ForensicAsia
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AFSN President's Address

Dear colleagues and friends,

It is my privilege and honour to be able to serve AFSN in the capacity of the President for the coming two years. I would like to thank all colleagues and friends who have put your confidence in me to lead this important network. I also like to thank my predecessor Mr Zhao Qiming, Director General of the Institute of Forensic Science, Ministry of Public Security, China, for being a good leader and an exemplary role model for me to follow.

AFSN held a successful 11th Annual Meeting & Symposium in Ho Chi Minh City, Vietnam, from 17th to 20th of September 2019. This meeting was hosted by colleagues from the Forensic Medicine Center of the Ho Chi Minh City and special appreciation goes to the Director, Dr Phan Van Hieu, and his team of dedicated staff who had put in a lot of efforts in the planning and running of the event. It was such a pleasure to connect with many colleagues from Asia who were attending this symposium. I would also like to thank all invited speakers and trainers who had travelled many thousands of miles to share your expertise with us.

I am very happy to announce the current AFSN Board that was elected by members at the Annual General Meeting held on the 20th of September 2019:

President	:	Dr. Angeline Yap, Health Sciences Authority, Singapore (also holding the position of International Liaison Officer)
Vice-President	:	Pol. BG Rolando Hinanay, Philippine National Police Crime Laboratory, Philippines
Board Members	:	Pol. Supt. Dr Lisda Cancer, Department of Police Medicine of the Indonesian National Police, Indonesia Dr. Namkyu Park, National Forensic Service, Korea Ms. Halimah Abdul Rahim, Department of Chemistry, Malaysia Mr. Zhao Qiming, Institute of Forensic Science, People's Republic of China (Past-President) Pol. Lt. Col. Wannapong Kotcharag, Central Institute of Forensic Science, Thailand

11th AFSN meeting for AFSN Board and Workgroup committee members

This Board is supported by Secretariat Ms. Nellie Cheng from Health Sciences Authority. I would like to thank the previous Board, led by Mr Zhao Qiming, and supported by Secretariat Dr Meng Qingzhen, for building up AFSN through knowledge sharing and training, collaboration and partnership, and promoting the use of quality forensic science to support the administration of justice.

I also extend my appreciation to the outgoing Chairs, Vice-Chairs and Secretaries from the various workgroups and Quality Assurance and Standards Committee, for their commitment in leading and strengthening the workgroups. As AFSN continues to grow in membership size, knowledge and expertise, my sincere hope is that we will continue to collaborate with one another in this journey. In this regard, ForensicAsia plays an important role for us to share our research and investigative findings and learn from others. I would like to encourage all AFSN members to actively submit your articles to ForensicAsia. This issue of ForensicAsia contains many interesting articles – enjoy reading it!

Dr Angeline Yap
AFSN President
Health Sciences Authority
Singapore

Editor's Address

Dear colleagues and members of AFSN,

Thanks to our members who have supported ForensicAsia by contributing good articles for this 10th Issue. We hope this will bring forth insights of their scientific work so as to continue to improve our professional expertise in the forensic community.

In this Issue, we have a total of 3 technical articles and 2 case studies, including, DNA/forensic biology, toxicology, questioned documents and crime scene investigation. We have received write-ups in the AFSN/Member's News on the 11th AFSN Annual Meeting & Symposium, QASC survey, DNA Workgroup Inter-laboratory Exercise and Reformation of forensic system. In addition, we have also received introduction of 2 new member institutes which joined AFSN last year.

Once again, I would like to thank our guest editors who spent time in reviewing the articles, and our editorial assistants who designed the artwork and facilitated the online publication of this new issue.

Enjoy the reading!

Dr Lui Chi Pang
Editor

Editorial Committee

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For contribution of articles, please read the guidelines at www.asianforensic.net.

Content

The 11th AFSN Annual Meeting and Symposium 2019

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Forensic Medicine Center of Ho Chi Minh City (FMC) is a new member of the Asian Forensic Sciences Network. With our determination and the support from Board members, we, FMC, could have a chance to hold the 11th AFSN Annual Meeting and Symposium 2019 at Convention Center 272, HCMC, Vietnam with Pham Ngoc Thach University of Medicine as a co-organizer.

We hosted this 4-day meeting successfully, from 17 – 20 September, with more than 400 overseas and local in terms of delegates from 17 countries and 56 member institutes. There were 13 invited speakers from all over the world including Australia, Germany, Spain, Austria, New Zealand, China, Hungary and Singapore. Six keynote lectures were presented in general session, while 83 oral presentations were shared in the respective workgroups session, along with 62 posters from AFSN members.

The first day saw one meeting from Board members and one DNA workshop. General session on day 2 provided not only new information and essential knowledge to participants, but also an overview of forensic science from past years to now.

With the formation of two new workgroups (WGs): Fingerprint and Questioned Documents, there were 8 WGs in total. Each WG saw a remarkable achievement with its scientific session, for instance “Bloodstain pattern analysis workshop” in Crime Scene Investigation, and “Bring a case session” in Trace Evidence WG brought to attendees the diversity of forensic cases, drew their attentions and hot debates as a result.

The event was supported by 11 sponsors who put up impressive booth displays and interesting games for participants.

Work hard, play harder! All delegates were able to enjoy a memorable dinner with delicious food and traditional Vietnamese musical instrument performance (Zither) from our staff so that we could build up friendships and unforgettable moments together before closing ceremony which took place on 20 September.

QASC-AFSN Progress Report: Accreditation Status of AFSN Member Institutes

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Quality Assurance and Standard Committee (QASC) is the only committee among the workgroups in AFSN. Its objectives are to provide information and create awareness of quality assurance in forensic laboratories in Asia and to coordinate training and other activities related to quality assurance under the mission "to be a focal point to promote development and delivery of quality forensic services in Asia". QASC has surveyed the accreditation status among AFSN members during 2018-2019. The data were collected within August 2019. The objective of this survey was to understand the gap of accreditation status among AFSN members and the needs for minimizing the gap. This article is the first QASC-AFSN progress report showing the accreditation status of 24 out of 56 current AFSN member institutes (42.9%) which responded to the survey. From the data, the AFSN member institutes can be divided into 2 main groups depending on accreditation status for ISO/IEC 17025 or 17020.

The first group is the accredited members. It is composed of 70.8% of the surveyed member institutes (17 of 24 labs). This statistics reflects a minimum percentage of accredited members is approximately 30.4% of current AFSN member institutes. A summary table below shows the disciplines which are accredited.

Disciplines	Biol	Chem	Tox	Drugs	Trace	FPrint	Doc	FT&T	GSR	CSI	DE	Others
No. of Institutes	11	10	12	12	8	5	10	9	7	4	7	7

It is noted that 23.5% of the accredited members (4 of 17) has been developing for extended scopes of accreditation. The extended scopes include most or all disciplines shown in the table above. Other disciplines that are also accredited, include, blood pattern analysis, traffic accident, impressions, serial number restoration, water testing and pharmaceutical products.

The second group is the un-accredited members. It is composed of 29.2% of the surveyed member institutes (7 of 24). This statistics reflects that the minimum percentage of un-accredited members is approximately 12.5% of current AFSN member institutes. These member institutes have been developing for the accreditation, and the disciplines aimed for accreditation are shown in the table below.

Disciplines	Biol	Chem	Tox	Drugs	Trace	FPrint	Doc	FT&T	GSR	CSI	DE	Others
No. of Institutes	4	3	5	5	4	2	2	2	2	2	1	1

The issues that need to focus for fulfilling the quality standard gap include: (i) providing mock accreditation to the un-accredited labs, (ii) gaining more knowledge and skill in how to prepare for accreditation of ISO/IEC 17025 or 17020, apply risk assessment in the laboratory, upgrade accreditation status to ISO/IEC 17025: 2017, and build up personnel capability, (iii) creating a work plan for getting accreditation, and (iv) participating in PT programmes and/or inter-laboratory comparison in various fields.

With this report, we hope to create an awareness among the AFSN Workgroups in their strategic work plans for promoting and strengthening the development of AFSN member institutes to deliver quality forensic services in Asia.

6th Asian Forensic Sciences Network (AFSN) DNA Workgroup Inter-Laboratory Exercise

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The inter-laboratory DNA testing exercises have been well received by the member institutes in the Asian Forensic Sciences Network (AFSN) since its launch in 2015 for process benchmarking purpose. Since then, the DNA Profiling Laboratory (DNAPL) has organised 6 comparative studies for its regional counterparts, covering a wide range of topics such as extraction efficiency and mixture interpretation approaches. The number of participants have grown from 11 laboratories in 7 countries in the first study to 18 institutes from 9 countries in the 6th exercise.

The 6th inter-laboratory exercise, titled "DNA profiling of buccal and semen mixed samples", aimed to compare the performance of the participating laboratories in the analysis of items with semen and DNA from multiple contributors. The exercise kit consisted of four items, which were prepared using buccal suspensions and semen samples from one female and three males. The items were sent to the participants for testing using their own casework methods and protocols. The laboratories were instructed to match the DNA results obtained from the items against the provided reference profiles.

The results returned from the participating institutes were collated and presented at the 11th AFSN Annual Meeting and Symposium in Ho Chi Minh City, held between 17 and 20 September 2019. More than 85% of the laboratories that took part in the study were able to separate sperms and non-sperm cells effectively and produce concordant DNA profiles from the samples, demonstrating the generally high standards of forensic DNA testing among the AFSN member institutes.

In addition to its objective of quality improvement and enhancement, participation in the inter-laboratory comparative exercise allows laboratories to demonstrate compliance to some aspects of the ISO/IEC 17025:2017 requirements for method validation and proficiency testing. Certificates of completion were provided to all laboratories who successfully completed the exercise. A summary report comprising the exercise information and collated results was also prepared. The 7th AFSN DNA Workgroup comparative study has been planned for 2020 and will focus on kinship analysis.

Colleagues of the DNAPL (Health Sciences Authority), Singapore, presented the results of the 6th AFSN DNA Inter-laboratory exercise at the 11th AFSN Meeting in Ho Chi Minh, Vietnam, 17-20 September 2019.

Participants of the 6th AFSN DNA Inter-laboratory exercise from China, Korea, Malaysia and Vietnam at the 11th AFSN Meeting in Ho Chi Minh City, Vietnam, 17-20 September 2019.

Reformation of Thailand Forensic System

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The result of forensic investigation is a crucial evidence during court trial. However, from the history of judgements in Thailand, it might not be used to indicate offense as shown in a number of verdicts. This is a big challenge in applying forensic sciences in court. Problems and causes have been widely discussed by related parties in Thailand forensic community. Guides for improving forensic investigation and administration system have also been proposed in order to get changes in Thailand forensic system.

During 2017-2019, at the beginning of Thailand reformation era, this issue was attended by the Thai Parliament. The Standing Committee to Propel Nation Reformation, Thai Parliament, appointed the Laws and Justice System Committee and the Forensic System Reformation Sub-Committee to study the root cause of the problems that have been occurred in Thailand forensic system. In November 2017, after many meetings and discussing with the major stakeholders of forensic sciences, the Sub-Committee launched the final report: the Reformation of the Entire Forensic System.

This report included the present situation of Thailand forensic system, problems, challenges, causes, and suggestions for reforming the entire system of Thailand forensic science. The problems, challenges and causes included disrupted forensic administration, outdated laws, immature-competent forensic practitioners, poor support and welfare for forensic practitioners, non-united standard of practices, aimlessly budget administration, limited applied research and inconsistent forensic courses. The Thai Parliament subsequently announced the "Reformation Plan of Forensic Science for the Completeness of Facts", which was a part of the "Nation Reformation Plan 2018".

The most important issue of the plan was to set up the "Commission of National Forensic Policy", which would be responsible for making up the policy for national forensic science administration and propelling forensic sciences to be more worthy in the justice system. This is a great opportunity for Thailand forensic community to get rid of all major problems and challenges, which have retarded the development of Thailand forensic science, and simultaneously to reform a new surpassing system.

It is suggested that while the essentials; administration, law, regulation, standardization, competency, research, courses, welfare, and budgeting, are being reformed in the new forensic system, national and international networking is a matter that must be concurrently proceeded for facilitating the forensic services to meet the quality standard.

Hopefully, the result of the forensic science system reformation will ultimately expedite and strengthen the process of Thailand justice system.

Introduction of the Institute of Forensic Science, Hangzhou Public Security Department

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The Institute of Forensic Science, Hangzhou Public Security Department, also named Hangzhou Public Security Department Criminal Science & Technology Institute (HZCSTI), was established in 1987. The HZCSTI Laboratory is currently one of the largest and most comprehensive crime laboratory in China.

The core functions of HZCSTI laboratory are Forensic examinations, Crime scene investigation, and Scientific research. HZCSTI laboratory uses state-of-the-art science and technology to conduct forensic examinations, including, Latent prints, Foot impressions, Firearms/Tool marks, Toxicology, Controlled substances, Drugs, Alcohol, Forensic pathology, Forensic clinical, Forensic biology, Imaging analysis, Voice recognition, Questioned documents, Cell Phone Forensic, and Digital evidence. The Laboratory comprises of Marks examinations unit, Forensic pathology unit, Chemistry unit, Imaging analysis and voice recognition unit, Questioned documents unit, DNA analysis unit, Digital evidence unit, and Automated fingerprint identification system (AFIS) center.

It was accredited by China National Accreditation Service for Conformity Assessment (CNAS) in 2008, and was certified by Certification and Accreditation Administration of the People's Republic of China in 2015. Today the HZCSTI Laboratory covers more than 7,000 square meters, employs 81 full-time employees, including 26 senior level analysts or examiners. Every year, the HZCSTI Laboratory completed more than 8,000 criminal cases, and more than 8,000 Laboratory reports. All the results are completed with strict QC procedures in order to ensure high quality.

Today, the HZCSTI Laboratory have executed more than 20 Forensic Science research and development projects from National and Province government. In the future, we plan to put more people and financial resources in the scientific research, keep in touch with the AFSN members and hope to collaborate with each other.

Introduction of the Forensic Examinations Centre, Republic of Kazakhstan

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The modern system of forensic science was established in Kazakhstan in the middle of the 20th century. Currently the public sector is represented by the Forensic Examinations Center of the Ministry of Justice, Republic of Kazakhstan.

The Centre comprises of:

- 19 institutes, which conduct forensic examinations across the whole of Kazakhstan;
- 1 scientific and research institute; and
- around 2800 staff members.

The private sector is represented by the Republican Chamber of Forensic Experts.

Forensic examinations are carried out in more than 57 disciplines, including, digital technologies, medicine, biology, criminalistics and religious studies.

The Center has scientific accreditation, which enables us to undertake scientific research through government funding. Our experts apply more than 400 methods in their work. One of the latest developments of the Center is a method for the analysis of electronic signatures created with a stylus and tablet.

In Kazakhstan, special attention is also paid to the continued training and development of our forensic experts. The Ministry of Justice together with the World Bank implemented a unique project to strengthen forensic science in Kazakhstan. As part of the project, more than 200 forensic experts undertook training in Great Britain, Israel, USA and Russia. 54 forensic experts were also able to participate in year-long scientific programmes in Great Britain funded by the Presidential Bolashak Scholarship. Additionally, more than 200 experts undertake training annually in foreign and local laboratories to improve their qualifications.

An integral part of our work is the implementation of the latest information technologies, for example, the electronic system «E-SARAPTAMA», which allows us to:

- automate the processes of chain of custody and exhibit handling;
- improve quality control of forensic investigations; and
- facilitate transition from paper to an electronic format.

The Forensic Examinations Center is actively involved in international collaboration, and is: a member of the working group for forensic examinations as part of the Shanghai Cooperation Organization; as well as a member of the Asian Forensic Sciences Network.

At the Forensic Examinations Center, our motto is “Law, independence and science”!

Performance of 24 STR Loci Multiplexes kit with Reduced Reaction Volume for Profiling Reference Blood Stained on Treated Paper

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Abstract

Internal validation of 24 short tandem repeat (STR) loci kit from GlobalFiler™ Express PCR Amplification kit were performed with reduced total volume reaction from the default manufacturer recommendation. A total of 43 reference blood stained on Flinders Technology Associates (FTA®) cards were directly amplified after been purified with Sodium Hydroxide, low Tris-EDTA (TE) buffer and deionised water. Amplification cycle was test at 25 and 24 cycle with additional 23 cycle while the data collection and data analysis were set at default. Amplification shows 100% first-pass rate at 24 cycle with a much lower overloaded electropherogram compare to 25 cycle. Comparison with direct amplification without purification of the disc demonstrate electropherogram with slope-like pattern. This result demonstrates that reduced reaction mix volume of GlobalFiler™ Express is still capable and successfully typing purified FTA® reference blood samples.

Introduction

Profile of reference samples are important to be type successfully to ensure DNA comparison made are assertively. There are many current database profiling kit available in the market providing assurance in typing reference samples with a good and interpretable profile as well as compliant with the CODIS loci expansion. These kits are made to be typing directly, especially reference blood specimen stained on FTA® card. GlobalFiler™ Express PCR Amplification Kit is one of the kits that has been developmentally validated to directly amplify reference blood samples on FTA® card with a 15 µL of total volume per reaction. The aim of this study is to internally validate the capability of GlobalFiler™ Express Kit with a reduced volume in profiling reference blood samples stained on FTA® card.

Materials and Methods

Sample preparation

A set of 42 reference blood specimen received in the Forensic DNA Division, Department of Chemistry Malaysia were randomly collected regardless of male or female contributor and stained on FTA® card. The dried blood stains were then punched using 1.2 mm sized micro puncher and the disc were subjected to purification using 10 mM Sodium Hydroxide, low TE buffer and deionised water to remove any inhibitor. Purified disc was then transferred to a sterilised 200 µL PCR reaction tube.

The same set of samples were punch and skip the purification step to compare the effect of modification in reaction mix volume for the non-purified samples. These samples amplified directly using the same optimum cycle determined from purified FTA® disc.

Reduced volume

Total volume of reduced amplification mix for a single reaction is 12 µL comprising 5 µL of Master Mix (additive added), 5 µL of Primer Set and 2 µL of low TE buffer.

Amplification and electrophoresis

ProFlex™ Thermal cycler was used to amplify all samples and amplified at 24 and 25 cycle. Data collection on Genetic Analyzer 3500xl setting was set according to manufacturer protocol. Data analysis was performed using GeneMapper version 1.5.

Results and Discussion

The default manufacturer reaction mix volume is optimized to amplify with high tolerance to inhibitors. Modification from manufacturer reaction mix volume will affect this tolerance. Thus, the purpose of purifying the bloodstained FTA® disc is to remove the hematein so that amplification process will not inhibited.

Purified samples amplified at 25 cycle number demonstrate massively overloaded signal in majority of the samples and these samples were omitted for further evaluation. Amplification at 24 cycle number demonstrate 100% first-pass success rate. Thus, the optimum cycle number for the purified FTA® disc of blood specimen is 24 cycle. Amplification of the non-purified samples at 24 cycle demonstrate 69% first-pass success rate (Table 1). However, 24 of the pass samples show electropherogram with slope-like pattern indicating inhibitors that relatively increase due to reduce in the reaction mix volume [1]. Figure 1, Figure 2 and Figure 3 show examples of two same samples amplified at 24 cycle with one of it amplified directly while the other amplified after been purified.

Average heterozygote peak height ratio for purified samples was observed to be more than 90% with its relative standard deviation 3.1%. There is only one sample observed to have lowest peak height i.e 467 RFU. Nevertheless, this sample still demonstrate a good heterozygote peak height balance with not less than 80%.

Conclusion

Successful profiling of 24 STR loci was achieved using reduced reaction mix volume of GlobalFiler™ Express amplification kit provided that the FTA® disc of reference blood were purified prior direct amplification to remove all possible inhibitors. The advantage on reduction of master mix volume is that more reaction can be performed to cater an increase number of reference samples submitted with relatively lower cost per sample. This new in-house method of reduced reaction mix volume had officially implemented in Forensic DNA Division of Department of Chemistry Malaysia Petaling Jaya and pending for upcoming accreditation.

References

[1] *R. Almheiri, R. Alghafri, Reduced volume for direct PCR amplification of blood reference samples using Identifiler® Direct and GlobalFiler™ Express assays, Forensic Science International: Genetics Supplement Series, Volume 6, 2017, Pages e340-e341*

[Table 1]: Number of samples for purified and non-purified FTA® disc and its corresponding first-pass rate.

[Figure 1]: Electropherogram of unpurified FTA® disc (a) shows a significant slope-like pattern compared to purified disc sample (b).

[Figure 2]: Note the large amplicons of red channel in unpurified FTA® (a) were dropped out compared to the same sample of purified disc (b).

[Figure 3]: Another slope-like pattern of electropherogram from (a) the unpurified sample indicating presence of inhibitors compared to the purified sample (b).

Sample Type	No. of samples		Success rate
	Full profile	Partial profile	
Purified	42	0	100%
Non-purified	29	31	69%

Table 1. Number of samples for purified and non-purified FTA® disc and its corresponding first-pass rate.

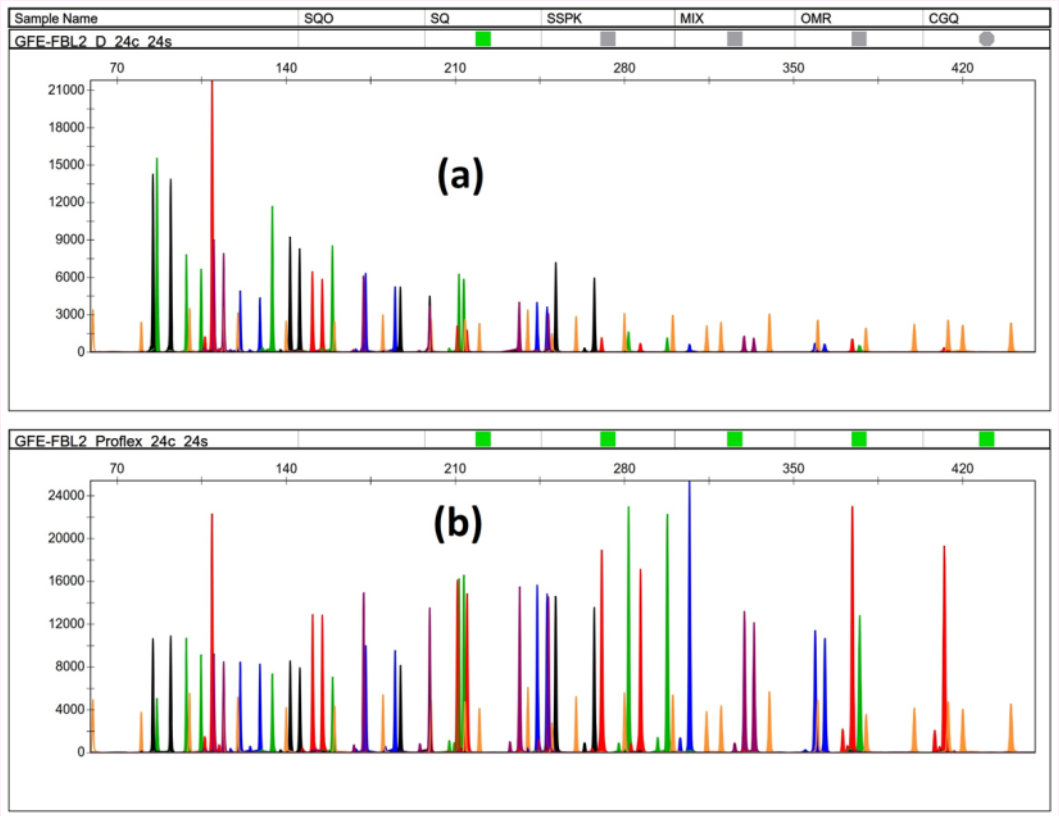

Figure 1

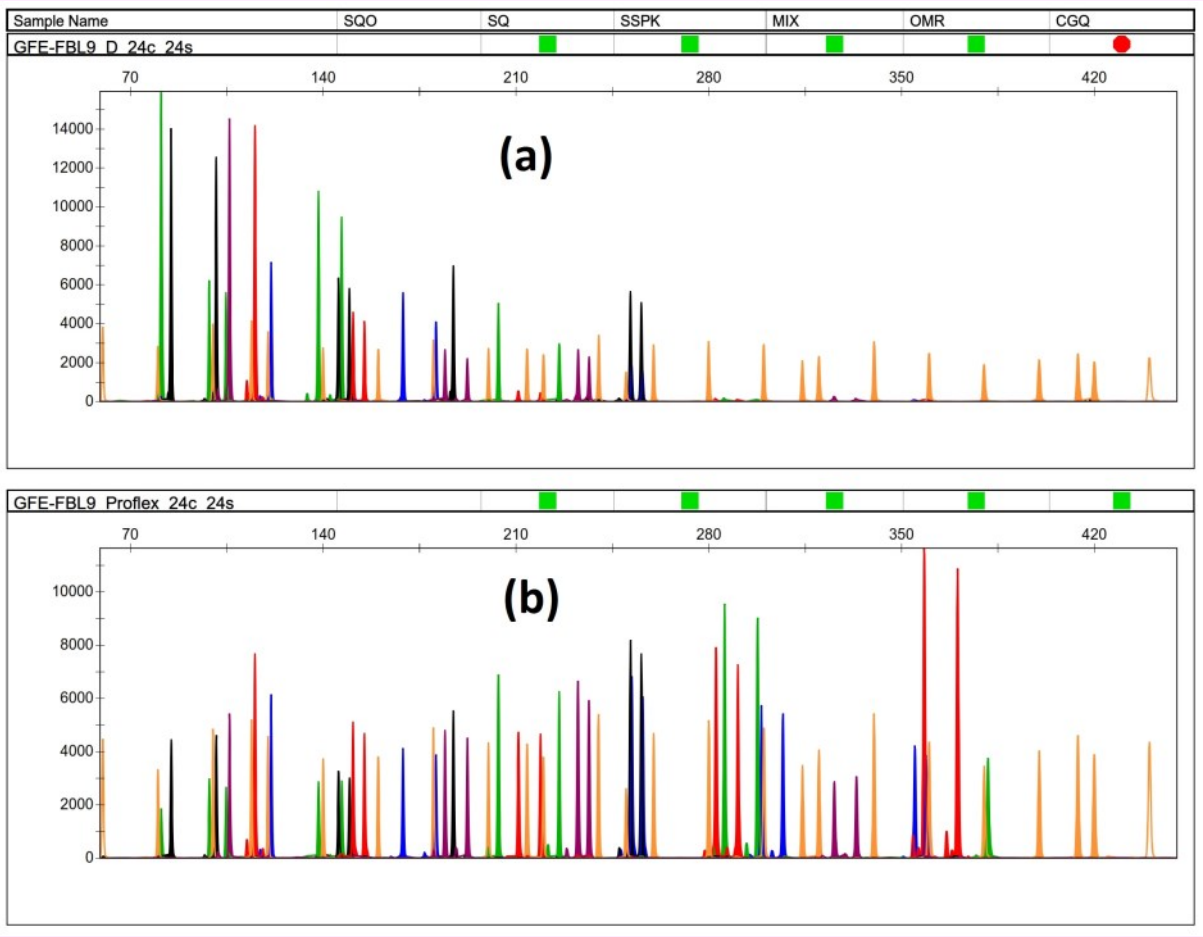

Figure 2

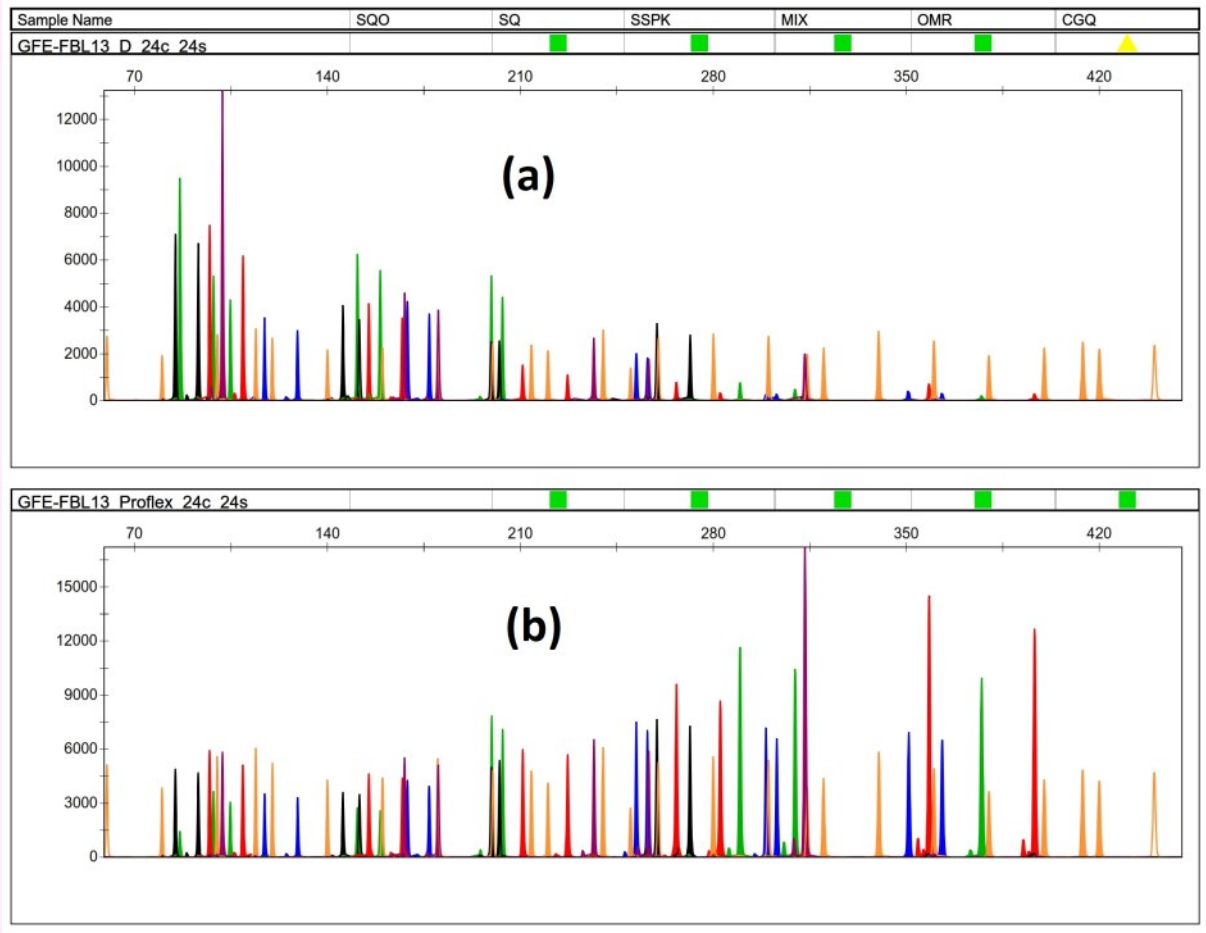

Figure 3

Confirmation of Toxic Alkaloids in Gelsemium Poisoning

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Introduction

Gelsemium elegans Benth is one of three species of Gelsemium (Loganiaceae) that contains highly toxic indole alkaloids such as koumine, gelsemine, gelsenicine, humantenine, humantenirine [1]. Of these, koumine and gelsemine are the most toxic alkaloids [2] and have been studied extensively. Figure 1 showed the chemical structure of these 2 alkaloids. The intoxication symptoms include rapid-onset dizziness, nausea, vomiting, blurred vision, limb paralysis, breathing difficulty, coma, and convulsion [3]. In severe poisoning, life threatening respiratory depression would lead to death [4]. *G. elegans* has been used for suicide. So, the detection of koumine and gelsemine are important evidences to establish the lethal cause in forensic medicine examination. Despite numerous studies on the gelsemium species, very limited information is available on the analysis of gelsemium toxins in viscera and biological samples [5]. Therefore, this paper studied samples from cases of suspected poisonings by the *G. elegans*.

Materials and Methods

1) Materials

Between December 2018 and May 2019, the laboratory received the specimens from fourteen suspected Gelsemium poisoning cases and analyzed these specimens by GC/MS. Eight cases of suspected death were from eating Gelsemium leaves. Postmortem gastric contents and blood specimens, and the leaves they took were obtained. Another three cases involved a mother and two children who drank wine in which Gelsemium leaves were immersed. They were admitted to the hospital and survived. The leaf wine was also submitted for analysis. The last three cases were from three children in a family who drank the liquid boiled with Gelsemium leaves. Two children survived but a 5 year old girl died and was autopsied. Gastric contents, blood and the liquid were submitted (Table 1).

2) Chemicals and Reagents

Gelsemine and koumine reference materials were obtained from the Forensic Chemistry Department, National Institute of Forensic Medicine, Vietnam. The solid phase extraction (SPE) of biological samples was performed on SampliQ Evidex 200 mg column (Agilent Technologies Co, USA). All other necessary chemicals and reagents were purchased from commercial sources in high purity and used with no further purification.

3) Gas Chromatographic/Mass Spectrometric (GC/MS) Conditions

All analyses were performed using a GC/MS, which consisted of an Agilent 7890B series GC connected to an Agilent 5977A mass detector. Data acquisition and data analysis were performed on the MassHunter software. The column used was HP-5MS (30 m×0.25 mm×0.25 µm), with a helium flow rate of 1 mL/min and splitless injection at an injector temperature of 270^{o}C . Agilent 7693 autosampler was used to inject 1 µL of the extracts into the GC. The oven temperature profile was 80^{o}C, held for 1 min, then increased 20^{o}C/min up to 290^{o}C and held for 20 min.

4) Sample Pretreatment by Liquid-Liquid Extraction (LLE)

Gastric content, vegetable, rice, juice, leaf water and unknown leaf wine, were extracted by LLE. Approximately 5g of sample was first diluted with water at suitable portion and adjusted to pH 2-3 with 1M hydrochloric acid. The mixture was then incubated at 60^{o}C for 6 hours to extract alkaloids from the sample and centrifuged at 4000 rpm for 10 min. The supernatants were then transferred to another tube and adjusted to pH 10-11 with 6 M ammonium hydroxide. The mixture was extracted with 10 mL of chloroform for 5 min and centrifuged at 4000 rpm for 10 min. The chloroform layer was transferred to another test tube and evaporated to dryness in a water bath at 40^{o}C under a stream of dry nitrogen. The extract was reconstituted with 100 µL methanol and injected into the GC/MS.

5) Solid phase extraction procedures using SampliQ Evidex Column

Blood and urine were extracted by SPE. Prepare the sample by adding 2 mL of blood or urine to 2 mL of 0.1 M phosphate buffer (pH 6) and vortex mix for 1 min. The SPE cartridges were solvated and equilibrated with 3 mL of methanol and 3 mL of 0.1 M phosphate buffer (pH 6). After sample addition, the cartridges were rinsed with 3 mL of distilled water, 3 mL of 0.1M hydrochloric acid and then 3 mL of methanol, after which the columns were dried. Alkaloids were eluted with 3 mL of ethyl acetate: methanol: ammonium hydroxide mixture (70:28:2). The extracts were evaporated in a water bath at 40^{o}C under a stream of dry nitrogen. The extract was reconstituted with 100 µL methanol and injected into the GC/MS.

Results

1. Results of mass spectra of koumine and gelsemine

The fragmentation of koumine and gelsemine under GC/MS electron-impact (EI) conditions leads to characteristic mass spectra as shown in Figure 2. Using the full scan mode, the retention times were 12.61 min for koumine and 13.73 min for gelsemine. The Selected ion monitoring (SIM) mode was set at m/z 306 and 70 for koumine, and at m/z 322, 297 and 108 for gelsemine. The SIM chromatograms for gelsemine and koumine at m/z 322.1 and 306.1 were showed in Figure 3.

2. Results of analysis of the samples

The samples were analyzed using the GC/MS method described here. The results showed that koumine and gelsemine were detected in the leaves, leaf wine and leaf liquid. Koumine and gelsemine were also detected in all the gastric content samples of the deceased but not detected in the urine sample of the deceased or in the blood samples of the survivor (Table 2).

Conclusion

Koumine and gelsemine in the leaf wine, leaf liquid, leaves, gastric contents and blood were detected using GC/MS. This is the first report on the detection of gelsemium alkaloids in gastric content and material specimens in the National Institute of Forensic Medicine, Vietnam. Determining the presence of koumine and gelsemine in gastric content provides strong evidence of acute poisoning by Gelsemium. Our study would be of reference value for future toxicological investigation of Gelsemium.

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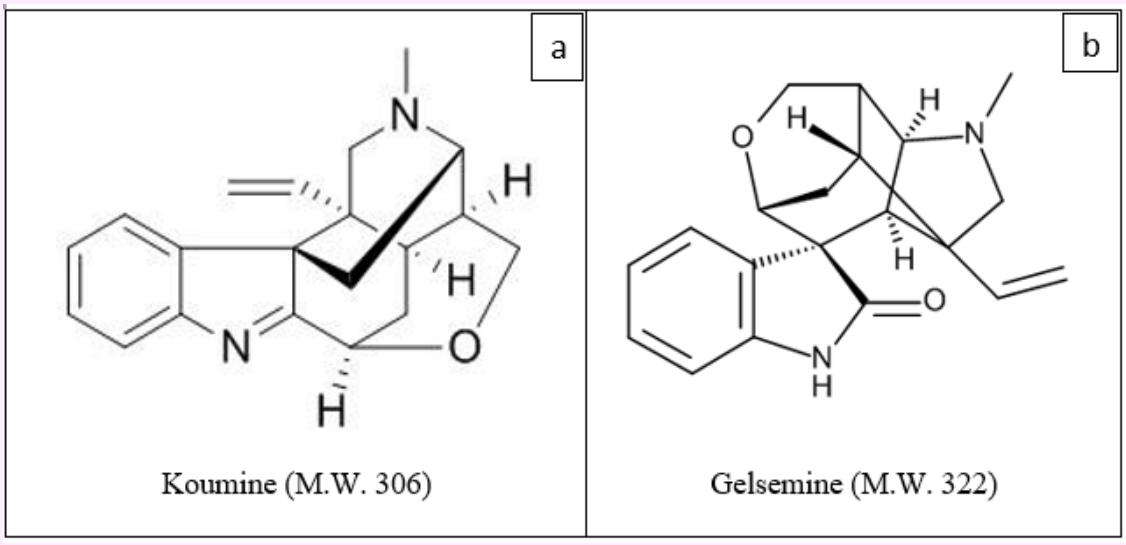

Figure 1. Chemical structures of koumine(a) and gelsemine(b)

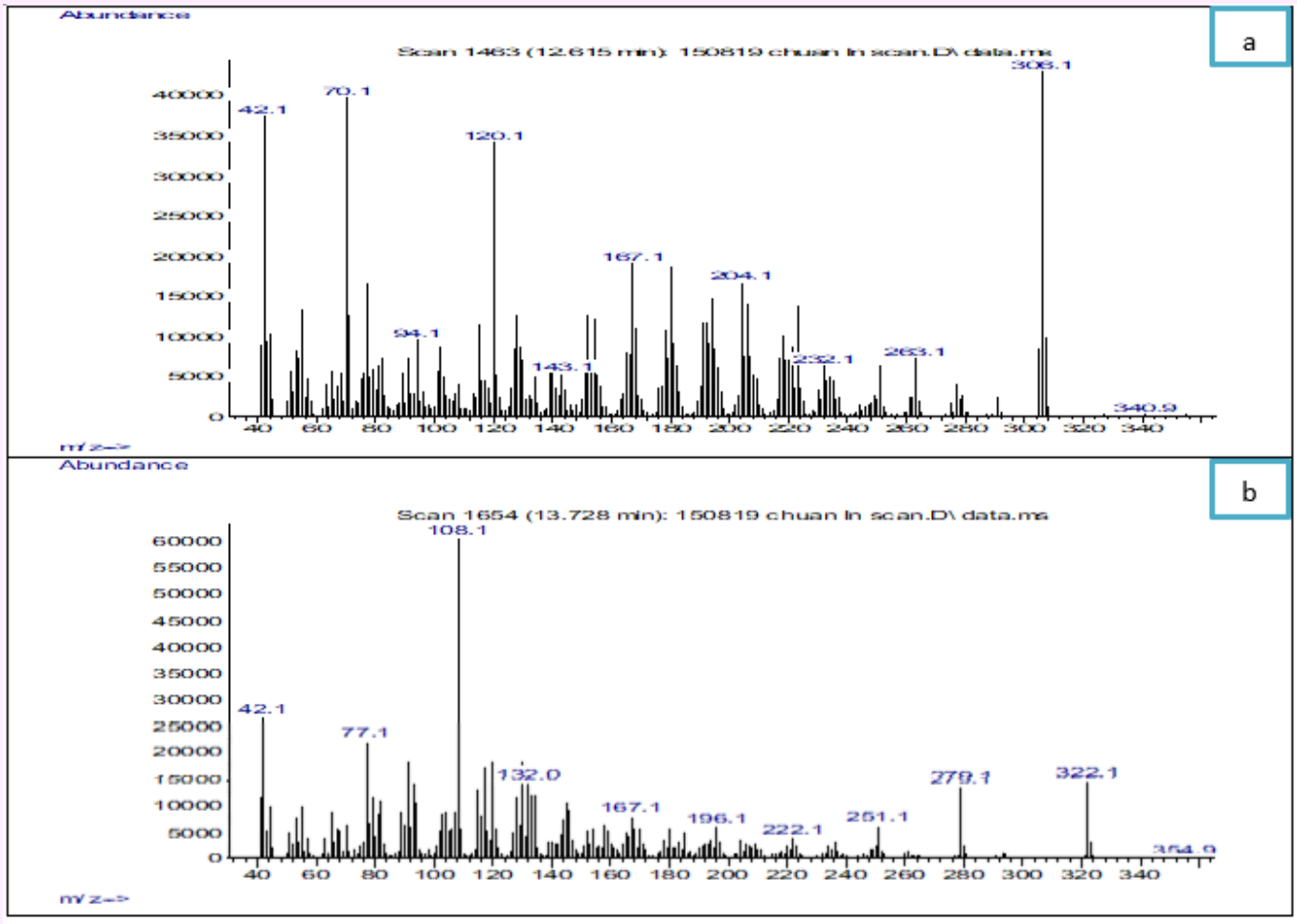

Figure 2. Mass spectra of analytes: koumine (a) and gelsemine (b)

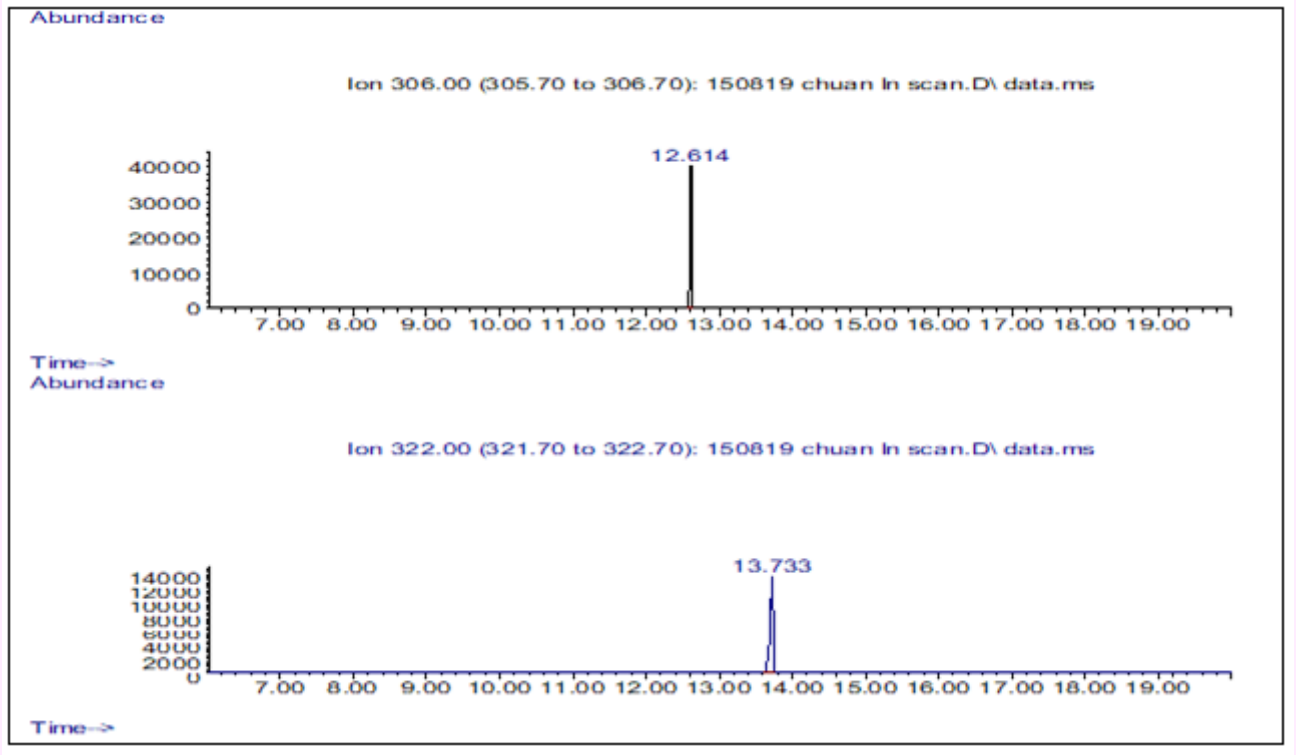

Figure 3. GC/MS data of koumine (ion 306.00) and gelsemine (ion 322.00)

Cas.No	Samples	History
1	Gastric content	Dead at home unknown causes
2	Unknown leaf wine	The mother and two children drink gelsemium leaf wine due to conflicts with the husband. They were alive after treatment in hospital.
3		
4		
5	Gastric content, blood, urine, vegetable, rice, juice.	Dead at home unknown causes
6	Gastric content, unknown leaves	Dead at the banana garden near the house unknown causes
7	Gastric content, unknown leaf wine	Dead at the construction shack after drinking
8	Gastric content	Dead at home unknown causes
9	Unknown leaf, leaf water	Dead at home unknown causes
10	Gastric content, unknown leaf, leaf water	Poison: The father was poisoned three children with the water to cook gelsemium leaves. A child was dead and two children was alive after treatment in hospital.
11	Blood	
12	Blood	
13	Gastric content	Dead at home unknown causes
14	Gastric content, blood	Dead at home unknown causes

Table 1. Materials and History of Intoxicated Cases

Cas.No	Samples	Alkaloids detected
1	Gastric content	Koumine, gelsemine
2	Unknown leaf wine	Koumine, gelsemine
3		
4		
5	Gastric content, blood, urine, vegetable, rice, juice.	Gelsemine in Gastric content, blood
6	Gastric content, unknown leaves	Koumine, gelsemine
7	Gastric content, unknown leaf wine	Koumine, gelsemine
8	Gastric content	Koumine, gelsemine
9	Unknown leaf, leaf water	Koumine, gelsemine
10	Gastric content, unknown leaf, leaf water	Koumine, gelsemine in gastric content, unknown leaf, leaf water
11	Blood	Not detected
12	Blood	Not detected
13	Gastric content	Koumine, gelsemine
14	Gastric content, blood	Koumine, gelsemine

Table 2. Results of GC/MS Analysis

Forensic Examination of Electronic Handwritten Signatures

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Introduction

The realities of our time dictate the need for the introduction of digital technologies in all spheres of society. The possibilities of forensic handwriting are constantly expanding due to the continuity of the process of accumulating expertise. Integration into the sphere of forensic handwriting of new achievements of science and technology, related fields of expertise, computer technology, requires a review of traditional approaches to the examination of documents. The need to develop the methodology described herein is due to the implementation of the "E-criminal case" project in Kazakhstan, which involves the translation of the procedural workflow into digital format and use of a Wacom STU-430 pad to authenticate electronic procedural documents with an electronic handwritten signatures ("EHS"). The developed integrated methodology allows to solve several problems related to the forensic examination of EHSs in questioned procedural documents. This article provides a general description and highlights the practical aspects of the methodology.

Summary of results

An analysis of the experience of foreign forensic document examiners in the field of analysis of electronic documents and EHSs [1-4] showed that EHSs captured using Wacom technology can be examined by forensic document experts using the Wacom Signature Scope software for identification purposes. Meanwhile, to date there is no information on the developed of an accepted methodology in this direction. In 2019 to solve the problem of developing a methodology for the study of signatures made using a tablet with a stylus pen, a commission was formed from the experts of the Center for Forensic Expertise of the Ministry of Justice of the Republic of Kazakhstan in the field of forensic handwriting and computer technology expertise.

The expert commission decided to develop a comprehensive methodology that combines elements of computer technology and forensic handwriting examination. The methodology developed was only applicable to Wacom equipment and Wacom Signature Scope software, as the Government of Kazakhstan plans to purchase this product from this manufacturer.

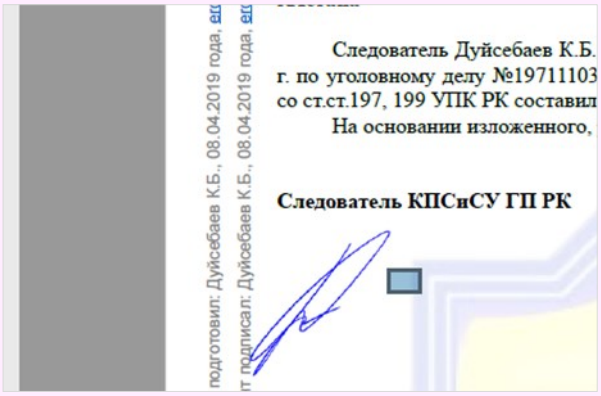
The tasks to be solved in the framework of a comprehensive methodology of forensic examination of EHS are:

- establishment of metadata of the investigated electronic document with the EHS;
- extracting the EHS from the electronic document under study using the Wacom Signature Scope software;
- establishment of metadata of the EHS;
- the establishment of data characterizing the written-motor skills of the performer;
- determination the genuineness of EHS;
- solving diagnostic problems (in what state was the performer)

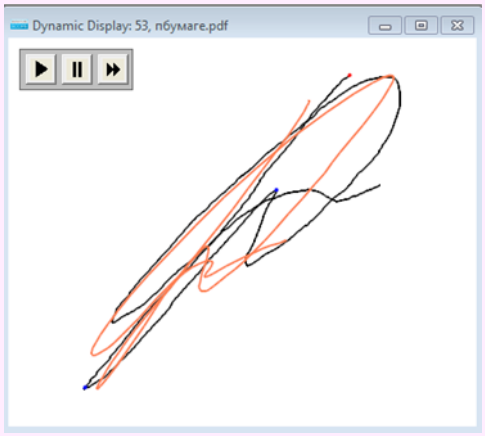
Basically, the section of the methodology relating to the study of computer technology is standard and involves a diagnostic analysis of the EHS included in the electronic document in order to obtain data that can be used to solve the tasks assigned to the expert and of interest for the investigation, including the establishment of any manipulations both with a text of an electronic document, and with a EHS image. Of great interest is the section of the methodology devoted to the forensic handwriting examination of a non-standard object - the EHS image containing its visual characteristics and quantitative data characterising the written-motor functional-dynamic complex of the person's attributes using a qualitatively descriptive method. An important issue when considering the EHS as an object of forensic handwriting is the solution of the question of how accurately the image of the EHS conveys the handwriting of the performer for identification purposes. The sampling frequency (at least 200 measurements per second) and the resolution of the tablet allows you to examine the EHS to solve both identification and diagnostic problems. Therefore, EHS, in contrast to the photocopy of signature, is not considered to be limitedly suitable for conducting handwriting analysis.

The most relevant and useful functionalities in the current version of the open source software are:

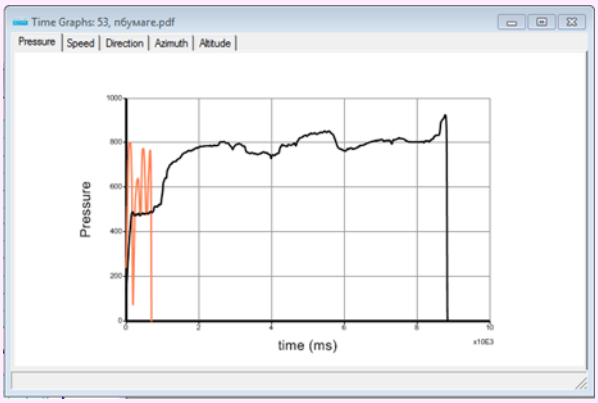
(1) The ability to playback the track of the signature in real-time (and the associated playback capabilities at reduced speeds) and, most importantly, to have all the detailed data related to the temporal characteristics of the signature, that is, speed data at any point in the EHS (Screenshots 1-4).



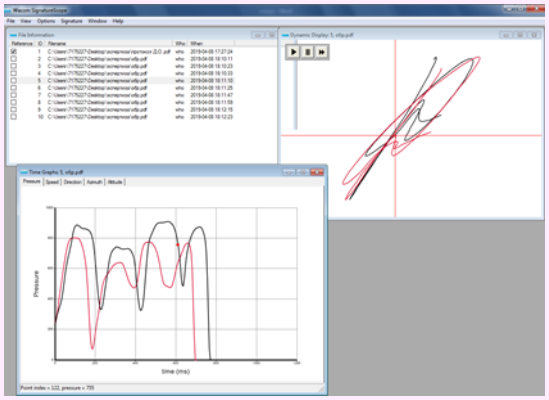
Screenshot 1



Screenshot 2



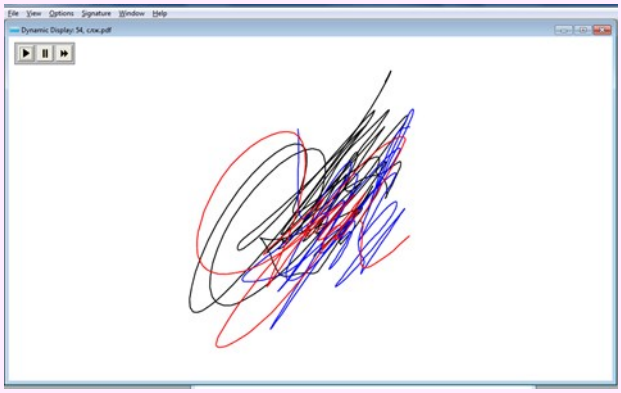
Screenshot 3



Screenshot 4

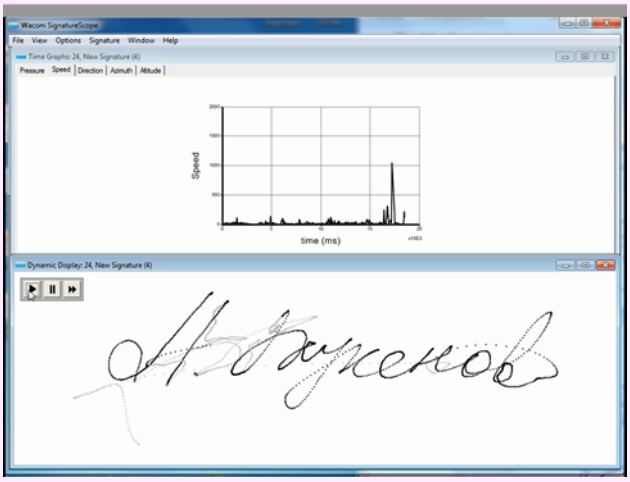
As it is known, the speed under the influence of imitation almost always tends to slow down or simply to slower execution in comparison with the samples. In the study of signatures made traditionally, the pace of signature execution by an expert handwriting expert is determined based on an assessment of the signature features established visually.

(2) The ability to unravel the system of movements of signatures complex in structure in cases where the stylus repeatedly intersects previously written signature elements. In practice, there are cases when, using traditional methods of signature research, it is impossible to unambiguously establish transcription and connectivity (with a complex system of movements), and this can seriously affect the ability of a handwriting expert in deciding on the authenticity of a disputed signature (Screenshot 5).



Screenshot 5

(3) The software allows to record and analyse the time and placement of the stylus tip above the surface of the pad. These data allow the handwriting expert to analyse a new plane of signature examination that is not available using traditional methods (Screenshot 6).



Screenshot 6

Conclusion

In the process of developing and discussing the draft methodology, we were faced with rather serious skepticism from practicing experts. However, after conducting training seminars and pieces of training, their unanimous approval and confidence in its effectiveness were obtained, and the draft methodology was adopted at the Scientific Council the first time, as it was provided with illustrative material. It should be added, that the methodology applies only to signatures made using the Wacom STU-430 tablet and Wacom SignatureScope software (v 1.44 and later). We suggest periodic refinement of the methodology and the inclusion of other Wacom products and products of other manufacturers, after conducting experimental studies.

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Decapitation : Homicide or Accident?

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Abstract

A beheaded of a male aged about 45 years was found in deep gorge in late evening in a remote area of Himachal Pradesh. The villager on hearing the thud rushed to the spot and saw a body with its head and torso separated. The police investigation was clueless. Then, the services of forensic experts were requisitioned. The scene of crime examination unravelled the cause behind the decapitation. This article describes in detail in-situ examination of crime scene and thereby solved the mystery.

Introduction

The death scene findings can provide a valuable evidence to distinguish between the homicidal and other modes of death. Decapitations can occur in homicidal, suicidal or accidental cases. Traffic accidents may also leads to decapitations. The failure to abide by motor vehicle rules including the non usage of seat belts, over speeding and parapets are amongst the contributing factors [1]. Decapitations may be either post-mortem or Ante mortem in nature. The Ante mortem decapitation occurred before the death or during the process of death whereas Homicidal decapitation associated with varied nature having fatal or near fatal injuries [2]. In case of suicide only the fatal injury of decapitations whereas in accidental and homicidal decapitations the fatal injuries can confined to other body parts [3].

Road accidents in India contribute a high degree of morbidity and mortality. Accidental decapitations or amputations are rare events but they contribute in high mortality and disability especially in younger age groups [4].

Case Report

A person aged about 45 years was reportedly returning late evening to home in his personal car in a remote area of Himachal Pradesh. But, did not return back. The local inhabitants on the way heard a loud sound and started searching the place from where the sound originated. On reaching the place a beheaded body lying near to a rock in a deep gorge was found. On the request of the police agencies, the forensic experts visited and examined the spot. The spot was inaccessible. The experts started moving on the road to have a view of the spot and while moving on the road near to a curvature observed two tracks/partial tyre marks passing through stones and soil on outer side of the road extending up to grassland. Thereafter, followed a trudged path down the hill towards the gorge (Figure 1) and observed followings:

- Broken pieces of glass, wheel covers, metallic pieces and plastic parts of the vehicle were seen scattered down the hilly slope.
- One grey, black and blue colour left foot shoe, with toe facing downwards, laces tied was seen stuck between the rocks with vegetation beyond broken parts of vehicle on the steep slope. The shoe tested negative with benzidine reagent indicating absence of blood.
- One boulder was found at about 8 meter away from the left foot shoe. On one side above the boulder, one double interwoven metallic barbed wire fence was seen running. Splash pattern of brownish stains was observed on the lower extremity of the boulder. The brownish stains were seen on the stones and soil adjacent to a boulder and were tested positive with benzidine reagent.
- Three loops of double interwoven metallic wire fence intermingled pressed downward and had entangled one red coloured sacred thread having a metallic locket embedded with green and orange colour gemstones and were about 5 feet away from the brownish stains. Brownish stains with tissue like material was seen stuck to the barbed wired and tested positive with benzidine reagent (Figure 3).
- At about 10 feet further down some brownish stains were seen on the soil and stones where the torso of deceased was reportedly found and tested positive with benzidine reagent (Figure 4).
- One Oak/ban tree with its bark peeled off was found about 40 feet below and found bent downward.
- One white colour car completely damaged with its front facing towards road, both front tyres and left rear tyre were resting on the rock and right rear tyre was found stuck in the rock downhill. Broken glass pieces were seen scattered inside the vehicle and some were resting on the side of beading of front wind screen.
- Gear of the vehicle was got checked from a mechanical engineering and was found to be in third gear which was suggestive of vehicle moving in high speed.

- The photographs taken by police investigation agencies were examined and a decapitated head with irregular tear margins and broken grass/ vegetation on the torn margins, and brownish stains on the head, which were more prominent on right side of the face lying in the vegetation on side of the boulders and one double interwoven metallic barbed wire seen above the decapitated head (Figure 2). The decapitated trunk was lying on the right lateral side in the vegetation (Figure 4).
- The clothes on the body of victim were intact except the shirt which was found torn with irregular margins and were around the neck area and had brown stains.

Results and Discussion

The decapitation of the head had occurred by the barbed wire during the process of rolling down of vehicle from the hilly terrain. The laboratory examination of blood revealed that the person was driving vehicle in inebriated condition as the large quantity of ethyl alcohol was detected in blood. The driving under the influence of alcohol especially in hilly terrain is resulted into the loss of control over the vehicle. The complete decapitation by car accident is a rare event. The literature on decapitations in cases of road accidents showed that decapitations was possible by impact of head with a tree [4], with a rope tied around the neck and moving a vehicle in opposite direction [5] with scarf caught in helix elevator machine [6]. Accidental decapitations could be possible by industrial or car accident, wires across road and as a result of explosion. The separation of neck from the body can occur by variety of reasons. In this case decapitation of neck had occurred between C2-C3 cervical vertebrae as per Post mortem findings. Hejna and Havel also reported vehicle assisted decapitation in C2-C3 cervical vertebrae [5]. Whereas Pilloud et al in their study showed that majority of decapitations occurred between C2-C5 cervical vertebrae [7].

Conclusion

The accidental decapitations are the rare occurrences. The careful observations at crime scene are necessary to differentiate between homicidal and accidental decapitations. Examination of evidences helped in reconstruction of the crime scene and resulted into conclusion of accidental decapitation.

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Figure 1. Schematic Diagram of Crime Scene

Figure 2. Decapitated Head and Barbed Wire

Figure 3. Testing of Blood on the Wire

Figure 4. Torso at the Crime Scene

A case study on sexual assault involving an individual with a large Y chromosome deletion and two X chromosomes genotype

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Introduction

Today, sex testing based detection amelogenin gene (AMELX and AMELY) is a part of many genetic profiling multiplex systems used by forensic scientists to decide if the sample being tested is of male or female origin [3]. Beside, Y-STRs marker is a powerful tool in current forensic DNA analysis. Both AMEL test and Y-STRs analysis are useful to distinguish between the victim and the perpetrator's evidence, sex determination of remains in mass disasters or missing persons' investigations and particularly in sexual assault cases. However, in some specific cases AMELY and some part of Y chromosome may be lost leading to change in the results. For example, typing of males as females due to insufficient markers in the Y-STR profiles. Such mis-profiling can have dire consequences if used in criminal investigations (forensic/rape cases) [1,4]. Here, we report a case of large Y chromosome deletion and absent of AMELY from a man in a rape case.

Casework

This case happened in 2007, the victim was a 14 -year-old girl who was raped by a 20-year-old male. The key evidence items were a tampon and underwear from the victim. The autosomal STR analysis from these samples was not successful because of sample degraded. However, an attempt was made to analyse Y-STR data from the above samples.

Materials and Methods

DNA extraction: Genomic DNA was extracted from hair sample of male suspect using the Chelex-100 method. DNA from the victim's underwear and tampon were extracted using QIAamp® DNA micro kit (QIAgen).

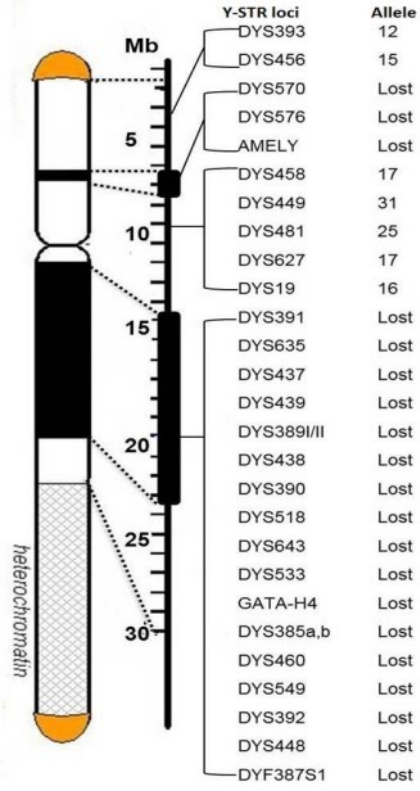
Genotyping of STR: Autosomal STR was typed using PowerPlex® Fusion System Kit (Promega). Y-STRs were evaluated with Yfiler™ Plus PCR Amplification Kit (Applied Biosystem) and PowerPlex® Y23 systems (Promega). Investigator® Argus X-12 PCR Amplification Kit (Qiagen) was used to amplify 12 X STR loci. The PCR products were separated by capillary electrophoresis using POP-4 polymer on ABI 3500 Genetic Analyzer (Applied Biosystems).

Results and Discussion

Amelogenin X was observed from hair samples collected from a male suspect with STR autosomal kit. However, we noticed the absence of the Amelogenin gene in Y chromosome (AMELY). According to previous studies, this may be due to a point mutation within the primer binding-site region in the AMELY homologue or deletion of the Y short arm encompassing the AMELY locus [4].

Y-STR profiles from this sample showed the absence of many loci. For this reason, it was suspected that this is a case of some part of Y-chromosome deletion. Based on Y-STRs position the result of deletion was mapped in Figure 1A. It should be noted that the X-STRs (Fig.1B) showed heterozygote genotypes in 7/12 locus X-STR, indicating the presence of two X chromosomes as in a female profile.

Our results suggested a case of Klinefelter's syndrome (47,XXY) plus the deletion of some part of Y chromosome. This hypothesis also matches some studies of Y chromosome deletions in patients with Klinefelter syndrome [2,5]. Regrettably, we do not have the condition to collect peripheral blood from a man to get a better molecular and genetic characterization of this case (such as Karyotype test, FISH).



X-STR	ALLELE
AMEL	X, X
DXS10103	18, 18
DXS8378	10, 12
DXS7132	15, 15
DXS10134	32, 35
DXS10074	16, 16
DXS10101	33, 33.2
DXS10135	20, 22
DXS7423	14, 15
DXS10146	30, 30
DXS10079	18, 20
HPRTB	11, 11
DXS10148	23.1, 26.1

B

Figure 1. Y-STR and X-STR profiles from a male sample. A. Physical map of Y-STR used PowerPlex® Y23 and Y-27, the deletion of alleles is given by black fill. B: X-STR observed in the male sample used Investigator Argus X-12.

In this case, although the Y-STR profile obtained on the victim's underwear sample was a partial profile with 7 loci Y-STR identical to male suspect, it was sufficient to support our conclusion that the man is the real culprit.

Our case also suggested that AMELY and Y-STR locus dropout may indicate more problems, especially in the mixed sample's interpretation. Therefore, accurate insight into deletion events on Y chromosome would be helpful for criminal investigations interpretation of the results of DNA profiling.

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AFSN Member Institutes

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Bangladesh	1	National Forensic DNA Profiling Laboratory
Brunei Darussalam	2	Department of Scientific Services
India	3	Centre for DNA Fingerprinting and Diagnostics
	4	Directorate of Forensic Science, Himachal Pradesh
Indonesia	5	Department of Police Medicine of the Indonesian National Police
	6	Eijkman Institute for Molecular Biology
	7	Forensic Laboratory Centre of Indonesian National Police Headquarters
	8	Indonesian Association of Forensic Pathologist
	9	Laboratory of National Narcotics Board
	10	Master Program of Forensic Science, Postgraduate School, Universitas Airlangga
Lao PDR	11	Food and Drug Quality Control Center
Malaysia	12	CyberSecurity Malaysia
	13	Department of Chemistry
	14	Malaysian Communications and Multimedia Commission
	15	Royal Malaysia Police Forensic Laboratory
Mongolia	16	Mongolian National Institute of Forensic Science
People's Republic of China	17	Forensic Science Center of Guangdong Provincial Public Security Department
	18	Forensic Science Division, Department of Fujian Provincial Public Security
	19	Guangzhou Forensic Science Institute
	20	Institute of Forensic Science, Ministry of Public Security
	21	Institute of Forensic Science, Dezhou Public Security Bureau
	22	Institute of Forensic Science, Hangzhou Public Security Department
	23	Institute of Forensic Science, Shandong Public Security Department
	24	Institute of Forensic Science, Suzhou Public Security Bureau
	25	Institute of Forensic Science, Tianjin Public Security Bureau
	26	The Institute of Evidence Law and Forensic Science, China University of Political Science and Law
	27	Forensic Science Division of the Government Laboratory, Hong Kong Special Administrative Region
	28	Forensic Science Department of Judiciary Police, Macau Special Administrative Region
Philippines	29	Laboratory Service, Philippine Drug Enforcement Agency
	30	National Bureau of Investigation
	31	National Reference Laboratory for Environmental and Occupational Health, Toxicology and Micronutrient Assay, East Avenue Medical Center, Department of Health
	32	Natural Sciences Research Institute, University of the Philippines Diliman Quezon City
	33	Philippine National Police
Republic of Kazakhstan	34	Forensic Examinations Centre of the Ministry of Justice
Republic of Korea	35	Daejeon Health Institute of Technology, Daejeon Health Sciences University
	36	Graduate School of Forensic Science, Soon Chun Hyang University
	37	Korea Coast Guard Research Institute
	38	National Digital Forensic Center of Supreme Prosecutors' Office
	39	National Forensic Service
	40	Scientific Investigation Center of Korean National Police Agency
	41	Scientific Investigation Laboratory, Ministry of National Defense

Country/Region	*No.*	*Name of Member Institute (as at December 2019)*
Singapore	42	Corrupt Practices Investigation Bureau
	43	Health Sciences Authority
	44	Ministry of Home Affairs
Sri Lanka	45	Government Analyst's Department
	46	National Dangerous Drugs Control Board
Thailand	47	Central Institute of Forensic Science
	48	Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University
	49	Department of Forensic Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University
	50	Department of Medical Sciences
	51	Department of Forensic Medicine, Thammasat University Hospital
	52	Faculty of Medicine, Chiang Mai University
	53	Human Genetics Unit, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital
	54	Institute of Forensic Medicine, Police General Hospital, The Royal Thai Police
	55	Office of Narcotics Control Board
The Republic of the Union of Myanmar	56	Defence Services Medical Research Centre
Timor-Leste	57	POLÍCIA CIENTÍFICA DE INVESTIGAÇÃO CRIMINAL - LABORATÓRIO DE POLÍCIA CIENTÍFICA
Vietnam	58	Forensic Medicine Center of Ho Chi Minh City
	59	National Institute of Forensic Medicine
	60	Forensic Science Institute Vietnam