCR inhibitor, the period of storage, and the sizes of DNA fragment being analyzed.

In case that substitution for sterile purified water is needed for bloodstain evidence collection, the following criteria are suggested for consideration. The substituted water should be in the single-use and airtight container, manufacture processed (purity and sterility), with good storage condition. From this study, although DNA extracted from bottled drinking water swab cannot be PCR-amplified after 12-month storage, it may be suggested for emergency substitution during field work because the physical properties are similar to that of highly purified water. It is bottled in a single-use container for consumption; therefore, cleanliness, freshness and storage condition would be better than other types of water in this study. However, the personnel should indicate the source of water used and the evidence collected must be immediately transferred to the laboratory for subsequence analysis.

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