ForensicAsia
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AFSN President's Address

My fellow members of the Asian Forensic Sciences Network,

Greetings to all colleagues in Asia and around the world!

It is an honor to share with you all our reflection on the past year's activities and look ahead to the promising future of our organization. The Asian Forensic Sciences Network has achieved remarkable milestones in advancing forensic science in our region, and I am proud of the dedication and hard work that each of you has contributed to our collective success.

Over the past year, we have seen tremendous growth and development in the field of forensic science across Asia. Our network has facilitated collaboration and knowledge-sharing among forensic experts, enhancing the quality and effectiveness of our work. Through conferences, workshops, and training programs, we have advanced our skills and expertise, contributing to the resolution of complex criminal cases and the delivery of justice to those in need.

As we embark on the next phase of our journey, I urge all AFSN members to stay committed to excellence and continue pushing the boundaries of forensic science. The challenges we face may be great, but our collective determination and passion for this field are even greater. Let us continue embracing innovation, explore new technologies, and strive for continuous improvement in our practices.

I am inspired by the dedication and professionalism of each member of the Asian Forensic Sciences Network. Your hard work and commitment to the pursuit of truth and justice are commendable, and I am confident that together, we will overcome any obstacles that come our way.

As we move forward, remember that our work has a profound impact on society, and our efforts can make a significant difference in the lives of others. Let us continue to uphold the highest standards of integrity, ethics, and professionalism in all that we do, and let us never lose sight of the importance of our mission.

In closing, I want to express my utmost gratitude to each and every one of you for your unwavering dedication to the Asian Forensic Sciences Network. Together, we can achieve great things, and I am excited to see what the future holds for our organization. Let us stand united, support one another, and strive for excellence in everything we do.

Thank you all for your hard work and commitment. Let us continue to inspire, innovate, and make a positive impact in the field of forensic science in Asia and beyond.

PBGEN CONSTANCIO T CHINAYOG JR
AFSN President
Philippine National Police
Forensic Group

Editor's Address

Dear colleagues and members of AFSN,

Thanks to all our colleagues who have contributed their research and studies in this Issue 14 of ForensicAsia.

We have published a total of 7 technical articles and 5 case studies. The content includes disciplines on illicit drugs, questioned documents, crime scene investigation, fires & explosions, forensic biology, forensic medicine and trace evidence. In addition, we have one AFSN News from the Health Sciences Authority, Singapore, to showcase a training course on Bloodstain Pattern Analysis, one International Scene article from the Government Analyst Department, Sri Lanka, on tackling the prevalence of methamphetamine in the country, and one Member Institute article from Mongolian University of Internal Affairs, sharing with us on forensic management.

Last year, the AFSN Board approved 5 new member institutes from Indonesia, Malaysia, People's Republic of China, Republic of Uzbekistan and Republic of Korea joining our Network. We are indeed very grateful to them in introducing their Institutes to us in this Issue.

Once again, I would like to take this opportunity to thank our guest editors' hard work in reviewing the articles, as well as our editorial assistants who have assisted in the administrative matters and the artwork design for the online publication of this Newsletter.

Happy reading and see you in Bangkok!

Dr Lui Chi Pang
Editor

Editorial Committee

Check out our new AFSN website: www.afsn.asia

Please remember to update any webpage links you have to the new url.

Content

Content

Discovering Bloodstain Pattern Analysis: A Course that Connects Colleagues from Across the Region

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The Forensic Chemistry and Physics Laboratory of the Health Sciences Authority (HSA) recently conducted its inaugural 40-hour basic Bloodstain Pattern Analysis (BPA) course for forensic scientists and crime scene investigators from the Asian Forensic Sciences Network (AFSN)'s Crime Scene Investigation workgroup, held from September 6 to 11, 2023. This course was held in conjunction with the 15th AFSN Annual Meeting and Symposium in Kuala Lumpur.

Participants included colleagues from the HSA, the Royal Malaysia Police Forensic Laboratory, the Department of Chemistry (KIMIA) Malaysia, National Institute of Forensic Medicine, Hospital Kuala Lumpur (Malaysia) and Polícia Científica de Investigacão Criminal – Laboratório de Polícia Científica, Timor Leste.

Group photo taken with the participants at the front lobby of Department of Chemistry (KIMIA) Malaysia.

What is BPA, and its Significance in Criminal Investigation?

BPA refers to the interpretation of the colour, location, size, shape and distribution of bloodstains and bloodstain patterns in association with knowledge of the underpinning sciences (chemistry, physics, mathematics, and biology). This analysis provides crucial information about the events that led to the deposition of these bloodstains, aiding investigators in reconstructing the sequence of events.

By analyzing bloodstain patterns, investigators can infer the positioning and movement of individuals involved in a crime. Additionally, the analysis can help determine the type of objects that produced the patterns and the mechanism responsible for the deposition of bloodstain patterns.

Furthermore, bloodstain pattern analysis can corroborate or challenge witness testimonies and suspect statements, providing an objective basis for evaluating the accuracy of accounts.

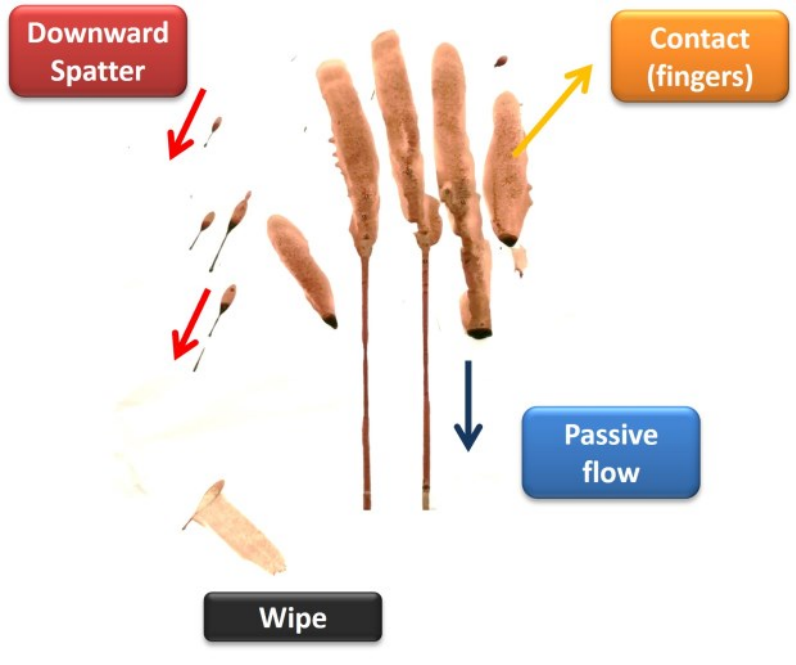

Figure showing the four types of bloodstains that could be encountered at a scene.

Hybrid Learning Approach that Provides a Distinctive Course Experience

The course began with a two-day theoretical session (6 and 7 September 2023) conducted via Microsoft Teams. This session provided participants with an understanding of the purpose, function and history of BPA, the biological properties of blood, the types of bloodstains and the use of different chemical reagents for detection and enhancement of bloodstains.

Additionally, international BPA case studies were discussed to help participants apply their knowledge and enhance their learning outcomes, demonstrating how BPA can assist in resolving critical investigative questions.

The practical sessions took place at the Department of Chemistry of Malaysia (KIMIA Malaysia) in Kuala Lumpur from 9 to 11 September 2023. During these sessions, participants utilized synthetic blood substitutes to understand how physics plays an important role in the formation and deposition of blood on different substrates. Participants also employed mathematics to analyze the shape, size, and distribution of bloodstains to determine the probable location of the blood source.

Towards the end of the course, the instructors set up a mock crime scene to assist participants in gaining confidence in recognizing, documenting, and presenting BPA evidence.

The practical sessions at KIMIA were facilitated by course instructors Louis, Zai Rong, and Grace. These sessions included exercises on documenting bloodstain evidence (left and centre) and depositing bloodstains from various objects (right).

Sharing their Newfound Knowledge during the AFSN Meeting and Symposium

During the meeting and symposium, participants shared their newly acquired knowledge with other attendees, fostering increased interest and understanding of the significance of BPA. This also further bolstered their confidence in presenting and articulating BPA evidence.

Photo on the left: Group 1 sharing their findings on impact pattern. Photo on the right: Group 2 sharing their findings on cast-off pattern.

Achievements and Accomplishment of this course

Zai Rong, one of the course instructors, noted that the participants represented diverse backgrounds and jurisdictions. It was fulfilling and encouraging to witness the participants' enthusiasm and openness to exchanging insights on forensic examination practices within their respective units. They shared valuable tips and gained knowledge from each other, promoting a mutual exchange of ideas that transcended the scope of bloodstain pattern analysis learning.

The key success of the BPA course was due to the various positive feedback received by the participants. Participants who successfully completed the course requirements could apply for a provisional membership with the International Association of Bloodstain Pattern Analysts (IABPA). The IABPA is a network of experts and resources to further develop expertise in BPA. This course also demonstrated HSA's commitment to develop our forensic science community regionally and globally.

> The structured design of the BPA course was commendable, carefully designed to cater to the learners, particularly through its immersive, hands-on practical approach.
>
> Jeyaganesan Pillay

> The instructors were very knowledgeable. Course was designed very well for beginners. Really appreciate the hands-on and practical component of the course. It gives me a clearer understanding and appreciation of the theoretical aspect of the course.
>
> Benjamin Tay

> The hands-on activities, particularly the mock-crime scene analysis were incredibly insightful. The instructors demonstrated a deep understanding of the subject matter, and their real-world examples added practical relevance to the theoretical concepts. Without the scene practical, I may not have pictured the patterns we have learnt.
>
> Khoo Lay See

Introduction to the Construction and Development of the Forensic Science Discipline at the Criminal Investigation School of Southwest University of Political Science and Law

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Southwest University of Political Science and Law (SWUPL) is located in Chongqing, the famous historical and cultural city and the only municipality directly under both central and western region of China. It is the earliest institution of higher education in law established in New China, one of the first batch of key universities in the country as determined by the State Council after the reform and opening up, and a commissioned institution for cultivating international students with Chinese Government Scholarships. SWUPL now has three campuses in Yubei, Shapingba and Baosheng Lake, covering a total area of more than 3,000 mu (more than 184 hectares). Adhering to the strategy of opening up education to the outside world, SWUPL has established exchange and cooperation relationships with more than 190 universities or institutions in nearly 40 countries and regions, and carried out more than 130 international exchange programs. SWUPL has been selected as one of the "Outstanding Undergraduate International Exchange Programs" and "National High-level University Postgraduate Programs" by the Ministry of Education, as well as selected as one of the "Internationalized Featured Universities in Chongqing" and "Bayu Overseas Program of Introducing Talents".

SWUPL at a Glance.

The history of forensic science discipline in SWUPL is closely linked to the first science of criminal investigation major in China. In 1956, the first criminal investigation research institution in China was born in the Criminal Investigation School (CIS) of SWUPL. In 1979, SWUPL established the first undergraduate program of criminal investigation in China. Relying on the nation's first science of criminal investigation major, the discipline of forensic science of CIS has developed rapidly in recent years. In the construction of forensic science discipline and specialties, teaching and educating program, scientific research and social service, the academy's achievements are consistently in the forefront of the country.

The CIS has four teaching and research departments related to forensic science: Trace & Document Examination Department, Forensic Chemistry & Medicine Department, Audio-Visual & Digital Evidence Department, and Evidence Technology Experiment Department. The academy offers two undergraduate majors: forensic science, and a dual degree in law and forensic science. With regard to the system for training high-level talents, the CIS sets up master's degree and doctoral degree authorization points for the secondary discipline of forensic science under the first discipline of jurisprudence independently in 2023, which is in leading position in our country. More excitingly, the academy will launch the first enrollment in forensic science program in 2024. Nowadays,

the CIS has established a system for training highly qualified forensic scientists that integrates undergraduate and graduate studies. The academy always adheres to the cultivation philosophy of “integration of law and technology, cultivation of both morality and law”, and cultivates eight innovation teams, including “forensic voice technology”, “intelligent governance of drugs”, “new technology of trace examination” and others. Apart from that, the CIS continues to build three academic brands with SWUPL characteristics, including “intelligent voice technology”, “intelligent evidence identification” and “intelligent drug identification technology”. All in all, the CIS strives to lead the development of the domestic forensic science academic community.

Currently, there are 31 faculties in the CIS including 4 doctoral supervisors, 20 master’s supervisors. 74% of the faculties have senior titles and 77% of them have doctoral degrees, which means the CIS has a cross-disciplinary faculty team with a reasonable structure of academic qualifications. The faculties have also made outstanding achievements in scientific research. In the past years, the faculty members have published more than 100 textbooks, monographs and translations, and participated in the formulation of more than 10 national technical specifications/standards for forensic identification, and published hundreds of high-quality academic papers in SCIE, CLSCI, CSSCI, and so on. In addition, our teachers have presided over more than 100 scientific projects, including the National Social Science Foundation, the National Natural Science Foundation, the Humanities and Social Sciences Program of the Ministry of Education and provincial and ministerial-level major or key scientific research projects, which have made a significant academic impact in forensic science.

Dr Liang Kun
Dean of CIS

Dr Zhang Cuiling
Chair Professor of Forensic Science

In terms of the construction of teaching and research bases, the CIS has established national-level platforms such as the Intelligent Justice Ministry of Education Engineering Research Center and the Evidence Technology Experimental Teaching Center. The academy also has provincial-level platforms like the Chongqing Institutes of Higher Education Forensic Science Key Laboratory and the Chongqing Institutes of Higher Education Evidence Technology Engineering Research Center. Apart from that, the academy has collaborated with many high-quality enterprises and research institutions to build various new laboratories, such as, the iFlytek Speech and Language Joint Laboratory, 3D Visual Recognition Joint Laboratory, Digital Forensics Laboratory, and Virtual Reality Laboratory, providing abundant experimental and training resources for cultivating talents who are proficient in both forensic science and evidence technology practices, as well as familiar with legal knowledge such as evidence rules and systems.

The CIS is committed to cultivating talents with broad perspectives. Over these years, the forensic science team of the CIS has actively carried out international exchanges and cooperation between Hong Kong, Macao and Taiwan, and has established regular cooperation and exchange relationships with many foreign universities. In addition, the CIS is also devoted to working with other institutions to promote scientific research and talent cultivation jointly. The academy has always kept in touch with the public prosecutors, lawyers and other practical departments, and cooperated with them through special commissioned projects, mutual assignments of personnel, part-time tutors, lectures and forums, and so on.

Indonesian Automated Fingerprint Identification System of the Indonesian National Police

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Indonesian Automated Fingerprint Identification System (INAFIS) was established in 1951, first under the name Dactyloscopy Center. INAFIS is led by the Chief of INAFIS, a Brigadier General in the Indonesian National Police.

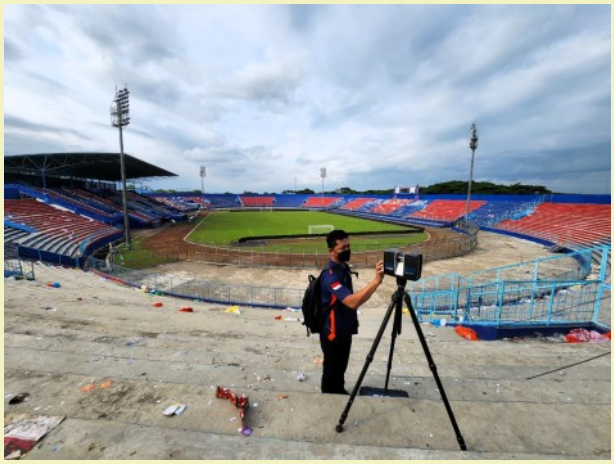
There are several divisions within the INAFIS organizational structure, three of which perform the main forensic tasks. These divisions are Criminal Dactyloscopy, Police Photography, and Public Dactyloscopy. Criminal Dactyloscopy is responsible for processing crime scenes, collecting evidence, conducting latent fingerprint enhancement and identification, as well as collecting tenprint from convicted criminals. Police Photography is mainly tasked with conducting forensic photography at crime scenes, creating police sketches, and performing facial identification. Common Dactyloscopy is in charge of collecting tenprint from civilians and storing civilian tenprint cards, both manually and digitally.

The Centre of INAFIS of the Indonesian National Police is the parent organization of numerous identification units in the Regional Police Force (34 identification units) and the Resort Police Force (501 identification units). The identification units perform similar duties as INAFIS, albeit with smaller organizational structures, resources, and capabilities. The Centre of INAFIS routinely conducting training for officers working in Regional and Resort Identification Units, criminal investigators, and counter-terrorism officers.

Evidence was collected from the scene of the Tangerang prison fire (September 2021). It was concluded that the fire was caused by short circuits, which caused sparking that ignited building materials.

Identification of victims of the Tangerang prison fire (September 2021). INAFIS managed to identify 7 out of 41 victims through fingerprints.

Detection of bloody fingermark using Rofin Polilight-Flare® Plus 2 450 nm on the vehicle in Mother and Child Murder Case in Subang, West Java (August 2021).



3D scene mapping using FARO® Focus Laser Scanner in the Kanjuruhan Stadium disaster case (October 2022). The 3D representations of the scene helped the investigators to determine or eliminate the events at the crime scene.

Introduction of Department of Forensic Sciences at Graduate School of Sungkyunkwan University

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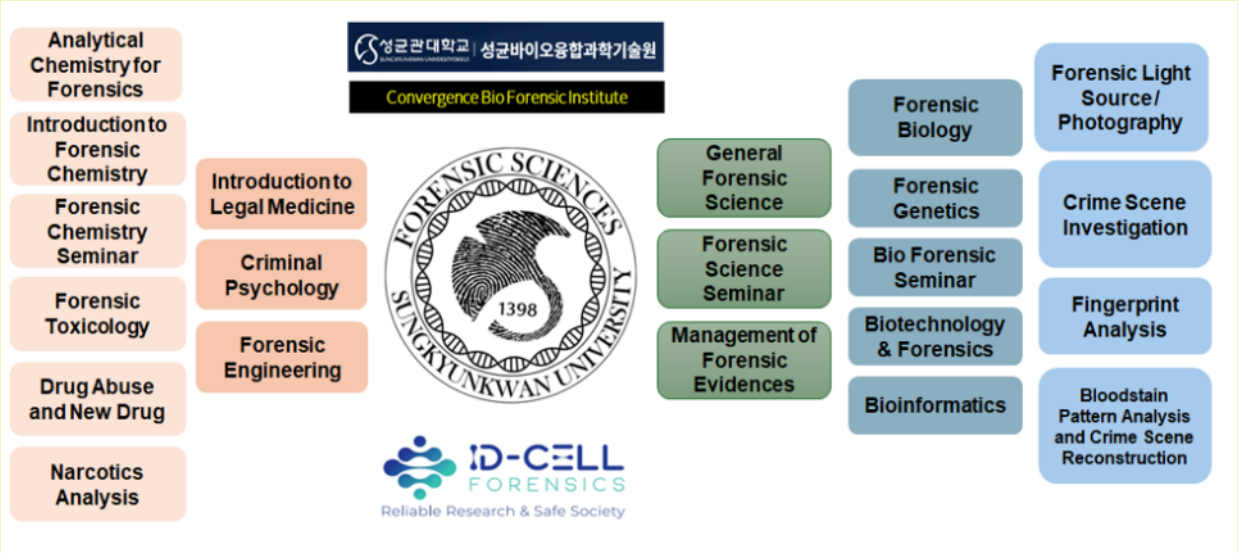
General Information

The Department of Forensic Sciences was established in 2017 at Sungkyunkwan University's Graduate School in South Korea. The Department of Forensic Sciences has a total of 114 full-time students and part-time students in master's and doctoral programs in the two majors of Bio and Digital forensics. It provides lectures allowing students to deepen their knowledge in this field, as well as an in-depth training in practical work. Here, we'll focus on Bio Forensics.

Bio Forensics' Curriculum



The Bio Forensics major encompasses a broad spectrum of subjects, including fundamental principles in forensic science, as well as specialized areas such as forensic biology, and forensic toxicology. Furthermore, it offers coursework in crime scene investigation (CSI).

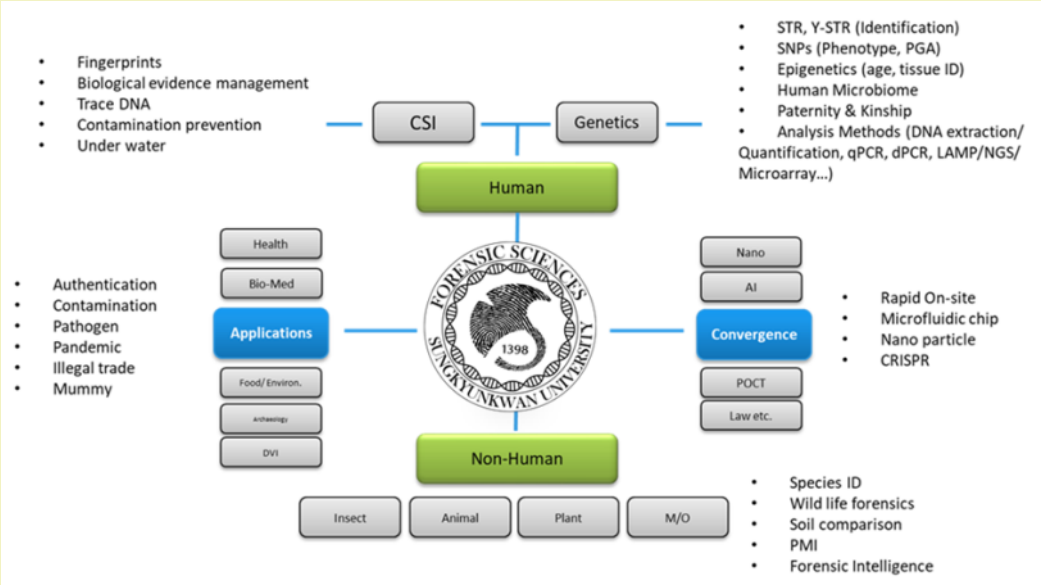
Research Topics

Bio Forensics

The laboratory of Bio Forensics is actively collaborating with the National Police Agency (NPA) on research project focusing on forensic biology and DNA analysis. Research is aimed at reducing time and costs while enhancing the precision and sensitivity of analytical procedures across all stages, from the crime scene to the laboratory.

The laboratory is developing kits such as evidence collection tools, preliminary/confirmation tests for body fluids, valid evidence screening kits, and conducting research on forensic genetics applications utilizing advanced technologies such as digital PCR and NGS.

Moreover, we are expanding our research into non-human fields such as animals, plants, and microbiome.



The Department conducts researches in Forensic Toxicology. The researchers are led by Prof. Heesun Chung, a distinguished professor who analyzes narcotics and studies drug trafficking.

The Convergence Bio Forensics Research Institute aims to achieve innovative and practical outcomes through interdisciplinary research, merging advanced science and technology such as nanotechnology and artificial intelligence, with cutting-edge life sciences such as genomics and bioinformatics.

ID-CELL Forensics Co., Ltd., a lab-based professor startup, was established on December 30, 2021, with the mission to advance the creation of a safe and equitable society through quality control of modern biopharmaceutical raw materials and finished products through the application of bioforensic technology, as well as offering private forensic services.

Digital Forensics

The laboratory of Digital Forensics, centers its researches on laws, policies and technologies related to cybercrimes and digital forensics due to development of ICT technology.

National Institute of Forensic Medicine Hospital, Kuala Lumpur

Dr Ahmad Hafizam Bin Hasmi
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The Institute is a department within Hospital Kuala Lumpur (HKL), a tertiary hospital in the capital city of Malaysia. Prior to 2002, the Forensic Unit was a part of the Pathology Department in HKL providing forensic pathology and mortuary services. Due to the rising importance of forensic medicine in assisting the judiciary within the legal framework, the National Institute of Forensic Medicine (IPFN) was established in April 2002 by the Ministry of Health Malaysia.

The Institute is headed by a Forensic Medicine Specialist. There are 60 staffs all together in the Institute consisting of Forensic Medicine Specialists, Medical Officers, Forensic Science Officers, Paramedics, Record Officers and Administration staff.

The Institute strives to be the Centre of Excellence in the field of Forensic Medicine through the provision of efficient, effective and professional forensic medicine services in accordance with Malaysian Law. The Institute will ensure that quality Forensic Medicine services being provided is based on a corporate culture of caring, teamwork and professionalism through personnel who are knowledgeable, competent and sensitive towards clients' needs in compliance with an established Quality Management System. The services provided in the Institute are:

1. Mortuary services and management of the dead
2. Forensic Pathology
3. Clinical Forensic Medicine
4. Forensic Anthropology
5. Forensic Radiology
6. Disaster Victim Identification

The Institute is the only Forensic Medicine department in the country that provides post mortem-Computed Tomography (PMCT) services. The aim is to provide an excellent and dynamic forensic service by applying state of the art technology in Forensic Medicine.

Objectives and Functions

1. To provide independent and impartial forensic medical services that meet the requirement of the Malaysian legal system.
2. To provide efficient mortuary services those fulfill clients' needs.
3. To conduct professional, efficient and effective examination of forensic medical cases.
4. To provide consultation services in the management of forensic medical cases.
5. To build on knowledge and skills through Continuous Medical Education and training.
6. To conduct research in the field of forensic medicine and to collaborate with relevant medicolegal agencies for that purpose.
7. To provide specialized services to manage Mass Fatality Incidences, CBRNE incidents (chemical, biological, radiation, nuclear and explosives) and infectious disease outbreaks (such as SARS, COVID).

National Institute of Forensic Medicine (IPFN), through its expertise in Forensic Medicine, training andforensic research, is determined to contribute and collaborate in advancing the goals and ideals of the AFSN in the pursuit of justice and humanity.

Overseas deployment with multinational DVI team in MH17 tragedy.

PMCT facility in National Institute of Forensic Medicine.

High risk autopsy conducted in the Biosafety Level-3 Post Mortem Suite.

Organizational, Methodological, Scientific, Educational and Expert Activities of the Republican Scientific and Practical Center of Forensic Medical Examination of the Ministry of Health - Republic of Uzbekistan

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The State Forensic Expert Service of the Republic of Uzbekistan currently represents an extensive structural network in the country's healthcare system, whose activities as a State medical institution, on the one hand, are related to the protection of public health and the prevention of pathological conditions, as well as improving the quality and effectiveness of treatment of injuries and diseases. The building of the Republican Scientific and Practical Center is shown in Figure 1.

Figure 1:
The building of the Republican Scientific and Practical Center.

The RSPCFME of the Ministry of Health of the Republic of Uzbekistan has the following structure: Director of the Center; deputy directors of the Center for scientific, expert work and work with regional branches; departments of the Center - scientific department, scientific laboratory research, information technology, international department; organizational and methodological, complex examinations, medical criminology with X-ray department, forensic biological, forensic chemical, forensic histological; as well as regional branches in the Republic of Karakalpakstan, the city of Tashkent and in 12 regional centers. Territorial district and inter-district points of forensic medical examination have been established as part of each regional branch.

The main purpose of the Center is the organization and planning of the forensic medical examination service of the Republic of Uzbekistan. Based on this, the Center provides organizational, methodological and expert guidance to the institutions of forensic medical examination of the country.

1. Organizational and methodological activities

Annual and prospective roadmaps are being developed to improve the activities of forensic medical examination, with schedules of visits to regional branches to provide methodological and practical assistance, as shown in one of the organizational and methodological activities in Figure 2.

Figure 2:
Organizational and methodological activities.

2. Research activities

RSPCFME is a scientific and practical institution, which includes both scientific and expert departments. In the scientific direction of the Center, the results of modern forensic and forensic chemical research aimed at improving the quality and effectiveness of forensic medical examinations are being developed and put into practice.

Over the past 5 years, 6 dissertations have been defended at the Center, 14 scientific events have been held, 12 scientific developments have been compiled, 12 educational and scientific materials have been published, 16 medical technologies have been put into practice, more than 120 articles have been published in cited foreign and national publications.

Scientific developments of forensic doctors of Uzbekistan are conducted in close connection with leading scientists and specialists from foreign countries and the boundaries of international cooperation are expanding year after year. Figure 3 shows the participation in an international conference.

Figure 3: Participation in an international conference.

Thematic improvement cycles of 36 and 72 hours (1.2 credits) are regularly organized on the basis of the RSPCFME of the Ministry of Health of the Republic of Uzbekistan. The theme of the cycles covers modern aspects of forensic medical examination of living persons, corpses and laboratory research. Figure 4 shows an advanced training course was being conducted.

Figure 4: Conducting an advanced training course.

3. Expert activity

One of the main tasks of the RSPCFME of the Ministry of Health of the Republic of Uzbekistan is to meet the needs of forensic investigative bodies for the production of forensic medical examinations.

The structure of the Center does not include morphological and outpatient departments. In this regard, the Center conducts repeated commissions. Comprehensive, forensic biological, forensic histological, medical and forensic examinations. The main part of the examinations carried out are in the nature of re-examination of the most complex cases, which were primarily carried out in regional branches.

4. Activities related to working with regional branches

The RSPCFME of the Ministry of Health of the Republic of Uzbekistan, as the main expert institution, coordinates the work of branches in the Republic of Karakalpakstan, the city of Tashkent and in 12 regions of the country, and provides them with practical and methodological assistance. The action plan and schedules of work with branches are approved annually by the Ministry of Health. According to this, the heads and leading specialists of the Center travel to the branches on a monthly basis.

The main conditions for improving the quality and effectiveness of expert research are the creation of a material and technical base of institutions that meet the standards. In the modern conditions of the functioning of the country's forensic medical service, quality issues are a priority.

The reliability of the obtained research result is an indicator of the effectiveness and efficiency of any type of forensic expert activity. In turn, a reliable, i.e. correct result can be obtained only during a properly conducted research process.

The prospects for the development of forensic medical examination in the Republic of Uzbekistan are to improve its structure and create genetic molecular (DNA) laboratories in the Center and 3 regional branches.

In addition, there is a question of amending the legislation of the Republic of Uzbekistan on the issue of cremation of the bodies of deceased unknown persons or foreign citizens who wished to cremate the corpse in order to facilitate its further transportation.

The priorities are also:

a) improvement of the regulatory framework for forensic medical expertise and the opening of non-governmental medical institutions;
b) development and implementation of new and modern medical technologies (virtopsies);
c) further development of the material and technical base of the Center and branches, construction of standard buildings in Andijan, Zhizzakh, Navoi, Samarkand and Surkhandarya regions;
d) professional training of specialists.

In general, our further prospects for the activities of the RSPCFME are related to the development of the material and technical base of expert institutions, the deployment of new structural, primarily laboratory units, the introduction of new high-tech and evidence-based research methods, training and advanced training of personnel, a significant increase in salaries of employees of the forensic medical service of the Republic of Uzbekistan.

Thus, in the Republic of Uzbekistan, judicial reform is being implemented in the context of deep ideological, socio-political and economic transformations.

The strengthening of all parts of the judicial, medical and legal system represents the necessary conditions for the successful implementation of judicial reform.

The expansion of the range of complex and high-tech cases in the courts dictates the need to use high information technologies in the production of forensic medical examinations.

Theoretical and Practical Value of the Conceptual and Terminological Apparatus of Forensic Examination

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Abstract

This article examines the usage of concepts and terminologies in the judicial examination procedures of Mongolia, evaluating how they are defined and applied, in light of theoretical scientific principles and the future development trends. The article suggests that in the context of theoretical and practical frameworks, it identifies issues that have arisen in this area and proposes grounded methods for resolving them.

Keywords: Forensic examination; forensic activity; examiner's conclusion; scientific language; professional language; conceptual and terminological apparatus.

As technology and science advance, new words, phrases, concepts, and terminologies are being introduced across all areas of social interaction. Concurrently, some of them become obsolete and fall out of use. For instance, terms currently used in Mongolian forensic practices such as “criminalistic registration” should be updated to "forensic registration," “fingerprint examination” to "fingerprint analysis," and “forensic medicine” to "judicial medicine," among other necessary terminology updates in the sector.

Forensic science is one of the fields experiencing this phenomenon. The concepts and terminologies of examination are not only integral for articulating precise scientific inquiry but are also closely linked with several disciplines such as law, deep science, medicine, technology, physics, chemistry, and economics. This is because the process of conducting examinations and developing expert conclusions involves complex and responsible work that demands a broad spectrum of knowledge from various fields, making the clarity of concepts and terminologies crucial.

The development and refinement of concepts and terminologies help to measure the progress and orderly functioning of a scientific field, as well as to evolve the professional language that is foundational to the discipline. Through professional language, various stakeholders like experts, professionals, scholars, educators, and students engage in communication, share knowledge and information, articulate thoughts clearly, tackle issues, and conduct analyses, fulfilling their fundamental roles.

By the end of the 20th century, Russian scientists had developed a common theory of forensic science, providing a favorable environment for the field to evolve into an independent discipline. Mongolia considers its judicial examination activities based on this theory and methodology as part of an integrated system. Despite this, there are significant unresolved issues at the theoretical level, particularly concerning the linguistic aspects that constitute the knowledge base of this field.

a) Theoretical Framework

We have previously mentioned several important requirements for defining forensic examination concepts and terminologies, including:

- *Being scientifically based;*
- *Uniformity;*
- *Systematic organization;*
- *Unambiguous;*
- *Named explicitly;*
- *Concise and clear;*
- *Stable;*

An inappropriate or imprecise use of terminology can lead to misunderstandings and, particularly, faulty decisions, which is especially unacceptable in the process of developing an expert's conclusion. Additionally, the manner in which an expert's conclusion is formulated can reflect the professional skills and knowledge level of the expert in question.

The position and significance of the language and concepts of examination in theory and practice have been approached from various perspectives by scholars in the field. For instance, Russian scholars A.I. Vinberg and N.T. Malakhovskaya considers "an expert's language as a system of definitions, signs, and annotations (terminologies) similar to the language of science, [4] " whereas R.S. Belkin argues that "the language of criminalistics should not be seen as a unique set of concepts and terminologies intended only for professionals in the field. Such a language does not and should not exist. Therefore, this issue should be considered both in terms of common usage and scientific accuracy, and as a unified system of concepts and terminologies suitable for the field of knowledge." V.A. Yumatov and O.A. Nesterov views "the language of forensic examination as fundamentally scientific. It should be based on scientific principles, contributing to success while being devoid of redundant, simple, and precise language [3]."

In the United States, efforts to establish a systematic and organized framework within this field of knowledge are recognized. For example, an international research conference organized in 2009 presented "Improving the investigative process: the way forward," highlighting 13 proposed actions to enhance the reliability of expert conclusions, standardised terminology, and improve outcomes in judicial practice. This recommendation states: "...create a standard terminology to use in expert conclusions and judicial practice, to develop tools that improve the reliability of expert conclusions, information exchange, and the results of examinations..[14].".

In Mongolia, for example, legal doctor Khurtsgerel J. defines "the system of concepts, definitions, and symbols expressed through the language of criminalistics, [10]" and professor Bat-Erdene B. describes it as "a system of concepts, definitions, and identifiers that can effectively communicate the content of science. [2]" Furthermore, legal doctor Lkhagvasuren B. describes "the language of examination as a system of precise, unequivocal definitions, terms, and identifiers necessary for both the accusatory process and the environment of forensic examination. [6]"

Despite the differences in scholars' viewpoints, it is evident that there is a consensus on the need to standardize and organize the concepts and terminologies of forensic examination into a unified system. Therefore, to develop the professional language of examiners, to systematically organize and shape concepts and terminologies, and to integrate them into a unified system, it is essential, first and foremost, to establish the definitions of these concepts and terminologies in the field according to the aforementioned requirements. This underscores the theoretical importance of the concepts and terminologies of forensic examination.

b) Practical Framework

To concretely determine the practical relevance of forensic examination concepts and terminologies, it's crucial first to identify and understand the challenges and obstacles encountered in their application. These challenges include misusing forensic concepts and terminologies, employing outdated terms that have fallen out of use, using imprecise terminologies, and conflicts between the principles of terminology designation and application [11].

An analysis of how Mongolia currently defines and utilizes forensic examination concepts and terminologies in practice reveals that higher education institutions, research units, and scholars within the legal field have variously named and engaged with the field, reflecting its interdisciplinary nature. The term "forensic examination" has been officially adopted in the 2023 legislation, indicating a departure from previously inconsistent naming conventions towards a more uniform and scientifically grounded terminology that is concise, clear, and precisely defined by word [1].

Some scholars have proposed naming the field "investigative science" to reflect its comprehensive and interdisciplinary scope more accurately. For instance, legal doctor Jargalsaikhan B. has suggested, considering linguistic nuances, that "forensic examination" might not fully capture the discerning and decisive aspects inherent in the Mongolian understanding of "judicial" and recommended reverting to a term used in the 1930s, which might be more fitting [5].

Legal doctor B. Tsolmon has proposed naming the field as "investigative science" and provided the following explanation regarding the term "forensic examination." He notes that the practice of interpreting and explaining the term "forensic" (Latin - *forensic*, Russian - *судебный*) in a way that defines the purpose, intention, and responsibility of the examination process has been observed to lead to misinterpretations regarding its scope and implications. According to Russian linguistic rules, compound words can be formed by connecting words with a hyphen for clarity. For example, "судебная экспертная деятельность"becomes"судебно-экспертная деятельность," and "научный технический процесс" becomes "научно-технический процесс." However, directly translating these condensed forms into Mongolian or choosing a version of "forensic" that conveys [12] action might lead to practical and disciplinary risks.

Tsolmon B. further states that the debate and research based on how to name and use the term within the field, as expressed by scholars and experts, are commendable. However, he warns against a superficial approach to such a complex issue, suggesting that any attempts to simplify or impose one's own view without thorough consideration can be detrimental [12].

To acknowledge any field of knowledge as an independent science, it is crucial first and foremost to have its theory (both general and specific), methodology, and techniques (technology) well-established and integrated. Only based on these foundations should the name of the specific science be finally determined, and this requirement also applies to forensic examination. However, according to the founders of this field of knowledge, under current conditions, while the general theory of forensic examination is being developed, the special theory, methodology, and certain types of examination techniques and technologies related to it are at the stage of development. Once these are comprehensively organized and recognized, the field can officially be acknowledged as an independent science, necessitating addressing issues related to its nomenclature. Therefore, any attempt to prematurely name the field without considering these foundational elements is deemed not significantly impactful.

However, since the general theory of the field has already been developed and accepted, it would be appropriate for scholars, researchers, and practitioners within the field to seriously consider issues related to the professional language of examiners and the concepts and terminologies that constitute it. For instance, as one of the founders of the theory of this field of knowledge, T.V. Tolstikhina suggests, just like any scientific theory, the general theory of forensic examination should be meaningful, comprehensible, and precisely defined with scientific concepts and terminologies. Based on this, it is advised that common terms and terminologies of practical importance from other fields should be developed in a way that aligns with the unique operations of this field, ensuring that they are integrated appropriately into forensic examination activities [9].

Standardizing and organizing the concepts and terminologies of examinations, integrating them into a unified system, and then applying them consistently are critically important. Additionally, for the practical application of any scientific terminology, it's essential to carefully examine and establish a clear and consistent foundation for the terms and concepts, considering their origins. This is particularly relevant when some concepts and terminologies are directly borrowed from foreign languages. In such cases, it becomes imperative to adapt these terms effectively within the Mongolian linguistic framework to ensure their appropriate and precise use.

Therefore, by diligently addressing these pressing issues and exploring ways to resolve them, it's possible to accurately define both the theoretical significance and practical relevance of the concepts and terminologies used in the field of examination.

Recommendations

Considering the above conditions, we aim to address these complex issues within the theoretical and practical dimensions and conduct a thorough analysis. Based on this situation, we propose establishing a "Unified Database of Forensic Concepts and Terminology" under the General Agency for Forensic Examination or the Academy of Internal Affairs. This database would integrate these concepts into a unified system and involve forensic experts, researchers, and linguists specializing in terminology and linguistics.

In summary, as the scope of specialized knowledge expands and the process of information dissemination accelerates, it is evident that the scientific language of forensic science, including its fundamental concepts and terminologies, needs to be clearly defined and applied both theoretically and practically. An effective way to address these issues would be to refine, integrate, and formalize forensic concepts and terminologies, incorporating them into a unified system.

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Methamphetamine Menace in Sri Lanka: Prevalence, Trends and Legislative Responses

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Introduction

Methamphetamine (MA) is a powerful and highly addictive central nervous system stimulant, poses a significant challenge globally [1]. In Sri Lanka, a SAARC region country, the issue of drug addiction and trafficking has escalated in the last five years. This article utilizes data from National Dangerous Drugs Control Board of Sri Lanka to shed light on the prevalence, trends and consequences of MA abuse in the country.

Background

MA is classified as an Amphetamine Type stimulant (ATS). The term 'ATS' was introduced at the WHO meeting in 1996 in Geneva to describe amphetamines, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA) and other psychostimulants [2]. ATS are a synthetic drug which constitutes on of the most significant drug problems in worldwide [3]. MA is the second most widely abused illicit drug after cannabis in the world [4]. Sri Lanka's geographical location, situated between the golden crescent and the golden triangle which are considered as two major poppy- growing areas. Due to that geographical situation, Sri Lanka become a transit point for illegal drugs entering country. The emergence of MA in Sri Lanka gained prominence in 2007 when a significant Customs detection at Colombo International Airport revealed a surge in widespread usage.

Manufacturing and Availability

In contrast to the intricate production processes of substances like heroin and cocaine, the manufacturing process of MA requires less effort in clandestine laboratories. This ease of production contributes to its widespread availability [5]. MA, known as meth, crystal meth or ice on the streets of Sri Lanka, has historical roots, having been first synthesized from ephedrine by Japanese pharmacologist, Nagayoshi Nagai in 1893 [6,7]. The turning point occurred in 1919 when a Japanese chemist Akira Ogata was successfully synthesized MA in a crystallized form [7]. There are several commercial medicines containing MA. Pervitin is a tablet containing MA that was widely used by German, Japan and American forces during World War II to increase alertness and decrease weakness and suppress the appetite of soldiers [8-10]. Oberto was introduced as a treatment for obesity in the 1950s [2], while Philopon was commercialized in Japan in 1941 [11,12]. MA has very limited medical use for the treatment of attention deficit/ hyperactivity disorder (ADHD) or weight loss, depression, its euphoric and asthma and is only available through a non-refillable, but it often illegally used as a highly addictive recreational drug. But invigorating effects were quickly identified by individuals seeking such experiences [13].

Recent Incidents and Trends

Recent incidents, such as the discovery of a clandestine drug laboratory in Kosgama in 2008 and the Sri Lankan Navy's historic seizure of 605 kg of crystal MA and 579 kg of ketamine in 2020, underscore the severity of the issue.

The primary categories of seized drugs in Sri Lanka, as outlined in Table 1, encompass Heroin, Cannabis, and MA, over the years spanning the years from 2018 to 2022. The drug report for Sri Lanka in 2023 highlights a significant surge in MA seizures in 2020, with a substantial escalation from 35.4 kg in 2019 to a concerning 818.8 kg. This spike indicates a troubling trend in MA trafficking and availability during that period. However, subsequent years saw a decline in seizures, plummeting to 377.3 kg in 2021 and further to 283.9 kg in 2022. Figure 1 shows Photographs of the seized bulk quantities of Methamphetamine received at the Government Analyst's Department in Sri Lanka.

	2018	2019	2020	2021	2022
Methamphetamine/(kg)	7.1	35.4	818.8	377.3	283.9
Heroin /(kg)	739.1	1741.9	1630.2	1630.1	1677.1
Cannabis/(kg)	4878.4	7071.1	16195.4	15628.2	17607.4

Table 1: Seized Quantities of Methamphetamine, Heroin, and Cannabis from year 2018 to year 2022 in Sri Lanka.

Figure 1: Photographs of the seized bulk quantities of Methamphetamine received at the Government Analyst's Department in Sri Lanka.

The main categories of seized drugs abuse in Sri Lanka, as indicated in Table 2, include Heroin, Cannabis, and MA, spanning the years from 2018 to 2022[14].

	2018	2019	2020	2021	2022
Heroin	40998	40970	51603	54412	69688
Cannabis	54686	45923	41080	44239	53579
Methamphetamine	257	2073	2387	13720	22631

Table 2: Number of drugs related arrests from year 2018 to year 2022 in Sri Lanka.

Compared to 2021, there is an observable upward trend in MA-related arrests in 2022, with law enforcement agencies detaining 22,631 individuals involved with MA. Over the past two years, the arrest records indicate a significant surge in MA usage. The percentages of increased usage related to MA, heroin, and cannabis in 2022 compared to the year 2021 were 64.95%, 28.07%, and 21.11%, respectively. This indicates that MA usage surpasses the percentages of heroin and cannabis abuse in 2022.

Government Analyst's Department and Testing Methods

The Government Analyst's Department is the official government laboratory in Sri Lanka responsible for analyzing illicit drugs received by the country as mandated for judicial investigations. Enforcement agencies like the Police Narcotic Bureau, Police and Excise Department submit seized drug samples to the laboratory for routine analysis. The laboratory employs both presumptive and confirmatory tests to identify MA in seized samples. Presumptive test can provide an indication of the presence or absence in the sample and eliminate negative results quickly. To identify MA in the samples, the laboratory uses presumptive tests such as the Marquis test, Simon's test, and Thin Layer Chromatography (TLC). The laboratory employs confirmatory tests, including Fourier Transform Infrared Spectroscopy (FTIR), Gas Chromatography with Flame Ionization Detector (GC-FID), Raman Spectroscopy, and Gas Chromatography Mass Spectrometry (GCMS). All MA seizures with a net weight of 2 grams or more submitted to the laboratory are quantitated using Gas Chromatography with Flame Ionization Detector.

Legislative Measures

The increase in MA distribution has led to the implementation of legislative measures aimed at regulating its availability. In Sri Lanka, MA is regulated under the “Poison Opium and Dangerous Drugs Ordinance” and “The Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances Act No. 01 of 2008”. Under these Acts, possession of MA in quantities exceeding 5 grams may result in the death penalty or lifetime imprisonment. These Acts criminalize the manufacturing, possession, sale, purchase, transport, import, and export of any listed narcotic drug. However, enforcement efforts have been challenged by the increasingly sophisticated tactics employed by traffickers, including transnational smuggling networks and clandestine manufacturing operations.

Conclusion

Methamphetamine's medical utility is constrained due to its propensity to induce severe psychiatric ailments and organ deterioration, often leading to fatal consequences. Additionally, its usage correlates with heightened risk -taking behavior and aggression, exacerbating the incidence of unnatural fatalities. The rise in MA distribution calls for a rapid and efficient analytical to prevent drug smuggling and trafficking. Contributing to the establishment of a drug-free society. Further, the future of the country is at stake, emphasizing the need for all relevant authorities to implement well-planned measures to address this escalating menace.

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Accreditation of Crime Scene Investigation under ISO17020:2012 Standard in Hong Kong

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Abstract

Crime scene investigation (CSI) is an important link in the criminal justice system as it serves as a bridge between establishing the happenings during an incident and possibly identifying the accountable persons, providing light in the dark. The International Organization for Standardization (ISO) and the International Electrotechnical Commission (IEC) collaborated to develop the ISO/IEC 17020:2012 standard to govern the quality of CSI, a branch of inspection activity. These protocols include the impartiality and competence of the crime scene investigators involved, contemporary recording of scene observations and data obtained, the correct use of resources during scene processing, forensic evidence collection and handling procedures, and the confidentiality and integrity of any scene information obtained from other parties etc. The preparatory work, the accreditation processes involved and the implementation of new quality measures to the existing quality management system in order to achieve the ISO/IE 17020:2012 accreditation at the Forensic Science Division of the Government Laboratory in Hong Kong are discussed in this paper.

Keywords: ISO/IEC 17020; crime scene investigation, CSI, on-site monitoring, critical findings check; independent check; scene of crime officer, SOCO

Introduction

ISO/IEC 17025:2017 is an international standard that sets out the general requirements for the competence, impartiality, and consistent operation of laboratories [1] whereas the ISO/IEC 17020:2012 standard specifies requirements for inspection bodies to demonstrate their technical competence, independence, and ability to provide reliable inspection services [2]. ILAC-G19 provides guidance for forensic science units involved in examination and testing in the forensic science process by providing application of ISO/IEC 17025 and ISO/IEC 17020 [3]. The Forensic Science Division of the Government Laboratory (FSDGL) in Hong Kong gained the ISO/IEC 17025:2005 accreditation for testing and calibration laboratories in March 2011 under the Hong Kong Laboratory Accreditation Scheme (HOKLAS) provided by the Hong Kong Accreditation Service (HKAS). In June 2020, FSDGL made a transition to ISO/IEC 17025:2017 standard. A Standard Operating Procedures (SOP) documenting the requirements set out in the ISO/IEC 17025 standards had been adopted by FSDGL ever since the accreditation in 2011.

Apart from providing crime-related laboratory testing services, FSDGL had been providing 24-hour comprehensive CSI service in Hong Kong for decades. To echo with the Laboratory's vision "To be recognised internationally as a laboratory providing world-class scientific services", the FSDGL had been preparing for ISO/IEC 17020:2012 accreditation for CSI since November 2020. This accreditation programme is under the Hong Kong Inspection Body Accreditation Scheme (HKIAS) operated by the HKAS. During the preparation, a gap analysis regarding discrepancies between the requirements in the ISO/IEC 17025:2017 and ISO/IEC 17020:2012 standards was performed. In addition, since FSDGL is seeking accreditation under the HKIAS, other quality standards [4]-[6] are also key documents to make reference to. As a result, a new chapter dedicated to CSI was incorporated into the SOP of FSDGL to provide general approaches and procedures for CSI under the framework of the standard, and definitions to note such as impartiality, independence, and confidentiality. A pre-assessment was conducted by an assessor from the HKAS in June 2021. FSDGL was found to be qualified for the on-site initial assessment for ISO/IEC 17020:2012 in August 2021. To avoid potential conflict of interest, it is desirable for an overseas technical assessor to conduct the assessment; however, due to travel restrictions enforced during the COVID-19 pandemic, it was postponed until June 2023. FSDGL gained accreditation to ISO/IEC 17020:2012 for the scenes of crime investigation under the HKIAS operated by the HKAS in August 2023.

Requirement Framework

The framework of the requirements set out for the operation of various types of bodies performing inspection in the ISO/IEC 17020:2012 standard is similar to that of the ISO/17025:2017 standard (Table 1). All these requirements apply to inspection bodies of type A, B, or C, as defined in the standard. Type A bodies are independent from the parties involved in the inspection, while type B and C bodies may have a different level of independence. FSDGL is a Type A body since crime scene investigations are independent and are not influenced by the client department. These requirements cover various aspects of an inspection body's operations, including its organisation structure and management system, personnel competence, facilities and equipment resources, inspection processes, and quality assurance practices.

Clause No.	Description
1	Scope
2	Normative references
3	Terms and definition
4	General requirements
5	Structural requirements
6	Resources requirements
7	Process requirements
8	Management system requirements

Table 1: Requirement Framework of ISO/IEC 17025:2017 and ISO/IEC 17020:2012 [1-2].

Structural Requirements

Regarding structural requirements, the ISO/IEC 17020:2012 standard defined that the inspection body shall have documentation which describes the activities for which it is competent, it shall be structured and managed in such a way to safeguard impartiality, the responsibilities and reporting structure of the organisation shall be defined and documented, and that one or more persons shall act as the technical manager who shall be technically competent and experienced in the operation of the inspection and has overall responsibility to ensure that the inspection activities are carried out properly [2].

FSDGL follows a two-tier casework reviewing system in which scene reports prepared by the scene of crime officers (SOCO) are reviewed by a technical reviewer, followed by an administrative reviewer, who also plays the role of the technical manager. Any observations or results that (i) have a significant impact on the conclusion reached and the interpretation and opinion provided; (ii) cannot be repeated or checked in the absence of the exhibit or sample; and/or (iii) could be interpreted differently, are classified as critical findings. In addition, if a check of critical findings is the only quality control measure, it shall be performed without knowledge of the original result. Since an incident scene may not be able to be re-visited and its original state may be disturbed or altered through time, any observations made at scene are classified as critical findings. The critical findings of a case are checked independently based on the scene observations made by the checking officer, namely the technical reviewer, against the observations made by the SOCO, in which the checking officer has no prior knowledge of, therefore the SOCO and the checking officer would process the scene in parallel but independently. During technical review, the technical reviewer will compare the scene observations and conclusions made by the SOCO and critical findings checking officer. If disagreement is found, the SOCO shall reconsider the scene observations and conclusions. If necessary, the technical manager would be consulted to determine the subsequent course of action. Further training on specific aspects with unsatisfactory outcome would be required to be taken by the SOCO.

Resources Requirements

Under resources requirements, the ISO/IEC 17020:2012 standard defined that the inspection body shall employ a sufficient number of persons with the required competencies, including the ability to make professional judgements, to perform the type, variety and volume of its inspection activities [2]. The performance of the SOCO during scene investigation are monitored through on-site witnessing by personnel familiar with the inspection methods and procedures. On-site monitoring for each SOCO is carried out at least one every year. A designated form for recording the on-site monitoring findings and comments in different aspects during CSI is included in the SOP. The areas to be monitored include:

1. General preparation – scene assessment; use of adequate and calibrated equipment;
2. Process of work – preventive measures against cross contamination; thoroughness in observation; proper and sufficient documentation; proper methods used in exhibit collection and packing; and
3. Presentation skills – impartiality; ability to convey observations to client.

Feedback of the on-site monitoring findings are provided to the SOCO and if the performance of the SOCO is found to be unsatisfactory, follow-up action such as further training in inadequate areas and more frequent monitoring would be required.

Apart from on-site monitoring, in-house mock scene assessment is arranged once every year to access the scene processing competency of the SOCO. The mock scenes included all the inspection methods under the scope of the ISO/IEC 17020:2012 accreditation FSDGL is seeking for:

1. Recovery of latent DNA; and
2. Location and recovery of blood/semen; fibres; glass fragments; paint/plastic fragments; shoeprints; and tool marks.

The aim of the in-house mock scene assessment is for SOCO to demonstrate the ability to locate and recover relevant forensic evidence at scene, evidence handling, scene processing and documentation, and to provide appropriate advice to client department. Prior to the mock scene assessment, information on the case scenario and the list of evidence and their location at scene were reviewed by the head of the SOCO and endorsed by the technical manager. The SOCO being assessed have no access to any of the information beforehand. Only on the day of the mock scene assessment, the SOCO were provided with the case scenario and required to process the scene and collect relevant forensic evidence (Figures 1 and 2). Professional officers from FSDGL acted as the police officers at scene.

Figure 1: OCO processing the mock scene.

Figure 2: SOCO processing the mock scene.

The Accreditation

The two-day assessment was conducted in June 2023 by the audit team comprised of a lead assessor from the HKAS and a technical expert from Mainland China. The assessment included review of documents in various aspects under the ISO/IEC 17020:2012 framework, on-site scene processing assessment, review of case records and interviewing nominated approved signatories, i.e. the SOCO. Examples of the documents reviewed during the assessment are shown in Table 2.

Clause	Examples of Documents Reviewed
Structural and Management System Requirements	
Organisation and Management	Organisation structure, code of ethics, reports on risk assessment, quality audit, management review, and corrective action, and quality manuals, e.g. Quality Assurance Protocol, SOP, Sectional Work Manuals and Miscellaneous Work Instructions etc.
Resource Requirements	
Personnel	Training records, competency test records including proficiency tests and in-house mock scene assessment, and on-site monitoring records
Equipment	Calibration and performance check records of instruments and scene equipment
Process Requirements	
Inspection methods and procedures	Critical findings check records
Inspection Records and Reports	Case files including notes and photos of scene observations, crime scene exhibits records, and scene reports

Table 2: Examples of documents reviewed during ISO/IEC 17020:2012 accreditation.

On-site assessment involved two mock scenes, *viz.*, sexual assault case; burglary and wounding case, and a real traffic accident case. The two mock scenes were processed by different SOCO while another SOCO on duty handled the real case. The two mock scenes were set up at a detective training facility owned by the Hong Kong Police Force. This facility was chosen owing to the availability of the environmental settings commonly found in Hong Kong such as residential flats, restaurant, and bar etc. but tailor-made for crime scene investigation training purposes. The mock sexual assault scene was set up in the bar setting (Figures 3 and 4) and the mock burglary and wounding scene was set up in the residential flat setting (Figures 5-7). To make the mock scene assessment more realistic, police officers were invited to join on the day of the assessment and acted as the officer-in-charge, exhibit officer and investigative officer, who are usually present during scene examination by SOCO. Information of the two cases and the list of evidence and their location at scene pre-approved by the assessment team were provided to the police officers prior to the day of assessment. Information of the cases were only provided to the respective SOCO on the day of the assessment. The vehicle involved in the real traffic accident case was examined by the SOCO at a Police Vehicle Detention and Examination Centre (Figure 8).

Figure 3: SOCO processing the mock sexual assault scene.

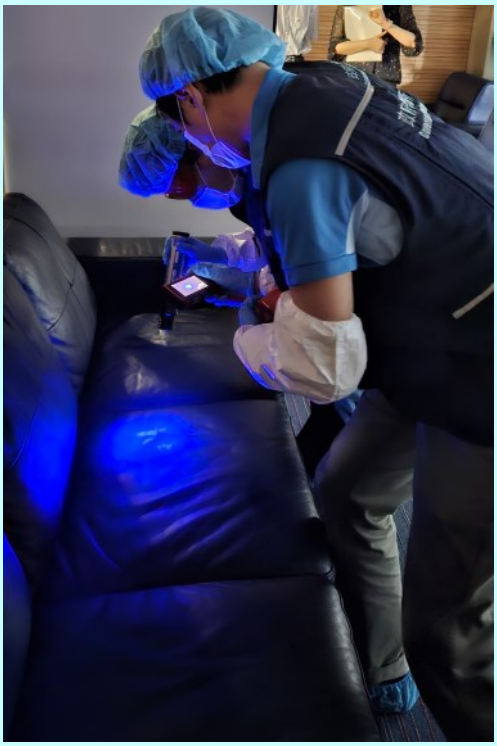

Figure 4: SOCO processing the mock sexual assault scene.

Figure 5: SOCO processing the mock burglary and wounding scene.

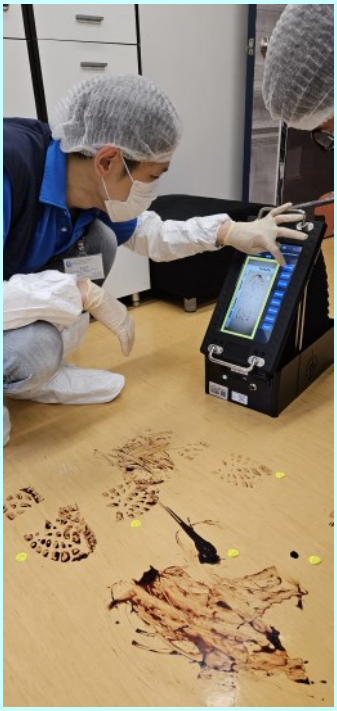

Figure 6: SOCO processing the mock burglary and wounding scene.

Figure 7: SOCO discussing the scene findings with the police officers and the technical assessor.

Figure 8: SOCO collecting evidence from the accident vehicle

During the interview sessions conducted by the assessment team, scene examination process, evidence collection and packing procedures, contemporary scene records from scene observation to evidence collection and transfer of exhibits to clients, and debriefing of the scene findings to clients were discussed. Special attention was drawn to the awareness of the risk of cognitive bias when communicating with clients since any unverified or incorrect information could mislead the SOCO during CSI, and relevant evidence of significant forensic value may be overlooked [7].

Conclusion

The accreditation assessment was with satisfactory outcomes. FSDGL gained accreditation to ISO/IEC 17020:2012 for the scenes of crime investigation under HKIAS operated by HKAS in August 2023. FSDGL had demonstrated its impartiality, the suitability of the methodologies and equipment being used, and the technical competence of the staff involved in handling various crime scene investigation activities, fully complied with the relevant accreditation criteria.

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Investigation of the Persistence of Diesel on the Fabrics

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Abstract

Arson often involves the use of ignitable liquids such as gasoline, kerosene, and diesel, which can accelerate the fire. In such a case, there is a possibility to spill the ignitable liquids onto the offender's clothing.

The current work aims to investigate the persistence of diesel on different types of fabrics by comparing the volatile residues remaining at different times. Diesel is used as an accelerant for arson because of its availability, inexpensiveness, and easy ignitability, and cotton and polyester which are some of the commonly used fabrics were selected as the substrate in this work.

These fabric samples were spiked with 0.5 ml of diesel separately and left to evaporate at different durations from 0 hour to one week. After that, the samples were placed in metal cans, and the passive headspace with activated charcoal strip extraction method was followed. The detection and identification of diesel constituents on each fabric were conducted via gas chromatography-mass spectrometry (GC-MS). Alkanes with higher carbon numbers in diesel can still be detected on both cotton and polyester fabrics up to one week after impregnation. Furthermore, results showed that the persistence capacity of diesel could be higher on polyester fabric than on cotton fabric.

These results can be helpful to the forensic analyst for the detection of diesel constituents on the clothing of the person carrying out arson.

Introduction and research problem

Arson can be defined as "the deliberate burning of property for malicious or fraudulent purposes and often involves the use of ignitable liquids (IL), which are flammable materials used to spread and increase the rate and intensity of burning" [1]. The commonly used ILs in Sri Lanka are petrol, kerosene, and diesel due to their availability, inexpensiveness and ease of transport. Considering diesel, it is used as the fuel in many vehicles and therefore, there is a chance to use this as an accelerant by common people. Further, with its slow rate of burning, fire can burn for a longer time without anyone discovering it. Then such a fire will cause much more damage to the properties than faster accelerants. When diesel is used as an accelerant by perpetrators, there is a possibility of spilling the used diesel on their clothing, shoes, and gloves etc. Therefore, there is a need to know how long diesel can persist on personal belongings. The detection of diesel constituents on the suspect's clothes after a few days, can provide information to the forensic investigators to identify the suspect, and it is helpful for judicial statements because evidence, such as DNA or fingerprints needed to identify the suspect is often destroyed at the origin of the criminal fire [2].

The chromatographic profile of Ignitable Liquid Residue (ILR) is dependent on the IL type, persistence time, substrate type and environmental condition, and may shift to the right with the longer weathering time.

There are a few experiments that have been undertaken for the persistence of petrol on various types of substrate materials such as cloth, carpet, wood, etc. [3,4,5], since they are good absorbing materials, and commonly available materials in the fire scene. Most of the research was done for petrol only.

Since there are limited studies on the persistency of ignitable liquids on different types of fabric material using passive headspace extraction with activated charcoal strip, the aim of this study is to determine the persistence of diesel residues on different types of fabric materials (polyester and cotton) using passive headspace extraction with activated charcoal strip. The weathered samples on fabrics at different time intervals were extracted using passive headspace extraction with activated charcoal strip and analysed using GC-MS.

Materials and Methods

Chemicals and Materials

HPLC (High-Performance Liquid Chromatography) grade acetone of Sisco Research Laboratories Pvt. Ltd with 99.9 %, Activated carbon strips (0.9 cm × 2 cm) obtained from ARROWHEAD FORENSIC in USA, Metal cans (medium size) purchased from Micro Industries in Sri Lanka, oven (DK-600DT) and GC-MS (Agilent Technology) were used in this study.

Two common fabric types used for T-shirts; dark blue dyed 100% cotton T-shirt material and blue-coloured polyester (4A) T-shirts material (synthetic fabric) were purchased from the local market, Galle. A test sample of diesel was purchased from the CEPETCO filling station, in Galle, Sri Lanka.

Method

A 0.1% diesel sample diluted with Acetone was prepared and injected (1.0 µl) into GC-MS to determine the classification of the ILs based on ASTM 1618. The GC-MS conditions are shown in Table 1.

Carrier Gas	Helium (99.99%)
Injector Temperature	250 ^{0}C
Flow rate	1.5 ml/min
Column	HP-5MS length 30m, 0.25 mm of internal diameter and 0.25µm of film thickness.
Injection volume and mode	1.0 µl, split less
Oven maximum temperature	300 °C
Oven temperature program	Initial 40 °C, hold 2.0 min 1st ramp 5 °C/min to 90 °C, hold for 0.0 min 2nd ramp 14 °C/min to 90 °C, hold for 10.0 min
Ionization method	Electron impact ionization (EI) 70 eV
Scan mode	Sim/scan
Source temperature	230 °C
Quadrupole temperature	150 °C
Scanning mass range	30-550

Table 1: GCMS conditions of temperature program.

Test fabric samples were prepared by pipetting 0.5mL of diesel on the fabric samples (10 cm x 10 cm) and placed in the open in an indoor air-conditioned environment (approximately 25 degrees Celsius) for different time intervals (0 hour (hr), 2 hrs, 4 hrs, 8 hrs, 16 hrs, 24 hrs and 7 days). The active charcoal strip was inserted into the metal can with fabric samples (Figure 1). Then they were kept in the oven at 80 ^{0}C for 8 hours for passive headspace extraction method based on the ASTM E1412 standard practice [6]. The carbon strip was eluted with 300µL acetone and analysed by GC-MS. Data interpretation was conducted by using pattern recognition of Total Ion Chromatogram (TIC) Gaussian shape recognition and Extracted Ion Chromatogram (EIC) where appropriate according to the ASTM E1618 [7]. The selected target compounds were identified using the NIST MS library.

Figure 1: Preparation of test samples by spiking different volumes of diesel.

Results and Discussion

The diesel was identified by comparing the Total Ion Chromatogram (Figure 2- top), Extracted Ion Chromatogram (considered ions are 43, 57, 71, 85) (Figure 2- bottom), and selected target compounds (Table 2) of diesel with the literature [1].

The classification of ignitable liquids in ASTM E1618 [7] indicates that the diesel is in the C_9- C_{20+} carbon range and this study obtained all alkanes in the range of C_{9+} - C_{20+} with pristain (2, 6,10, 14-tetramethyl pentadecane) and phytane (2,6,10,14-tetramethyl hexadecane).

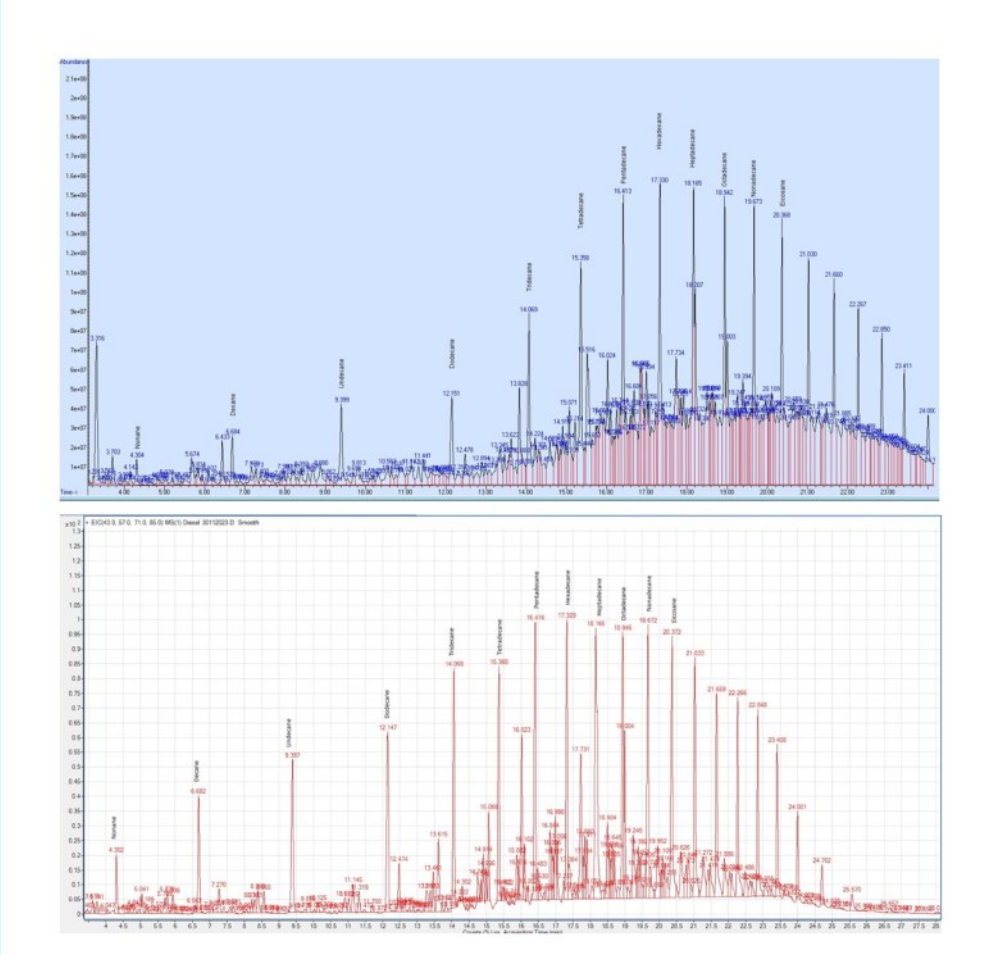

Figure 2: Total Ion Chromatogram (top) and Extracted Ion Chromatogram (bottom) of Diesel.

Analysis of control samples indicated there is no effect on the data interpretation of test fabric samples from the solvent, both fabric types, metal can and carbon strip.

Target compound	Retention Time/min
Nonane	4.31
Decane	6.68
Undecane	9.37
Dodecane	12.13
Tridecane	14.05
Tetradecane	15.35
Pentadecane	16.4
Hexadecane	17.31
Heptadecane	18.15
Octadecane	18.92
Nonadecane	19.65
Eicosane	20.15

Table 2: Diesel target compounds and retention time for reference diesel (0.1%) sample.

Among the components eluting during the runtime of the temperature program, twelve (12) major components; Nonane (C_9), Decane (C_{10}), Undecane (C_{11}), Dodecane (C_{12}), Tridecane (C_{13}), Tetradecane (C_{14}), Pentadecane (C_{15}), Hexadecane (C_{16}), Heptadecane (C_{17}), Octadecane (C_{18}), Nonadecane (C_{19}), and Eicosane (C_{20}) were selected for checking the presence or absence of diesel on each fabric sample.

According to the United Nations Office on Drugs and Crime[8], minimum requirement for signal-to-noise of 3 is widely accepted. Using that as the basis for detection, the peaks for targeted compounds were identified in both TIC and EIC. Figures 3 and Figure 4 show the persistence of diesel constituents on cotton fabric and polyester fabric at different times respectively.

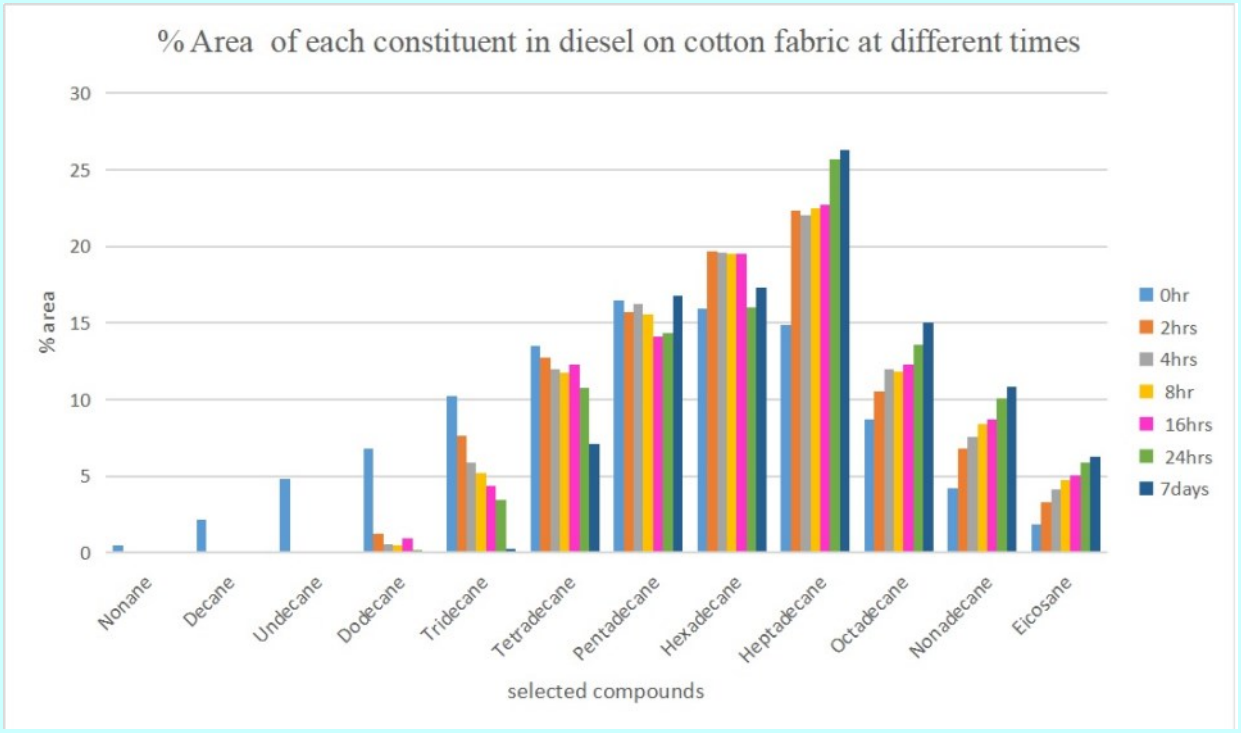

Figure 3: Diesel constituents retained on cotton fabric at different times.

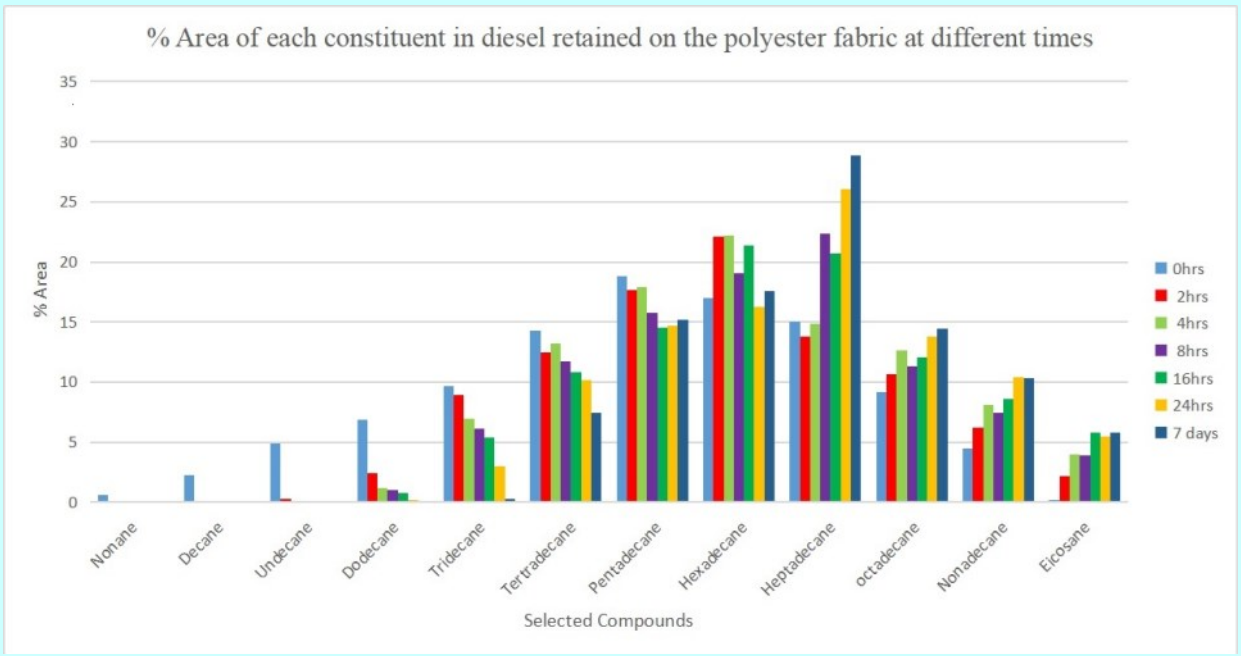

Figure 4: Diesel constituents retained on polyester fabric at different times.

According to Figure 3, all selected target components of the diesel were present in the cotton fabric at 0 hours. The first three selected hydrocarbons in diesel (nonane, decane and undecane) had been evaporated after 2 hours of impregnation on cotton fabric. While the highest abundance peak at 0 hours on cotton fabric impregnation was pentadecane, for other remaining hours samples, heptadecane was the most abundant peak. According to this figure 3, the low molecular weight components had been evaporated with the evaporation time and spectrum was shifted to the right hand side (high molecular end).

Figure 4 shows all selected targeted compounds of diesel present on polyester fabric at 0 hours. The peaks for first two selected compounds (nonane and decane) had been evaporated on the polyester fabric after 2 hours, and after 4 hours undecane had been also evaporated. The most abundant peak on polyester fabric at 0 hours was pentadecane and hexadecane was the most abundant peak at 2 hours and 4 hours samples. For other remaining samples, heptadecane was the most abundant peak. The remaining components of diesel on the polyester fabric at different times decreased with the evaporation time and chromatograms were shifted to the high molecular end with the time.

The low molecular weight components in the diesel evaporate with time, high molecular components retain on the fabric and the chromatograms shift to the high molecular side. Theoretically, the concentration of low molecular weight components decreased gradually with time, and the concentration of high molecular weight components increased gradually. The obtained results of variation of some targeted compounds in diesel on each fabric with time are shown in Figure 5.

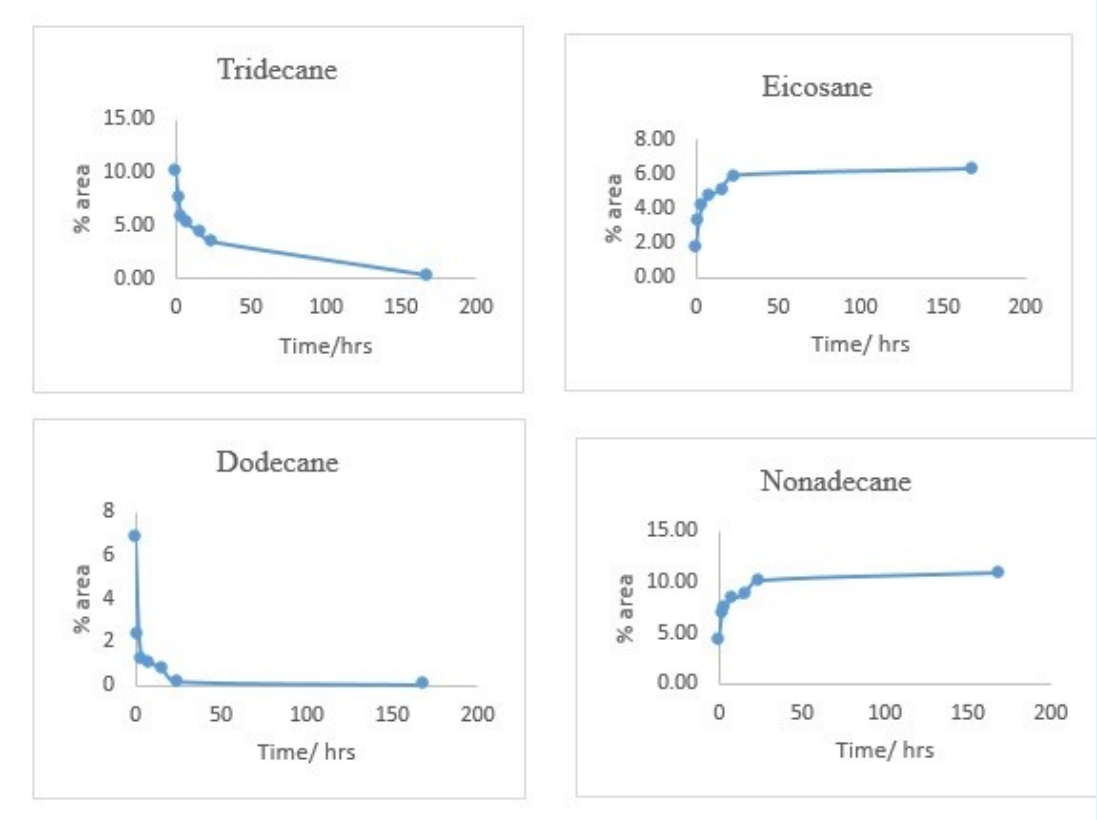

Figure 5: Variation of abundancy of diesel constituents with time.

However, according to the Figure 5, the graphs for variation of abundance of the selected target compounds did not change gradually as expected. At 16 hours, each of the target compounds showed some variations when compared with the other samples, and this type of inconsistency with the theoretical expectations could be due to the variations in environmental conditions.

By considering the results of diesel, the residues of diesel on cotton and polyester fabric can be identified after one week of weathering in an indoor environment.

Conclusion

The diesel residues were detected on both fabrics after one week of impregnation. Although the low molecular weight components evaporated with time, high molecular weight components were retained on both fabric samples. The study also indicated that diesel may have higher preservation capacity on polyester fabric than cotton fabric. However, no repeatability studies were carried out and it can be considered as a limitation of this study. The obtained results can be helpful to investigators to interpret the results of the fire investigation.

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Fundamental Study of the Sensitivity and Selectivity of Two Presumptive Tests for Blood

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Introduction

Blood is a connective tissue and a liquid, which can be found within the cardiovascular system in humans and other vertebrates [1]. Like other mammals, human blood that is composed approximately 55% blood plasma and 45% cellular material by volume. Blood plasma is a slightly yellow colour fluid that contains 90% water and 10% dissolved substances like enzymes, hormones, antibodies, blood proteins, waste products, and nutrients. Blood serum is blood plasma without any protein. Red blood cells, white blood cells, and platelets are the major three cellular components of blood [2].

Blood is usually encountered at crime scenes due to the violent nature of criminal events. Most of the crime scenes frequently contain trace amounts of blood due to their small size, may even go unnoticed [3]. Thus, identification of trace quantities of blood found in criminal events may play a crucial role in forensic investigations [4].

Presumptive testing can be conducted to find the evidence of blood at a scene of a crime [5]. Due to their high sensitivity, they can quickly detect even the tiniest amounts of blood. Phenolphthalein also known as Kastle-Meyer (KM), Leuco-malachite green (LMG), Ortho-toluidine, and Tetramethylbenzidine are a few of the chemicals that have been commonly employed as presumptive tests [6]. The underlying mechanism of all these presumptive tests is the haemoglobin's peroxidase activity which changes a colourless molecule into a coloured component [7].

There is frequently insufficient stain in circumstances in which there is suspected of blood to get beyond the screening method. In such cases, the selectivity and sensitivity of the presumptive test used are given more weight [8]. The findings of numerous blood identification methods are reported to have varying sensitivities in the most recent research, which is frequently contradictory. Thus, detecting the reagents' selectivity and sensitivity towards various interfering compounds will be an advantageous for further DNA comparison [9]. Past developments have seen a numerous study on the sensitivity and selectivity of presumptive blood tests. As shown in a study by Grodsky et al. Phenolphthalein and Luminol were found to be more sensitive than the other two reagents, LMG and Benzidine, whenever used often as presumptive reagents on blood [10]. Prior to presumptive testing, Proescher and Moody treated the samples with diluted hydrochloric acid and demonstrated how this could enhance the sensitivity of degraded blood [11]. Cox examined the sensitivity of Ortho-Toluidine,Tetramethylbenzidine,Leuco-Malachite Green, and Phenolphthalein. Out of above four reagents, he saw that Ortho-toluidine and Tetramethylbenzidine were more sensitive [8].

Higaki and Philip used the peroxidase activity of plant extract to evaluate the specificity of presumptive reagents. Using the Benzidine test, they demonstrated how plant peroxidase can induce false positive responses [12]. An evaluation conducted by Ponce and Pascual produced false negative results by adding lemon juice into presumptive test which could lower the acidity [13].

To the best of author's knowledge, no single article that critiques and compares presumptive studies by reagent volume modification rather than dropwise addition. Standard crime scene investigations do not use presumptive procedures in specified amounts. Numerous studies on the selectivity and false positive results in phenolphthalein and leuco-malachite green have produced inconsistent results. The purpose of this study is to optimise the reagent quantities by using absorbance measurement in the two tests stated above and study the successfulness of the method for subsequent forensic presumptive blood testing.

Materials and Methods

Substrates

Defibrinated horse blood was used. White cotton cloths were purchased from the local market.

Preparation of presumptive reagents

The stock solutions for the Kastle Meyers and the Leuco-Malachite green reagents preparation was done as described by Webb et al. [6].

Preparation of blood dilutions and stability testing

A ten-time succession of blood dilution was created, ranging from neat (whole blood) to 1×10^{-7} using both blood and deionized water and they were mixed thoroughly to assure an even concentration of the blood's constituent parts.

A range of 2×10^{-3} to 9×10^{-3} dilution series was prepared from the 1×10^{-3} dilution solution. 2000 µL of each diluted sample from the each of above series was mixed with 200 µL of both KM reagent. Finally, 1000 µL of 3 % H_2O_2 was added and mixed thoroughly. A cuvette was filled with each separately prepared solution and then the initial absorbance was determined at 553 nm by the UV-visible spectrophotometer (WPA-Biowave II, biochrom, USA).

Then the absorbance of the same sample was measured for 5 minutes at each 30 seconds intervals. Same procedure was followed for the LMG reagent at 618 nm.

Optimization of reaction volumes

Kastle-Meyers

From 1×10^{-3} dilution series, 2000 µL each diluted blood sample was mixed with different volumes (200, 500,1000,1500 and 2000 µL) of KM reagents in 10 mL separate volumetric flasks respectively. Then 1000 µL fixed volume of 3% H_2O_2 was added each flask and mixed vigorously. Next, the volume was adjusted to 10 mL with deionized water. Finally, the flask was mixed thoroughly, and a cuvette was filled with the solution and following absorbance was measured at 553 nm. The same procedure was followed for 3,5,7,9 and 10×10^{-3} dilutions respectively. The graph of absorbance versus volume of KM was plotted (Figure 1). From the above graph, most suitable volume of KM was determined.

From 1×10^{-3} dilution series, 2000 µL each diluted blood sample was mixed with 1500 µL fixed volume of KM in a 10 mL volumetric flask. Different volumes (200,500,1000,1500 and 2000 µL) of 3% hydrogen peroxide was added to each flask respectively and mixed thoroughly. The final volume was adjusted to 10 mL with deionized water and the absorbance was measured at 553 nm as mentioned previously. The graph of the absorbance versus volume of 3% H_2O_2 was plotted (Figure 3).

Leuco-malachite green

Similar steps were followed for the LMG, and absorbance was measured at 618 nm (Figure 2). Finally, the graph of the absorbance versus volume of 3% H_2O_2 was plotted (Figure 4).

Testing of sensitivity

Volume of 40 µL sample from neat blood was transferred on to a midpoint of a piece of cotton cloth (3 cm × 3 cm). Then it was tested by adding 30 µL of KM reagent followed by adding 10 µL of 3% hydrogen peroxide. A positive response was noticed by a pinkish colouration at the centre of the cotton stain. Same steps were repeated for each of diluted series. To test LMG, 30 µL of LMG reagent followed by 30 µL of 3% H_2O_2 and a greenish colouration was indicated as the positive reaction for all dilutions.

Testing of selectivity

To examine the reactions with oxidizing and anti -oxidizing agents, 10 µL of 5% hypochlorite solution and 0.1 M ascorbic acid were added respectively to the piece of cotton stains prior to the admission of blood. The stains were then subjected in the way previously done for both KM and LMG respectively. Additionally, the reactivity of both presumptive reagents in the absence of blood with 10% ferrous sulphate was also examined.

Results and Conclusions

Optimization of reaction volumes

The stability test showed that both presumptive coloured complexes are stable for measuring absorbance during the tested time period.

The graph of absorbance versus volume of KM and LMG can be found (Figure 1 and Figure 2) respectively. Both KM and LMG have given a similar trend with fixed volume of H_2O_2 and shown maximum absorbance for all dilutions near 1500 µL for both presumptive volumes.

The graph of absorbance versus volume of H_2O_2 at selected fixed volumes for both KM and LMG (Figure 3 and Figure 4) respectively shown maximum absorbance in their all dilutions.

As KM volume fixed at 1500 µL, maximum absorbance showed at 500 µL of H_2O_2 in the detectable range of the instrument (Figure 3). Thus, the ratio of reactants can be stated as blood:KM:H_2O_2 is 2000:1500:500. Even though highest absorbance has showed from the lowest volume of 200 µL, this point cannot be considered as it exceeds the maximum detectable level of the instrument. This may be a limitation. In that volume ratio, the three components (blood, KM and hydrogen peroxide) react completely and forming most intense pink coloured complex. When the LMG volume fixed, peroxidase reaction entirely occurred at the 1500 µL (Figure 4) in all dilutions showing minimum and maximum absorbance fluctuation. Thus, the ratio of reactants can be stated as blood: LMG:H_2O_2 is 2000:1500:1500. It could be predicted that completion of reactants would occur and produce a blue complex when the absorbance marked highest.

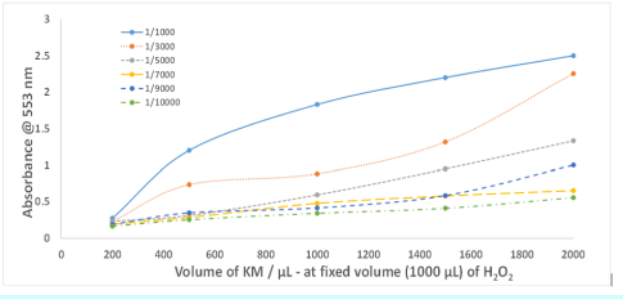

Figure 1: The graph of absorbance at 553 nm versus volume of Kastle-Meyers at fixed volume (1000 µL) of hydrogen peroxide with 2000 µL of different blood dilutions.

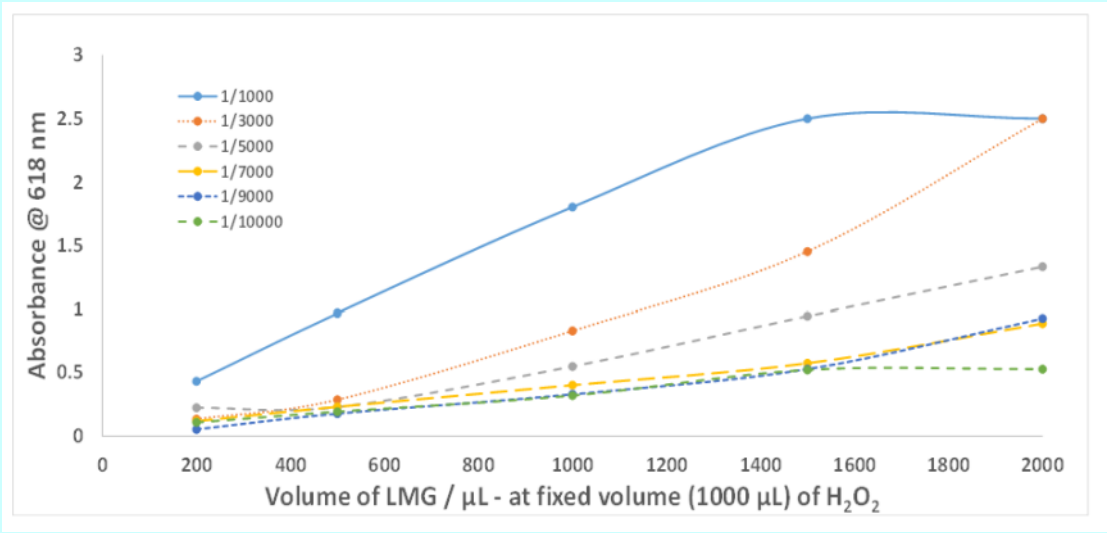

Figure 2: The graph of absorbance at 618 nm versus volume of Leuco-malachite green at fixed volume (1000 µL) of hydrogen peroxide with 2000 µL of different blood dilutions.

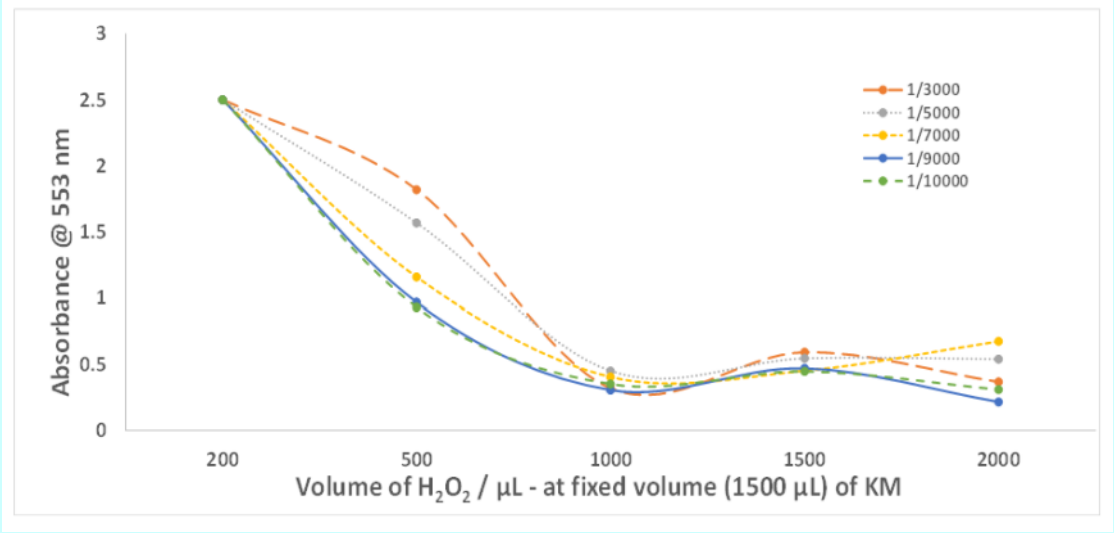

Figure 3: The graph of absorbance at 553 nm versus volume of hydrogen peroxide at fixed volume (1500 µL) of Kastle-Meyers with 2000 µL of different blood dilutions.

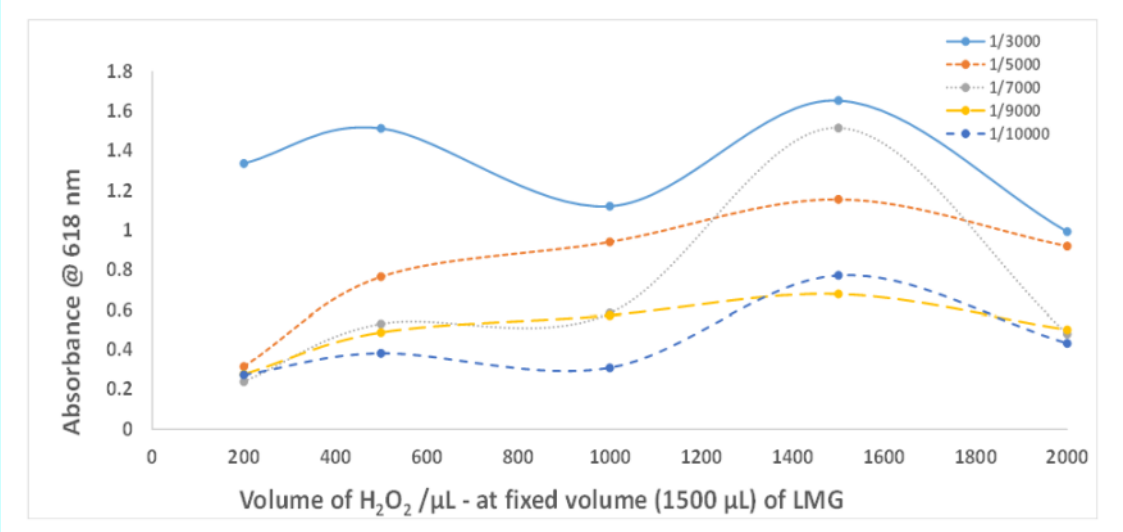

Figure 4: The graph of absorbance at 618 nm versus volume of hydrogen peroxide at fixed volume (1500 µL) of Leuco-malachite green with 2000 µL of different blood dilutions.

Testing of sensitivity

At the maximum attempted dilution factor (1×10^{-7}), KM still exhibited a percentage of 56.3 positive reactions, while that of LMG did not show any colour change (Table 1). A similar trend but different dilution factors have been observed in blood-soaked cloths in a previous study [6]. According to the results, The KM test appears to be marginally more sensitive than the LMG test, this difference is mostly seen at higher dilutions.

Testing of selectivity

KM reagent started to show an inhibition at the dilution of 1×10^{-2}, showing 87.5% positive results. While that of LMG began to show 56.3% positive responses at the dilution of 10^{-3}. The inhibition caused by an ascorbic acid progressively increased in both tests when the blood dilution is increased throughout the studied range. It is important to know, that the inhibition may be a result of anti-oxidizing property of ascorbic acid [14]. The trace blood found in crime scenes such as a kitchen or food processing (fruits and juices) areas are more susceptible to mixed with ascorbic acid and hence there is a possibility to inhibit the presumptive testing.

Dilution factor	Blood		Blood with ascorbic acid		Blood with hypochlorite	
	KM (%)	LMG (%)	KM (%)	LMG (%)	KM (%)	LMG (%)
Neat	100	100	100	100	100	100
1×10^{-1}	100	100	100	100	100	93.75
1×10^{-2}	100	100	87.5	100	87.5	56.25
1×10^{-3}	100	100	68.75	56.25	81.25	43.75
1×10^{-4}	100	100	50	31.25	68.75	37.5
1×10^{-5}	100	56.25	37.5	18.25	56.25	18.75
1×10^{-6}	81.25	31.25	25	12.5	37.5	18.75
1×10^{-7}	56.25	0	18.75	12.5	37.5	12.5

Table 1: Percentage of positive screening results for the Kastle-Meyer (KM) and Leuco-malachite green (LMG) tests by observing immediate colour change, performed on piece of cotton samples stained with defibrinated horse blood produced through successive dilutions, in the presence of 1 M ascorbic acid and 5% hypochlorite solution, respectively.

Due to oxidizing properties of hypochlorite, KM also started to show inhibition at 1×10^{-2} resulting 87.5% positiveness. The KM always showed a higher positive result compared to LMG in all the dilutions from neat to 1×10^{-7}. These results agree with previous study done by Vennemann et al. [15] by using filter papers. As no positive responses for the untreated diluted (1×10^{-7}) blood with LMG, it still showed 12.5% positive results in the presence of hypochlorite could be explained because of bleached interacting with LMG and oxidising it to produce the green coloured complex. Hypochlorite is an ingredient of many cleaning materials and some laundry detergents [16]. The impacts of hypochlorite must be considered to assess presumptive examinations. For instance, specimens collected from stains on white cloths or toilet and bathroom floors, as these things perhaps may have been allowed to treat with hypochlorite.

The KM tests revealed 87.5% false positive results is when 10% ferrous sulphate was applied without blood, whereas the LMG study showed 68.75% false positive results. It was noticed that before hydrogen peroxide is introduced, the colour was brownish orange. It is important not to misunderstand this phenomenon as the anticipated reaction. It was evident that the pink and blue colours of KM and LMG, respectively might be seen shortly after hydrogen peroxide is introduced.

Conclusions and future work

This study has suggested two key factors for both KM and LMG, which are conducted to be more sensitive. It has been demonstrated that mixing diluted blood, KM and hydrogen peroxide in a 4:3:1 ratio result in the highest absorption, while that of blood, LMG and hydrogen peroxide in a 4:3:3 respectively. Compared to the Leuco-malachite green test, the Kastle-Meyer test for blood appears to be a little more sensitive and less susceptible to false positive reactions driven on by the strong oxidizing hypochlorite. It is essential to always compare colour change with the control sample (reagent blank), because that misinterpreting colour is a frequent cause of human error.

In future research it is recommended to use more replicates so that conclusions can be drawn with greater accuracy. It will also be important to investigate the dissolving forensic blood stains in solution and estimate the absorbance.

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Technical Article

Use of DNA Barcoding to Control the Illegal Hunting in Mongolia

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Abstract

Recent studies have revealed a concerning decline in the variety of wildlife species in Mongolia. This trend is alarming and requires immediate attention from conservationists and policymakers. In 2020, the Ecological Police Unit was founded with the primary mission of protecting wildlife and investigating wildlife crimes. The National Forensic Agency of Mongolia received 7 animal flesh samples that were suspected to be Felidae family species from the Ecological Police Unit. We utilized DNA barcoding with COI gene primers to determine the source of samples. The sequences obtained from the samples were found to match (99-100%) with Eurasian lynx (*Lynx lynx*).

Introduction

Conservation of wildlife in Mongolia is facing serious challenges due to its different ecosystems and sparsely populated areas. Mongolia's unique ecosystems, ranging from the Gobi Desert to the Altai Mountains, provide a habitat for a wide variety of wildlife. However, the sparse population in these areas makes it difficult to monitor and protect wildlife effectively. This lack of oversight has led to an increase in illegal hunting, as poachers take advantage of the remote and unguarded nature of the land.

The Eurasian lynx is currently regionally assessed as Least Concern under the International Union for Conservation of Nature (IUCN) Red List. However, there is very little data available on population trends for this species in Mongolia and it is protected as Rare under the 2001 revision (Mongolian Government Act No. 264) of the 2000 Mongolian Law on Fauna (Badam and Ariunzul, 2005). Without precise information on the size, distribution, and trends of the population, it becomes difficult to implement effective conservation strategies to protect this species.

To combat illegal hunting, the National Forensic Agency of Mongolia utilizes DNA barcoding for species identification. This approach aims to improve the accuracy of identifying wildlife species involved in illegal hunting activities, ultimately aiding in the enforcement of wildlife protection laws and the preservation of biodiversity in the region.. DNA barcoding is a powerful tool that enables the identification of species based on their unique genetic markers (Hebert et al, 2003). Through the analysis of the DNA samples collected from confiscated wildlife products or crime scenes, researchers can accurately determine the species of origin. This information can then be utilized to prosecute individuals involved in illegal hunting and trafficking of protected species.

Materials and Methods

Tissue samples were collected in 7 different locations in Mongolia. Genomic DNA was extracted from samples by phenol-chloroform extraction following published protocols (Joseph Sambrook and David W Russell, 2006). The DNA barcode region (~700bp) of the COI gene was amplified by using the following universal primers, 5'-GGTCAACAAATCATAAAGATATTGG-3' and 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Hebert et al, 2003). The 25 µL PCR reaction comprised 2.5µL of 10X PCR buffer, 3µL of 25 MgCl2, 0.2mM each dNTP, 1.25µL of 10µM each primer, 2.5 U of Taq polymerase, 100ng of DNA template, and PCR water. Amplification was performed following PCR cycling protocol (pre-denaturation at 95°C for 5 minutes, 35 cycles of 95 °C for 1 min, 55°C for 1 min, and 72°C for 2min, post extension at 72°C for 5min). The amplicons were sequenced by the Sangers Method (Sanger et al., 1977) by an ABI 3500 genetic analyzer. The obtained sequences were aligned using NCBI's BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and searches conducted in both the NCBI GenBank database and the BOLD system database.

Result and Discussion

The DNA barcoding analysis of the ~700 bp COI gene region from the unidentified tissue samples collected across Mongolia revealed a striking 99-100% match with Eurasian lynx (Lynx lynx) in both the NCBI GenBank database and the BOLD Systems database (Table 1). This cross-referencing approach with established databases strengthens the accuracy and reliability of species identification. The obtained sequences have been uploaded to GenBank for future reference (Accession numbers: KU527900, KU527902, KX882035, OL589565, ON715825, ON715826, ON715827).

Sample name	DNA accession number	COI sequence length	NCBI Blast result /%/	BOLD system result /%/	Species identified
MGL-09	KU527900	654 bp	100	100	Lynx lynx
MGL-14	KU527902	619	100	100	Lynx lynx
MGL-28	KX882035	460	99.4	100	Lynx lynx
MGL-149	OL589565	663	99.5	100	Lynx lynx
MGL-390	ON715825	630	99.05	99.2	Lynx lynx
MGL-389	ON715826	657	100	100	Lynx lynx
MGL-	ON715827	690	99.27	100	Lynx lynx

Table 1: NCBI and BOLD system BLAST search results.

Despite the Eurasian lynx holding a "Least Concern" status globally according to the IUCN Red List, it faces specific threats in Mongolia. Illegal hunting and a lack of population data pose significant challenges to its conservation. To address these issues, efforts are underway to raise public awareness about the importance of lynx preservation and implement effective conservation measures.

The scientific analysis of these samples is crucial in determining the species of the animals and uncovering any potential illegal activities. The National Forensic Agency of Mongolia plays a pivotal role in investigating such cases and by providing robust scientific evidence of the species involved, the forensic agency strengthens the case against poachers. This evidence can help ensure harsher penalties and deter future poaching attempts, ultimately aiming to protect the endangered species and wildlife populations in Mongolia.

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A Preliminary Study on Comparison of Bone DNA Extraction Yields using Four Commercial Kits

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Introduction

Forensic cases often deal with bodies found in advanced stages of decomposition. In such cases, soft tissue and bodily fluids, which are the most common sources of DNA, are likely to be heavily compromised due to environmental factors as well as intrinsic factors associated with the process of decay. Bones and teeth, which are collectively known as hard tissues, are most likely to remain even in the latter stages of decomposition due to their mineral composition and robust structure. These characteristics also allow for higher chances of survival and better preservation of DNA over extended periods of time [1,2]. However, this also means that extraction of the remaining DNA becomes more challenging and requires more advanced methodologies [3–5]. Therefore, efficient DNA extraction methods of hard tissues become essential, particularly with investigations concerning longer post-mortem intervals.

DNA extraction can be considered as the most important step in the process of DNA identification as the quality and quantity of the DNA yield in this initial step greatly influences the success of the subsequent processes in the DNA workflow. Thus, the goal of positive identification through DNA profiling is only possible through the efficient extraction of DNA [6]

There have been countless methods applied to bone DNA extraction—with many of them having further modifications to optimize DNA yield. What seems to be apparent thus far is that bone DNA extraction methods do not seem to be a 'one-size-fits-all' process given the many factors influencing the preservation of DNA in tissue [7,8] Despite this, several companies have introduced DNA extraction kits that simplify the process and posit for higher DNA extraction success. In fact, continuous development of these kits has put forth products that are specific to certain evidence types; one of these evidence types is hard tissue or more specifically, bone.

Given the various methods proposed in dealing with hard tissue samples, there is a need to determine which approach yields optimal DNA quality and quantity. Many forensic cases work with limited amounts of sample; thus, selection of a highly efficient DNA extraction method continues to be crucial in performing successful DNA identification.

This research aims to assess the utilization of four commercially available DNA extraction kits and their success in yielding viable DNA from bone samples for potential use in subsequent steps of the DNA workflow such as polymerase chain reaction (PCR), short tandem repeat (STR) profiling, and even next-generation Sequencing (NGS). The results of this research will reveal the best-performing kit among the selected four, which can ideally serve as a guide when selecting commercial extraction kits for bone samples in future research studies, forensic casework, and other applications.

Materials and Methods

Sample preparation

Cow rib bones were collected from a dish locally known as '*galbi-tang,*' which is often made by blanching the ribs to remove blood, and then boiling them to release flavor and tenderize the meat. After collection, the bones were wiped and air-dried before being taken to the laboratory. The bone surface was cleaned by grinding using a Black+Decker® multi-purpose rotary tool with a grinder bit. The same tool attached with a cutting disc was then used to separate the anterior and posterior portions of the rib bones to access the cancellous bone for further cleaning. Samples were made using only cortical (compact) bone.

Two sets of samples were prepared for the experiments: bone powder and sliced bone samples. Inside a makeshift fume hood (Figure 1), the previously cleaned cortical bones were grinded with the rotary tool and powder collected at the bottom were transferred to 1.5ml or 2ml sample tubes, depending on the kit protocol. Sliced bone samples, on the other hand, were prepared by submerging a different piece of the cleaned cortical bones in a 0.5M ethylene-diamine-tetraacetic acid (EDTA) solution. After 2 days, the outer surface of the bone was scraped and then sliced into tiny pieces using a sterilized scalpel. These bone slices were transferred to sample tubes.

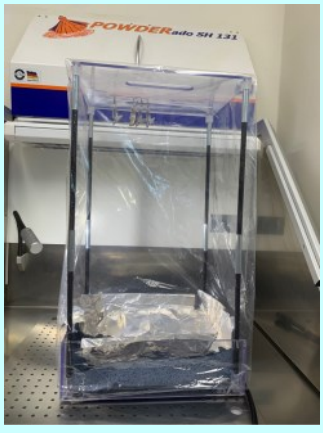

Figure 1: Makeshift fume hood used for surface cleaning, cutting and grinding of bones. It consists of an acrylic frame with a plastic sheet placed over it to limit airflow. Foil is also at the bottom of the frame to catch debris. The plastic sheet and foil are replaced with each use.

The four commercial DNA extraction kits used were the ARA MagNA Tissue DNA Isolation Kit (LAS Inc.), QIAmp® DNA Mini Kit (QIAGEN), PrepFiler™ BTA Forensic DNA Extraction Kit (ThermoFisher), and Bone DNA Extraction Kit (Promega). Maximum sample input quantity for each kit were 20mg, 25mg, 50mg, and 100mg, respectively.

A total of 32 samples were prepared with 16 bone powder samples and 16 sliced samples. Each kit was used to extract DNA from 20mg, 25mg, 50mg, and 100mg samples of both sample preparation types (i.e. bone powder and sliced bone).

DNA Extraction

As not all kits were developed specifically for bone or hard tissue samples, slight modifications were made to some extraction protocols, specifically the ARA MagNA Tissue DNA Isolation Kit and QIAmp® DNA Mini Kit. A full demineralization protocol suggested by Zupanič Pajnič, et al. [9], which included incubation in EDTA at 37°C, mixing at 950rpm overnight via a Thermomixer prior to the extraction steps indicated in the kit protocols was followed for the powder samples extracted using these two kits. Additionally, incubation at 56°C after addition of the lysis buffers also utilized the Thermomixer for constant mixing to promote more thorough lysis. All sliced samples of the four kits, as well as powder samples of the PrepFiler™ BTA and Promega Bone DNA extraction kits, were extracted following their respective protocols. In some cases, incubation at 56°C was done overnight or longer to allow the samples to lyse completely despite protocols indicating shorter incubation periods as well. When samples were still incompletely lysed after this longer incubation, the sample was centrifuged, and the supernatant was transferred to a new tube which then served as the sample. This was particularly true for higher input QIAGEN samples since the QIAGEN protocol calls for complete lysis but did not include a step to separate the liquid lysate or supernatant.

Quantification

To assess the success of bone DNA extractions using the commercial kits and to compare their results, the extracted DNA samples were quantified using three methods: NanoDrop™ spectrophotometer (ThermoFisher), Quantus™ Fluorometer with the QuantiFluor® ONE dsDNA System (Promega), and real-time polymerase chain reaction (qPCR) using the CFX Opus 96 PCR System (Bio-Rad). The primer and probe sequences, as well as qPCR amplification protocol used to quantify the samples follow the results of a study in the development of a Human-Animal Quantification Kit (HAQ kit) within the laboratory [10]. The ß-actin (ACTB) gene was selected as the target and the SensiFAST™ Probe No -Rox Kit was used for amplification. Primer and probe sequences and amplification protocol can be seen in Tables 1 and 2.

Target	GenBank accession no.*	Expected product size (bp)	Sequence position*	Primer / probe	Sequence 5'-3'
ACTB	NC_000071.7	168	2169 – 2190	Forward	ACT ACC TCA TGA AGA TCC TCA C
			2319 – 2336	Reverse	GCA GCT CGT AGC TCT TCT
			2270 – 2292	Probe	6FAM-CTG GAC TTC GAG CAG GAG ATG GC-BHQ1

*mouse (Mus musculus) as standard

Table 1: Primer and probe sequences used in qPCR designed by Bae (2021).

Step	Temperature (°C)	Time	Cycle
Initial denaturation	95	3 m	1x
Denaturation	95	10 s	40x
Annealing & Extension	62	45 s	
Hold	12	5 m	1x

Table 2: Optimised amplification protocol used in qPCR designed by Bae (2021).

Results & Discussion

Four commercially available DNA extraction kits were utilized to obtain DNA from powdered and sliced bone samples at 4 sample input quantities (20mg, 25mg, 50mg, 100mg). In determining the best-performing commercial kit for bone samples, optimal sample input quantities for each kit also need to be identified. According to a literature review conducted by Finaughty, et al., sample input weight and DNA yield were found to have strong correlations in the extraction of human hard tissue DNA [8]. However, kit protocols indicate maximum sample input quantities assumed to be optimized specifically for the protocol. As such, the expectation was that kits calling for lower sample input quantities (i.e. ARA MagNA and QIAGEN) might yield better DNA yields at lower inputs – which was not necessarily the case.

Results from the three quantification methods can be seen in Figures 2 to 4: NanoDrop™ (Figure 2), Quantus™ Fluorometer (Figure 3), and Bio-Rad qPCR (Figure 4). Figures have shown that the DNA yield had positive correlations with the sample input quantity in most cases; and this is especially true for the Promega Bone DNA Extraction kit. Across all three quantification methods, the Promega Bone DNA Extraction Kit had increased DNA yield with higher sample input quantity.

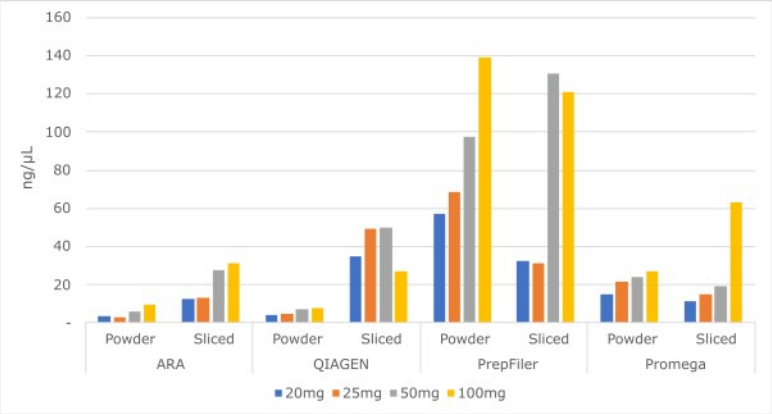

Figure 2:
DNA yields measured using NanoDrop™ spectrophotometer for all samples. Each color represents each sample input quantity and results are categorised by commercial kit and sample preparation method. The PrepFiler™ BTA Forensic DNA Extraction kit obtained the highest DNA concentration values of over 120ng/uL.

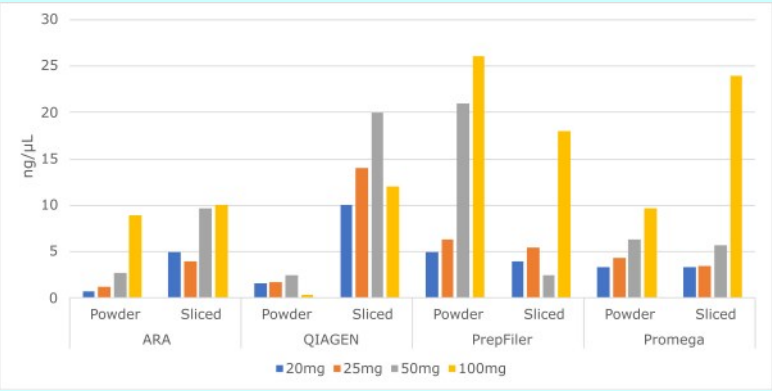

Figure 3:
DNA yields measured using Quantus™ Fluorometer with the Quantifluor® ONE dsDNA System. Results from each kit are shown at each kit are shown at each sample input quantity. The PrepFiler™ BTA Forensic DNA Extraction kit used on a 100mg bone powder sample yielded the most DNA at 26ng/uL.

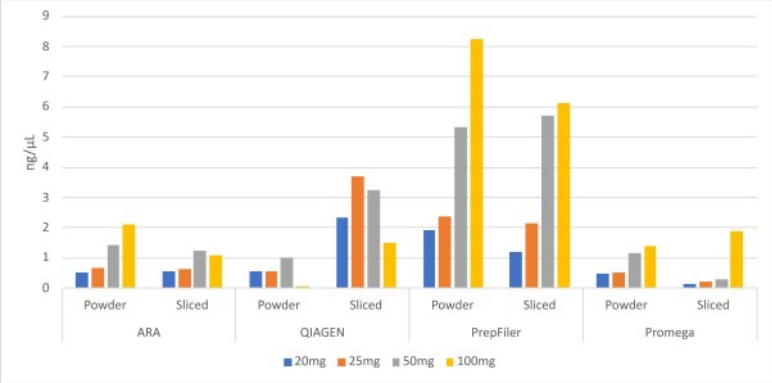

Figure 4:
Quantification values obtained through real-time PCR (Bio-Rad CFX Opus 96 PCR System). The bars represent DNA concentration values in ng/uL units. The highest values were obtained from the PrepFiler™ BTA Forensic DNA Extraction kit using 50mg and 100mg sample input quantities for both powder and sliced samples.

Alternatively, the QIAGEN QIAmp® DNA Mini Kit which indicates a 25mg sample input in its protocol, was found to have increased DNA yield only up to an input quantity of 50mg; at 100mg of sample input, DNA yield dropped substantially in all QIAGEN samples as observed across all three quantifications. The QIAmp® DNA Mini Kit utilizes a lysis mix of 1.0M dithiothreitol (DTT), Proteinase K, and buffer ATL at a total volume of 220µL. In initial trials of the extraction, this volume was not able to fully saturate the 100mg sample and was ultimately discarded. This incomplete saturation could be the reason for the significant drop in DNA yield. Although the addition of the full demineralization step using EDTA was eventually applied, DNA yield remained low at this input quantity.

The advantage of using powdered versus sliced bone samples does not have obvious patterns given the differing results from the quantification methods. Out of the four commercial kits, only QIAGEN had noticeably higher yields using the sliced samples as compared to the powder samples in all three quantifications. The PrepFiler™ BTA kit on the other hand, consistently had higher yields for powder samples which can be observed especially in the higher sample input quantities (i.e. 50mg and 100mg); but this difference is minimal for the lower sample input quantities.

Despite being a bone-specific kit, the Promega Bone DNA Extraction kit did not perform as well as expected. In several of the samples, it yielded comparable results to the ARA MagNA and QIAGEN samples which are not specific to hard tissue. However, the Promega Bone DNA Extraction kit did yield visibly higher results when using 100mg of sliced bone as opposed to the bone powder samples and other input quantities of the sliced samples. Thus, a 100mg sample input as indicated in the kit's protocol is best followed when using the Promega Bone DNA Extraction kit.

Looking at the values from all three quantification methods, NanoDrop™ was observed to overestimate DNA concentration values considerably as brought into light by earlier studies [11]. As Sedlackova, et al. [12] suggested, this overestimation could be due to the amount of DNA fragmentation in the samples, as well as the device's low sensitivity, inability to discriminate DNA from RNA, and other issues. Despite these, the simplicity in the NanoDrop™ quantification process allows it to remain as a common method in practice.

Conclusion

This study has further confirmed that higher sample input also generally has a positive correlation with DNA quantities. In fact, the ARA MagNA kit provided increased DNA yields at higher sample input quantities beyond the parameters specified by its protocol. In the case of the Promega Bone DNA kit, the highest yields were obtained from 100mg sliced samples than from any other sample quantity when extracted using the same kit. Overall, the PrepFiler™ BTA Forensic DNA Extraction kit yielded the most DNA especially for 50mg and 100mg bone powder samples. For sliced samples, the QIAGEN QIAmp® DNA Mini Kit also provided higher quantity DNA in comparison to the other three kits.

Scope and Limitations

Due to limitations in the amount of sample and time, samples could not be prepared in duplicates or triplicates for verification. As of writing, experiments are ongoing to further verify the results of this experiment. Additionally, bone samples used in this study were sourced after they had undergone long-term boiling for consumption purposes; as such, bone collected from different environments or circumstances may yield different results. Further research is needed to assess utilization of these kits with other types of bone samples.

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The Evaluation of Magnetic Bead and Silica Column Technologies in DNA Extraction from Human Bone and Tooth Samples

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Abstract

Bones and teeth are challenging sample sources for human identification due to their complex processing requirements. The aim of this study was to evaluate the effectiveness of two DNA extraction methods: magnetic bead and silica-based column technologies. These methods were applied to bone and tooth samples to obtain DNA profiles suitable for forensic analysis. Simultaneously, 10 bone samples and 10 tooth samples were processed using the Bone DNA Extraction kit and QIAamp® DNA Investigator kit. The evaluation criteria included extracted DNA yield, the number of established a-STR loci, successful amplification of the mitochondrial D-Loop region, and the time required for each method. The results revealed a statistically significant difference ($p<0.01$) in DNA recovery and a-STR profiling between the two technologies. However, no statistically significant difference ($p>0.1$) was observed in the amplification of the D-Loop region. Regarding the time range, magnetic bead technology demonstrated a shorter incubation period, thereby reducing the overall extraction time compared to silica column technology. Overall, this study showed that the magnetic bead technology is a reliable and robust method for DNA extraction from bone and tooth samples.

Keywords: forensic science, DNA extraction, bone, teeth, magnetic bead, silica column

Introduction

In certain criminal cases, reference samples often consist of teeth or bones, which may be exposed to environmental conditions. Additionally, the aftermath of war has resulted in an increased demand for skeletal identification. Human teeth and bone samples have a high resistance to postmortem changes, making them the preferred source of DNA in such situations. However, approximately 70% of human teeth and bones are composed of inorganic material, which poses a physical barrier for DNA extraction reagents [1]. Furthermore, most of the forensic bone and tooth samples are often highly degraded, poor quality, and contain inhibitory substances, which make DNA analysis more difficult [2]. Therefore, bones and teeth are considered highly challenging DNA sources in forensic science and human skeletal identification, requiring multiple processing steps and significant effort.

Optimizing the DNA extraction process holds the greatest potential for improving the success rate of DNA profiling from human remains. In the market, several chemical companies have developed commercial kits specifically designed for extracting DNA from bones and teeth. Numerous studies have also compared methods for extracting DNA from these challenging sample types [3-7]. However, to select an effective extraction method suitable for the facilities and resources available at the Forensic Medicine Center of Ho Chi Minh City, this study was undertaken to evaluate two DNA extraction approaches - magnetic bead and silica-based column technologies - when applied to human bone and tooth samples for forensic DNA profiling and identification purposes.

Materials and Methods

Materials

. A total of 20 samples (10 femur bone samples and 10 tooth samples) were collected from unidentified human skeletal remains for personal identification in the Forensic Medicine Center of Ho Chi Minh City. The individuals were mostly adults (18-50 years old). There was no exact information available regarding the age, gender of the victims, as well as the burial time and the degree of sample decomposition. The samples were found in conditions where they were buried directly in moist soil, with most of the samples being fragile (Figure 1).

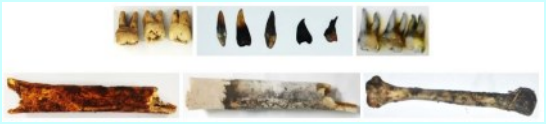

Figure 1: Bone and tooth samples with different degradation levels.

Methods

Sample treatment: The most solid and rigid part or structure of the bones and teeth was identified. The selected samples were cleaned with a 5% NaClO solution (Sigma, Germany) and then rinsed with distilled water to remove NaClO. The samples were left to dry, frozen in liquid nitrogen for about 3 minutes and ground into a fine powder using a mixing mill machine (Retsch MM400, Germany).

DNA extraction: 100 mg of bone/tooth powder was subjected to DNA extraction using two different technologies: silica column, using the QIAamp DNA Investigator kit (Qiagen, Germany) and magnetic bead, using the Bone DNA Extraction kit (Promega, USA). Both procedures were carried out following the manufacturer's instructions.

DNA quantification: The concentration of extracted DNA was quantified by using the Qubit 2.0 fluorometer (Thermo Fisher Scientific, USA) and the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, USA), following the manufacturer's recommended protocols. For each sample, 2 µl of the extracted DNA was mixed with 198 µl of the Qubit working solution before measurement.

Typing a-STR profile: The DNA samples were amplified through PCR using the PowerPlex® 18D System kit (Promega - USA). The amplification was performed on a Veriti™ 96-well thermal cycler (Thermo Fisher Scientific, USA). The reaction set-up and thermal cycling conditions were performed in accordance with the manufacturer's instructions. The PCR products were subjected to capillary electrophoresis on ABI 3500 Genetic Analyzer (Applied Biosystems, USA), following manufacturer's recommendations. Data was collected with Data Collection v1.0 software. Electrophoretic results were analyzed using GeneMapper® ID-X software v1.4 (Applied Biosystems, USA).

Amplification of the D-Loop region: The whole D-loop region was amplified with the primer sets of F15973 (5'-AACTCCACCATTAGCACCCAAAG-3') and R632 (5'-GTGAGCCCGTCTAAACATT-3') (Sigma, Germany). This primer pair amplified the extended D-loop region from nucleotide position 15973 to 632 to generate a band of approximately 1.3kb in size. PCR was performed in 25 µL of a volume containing 6.75 µL of DNA solution (1-5 ng), 2.5 µL of each 5 µM primer, 0.75 µl DMSO 10%, and 12.5 µl Phusion® Hot Start Flex 2X Master Mix (Thermo Fisher Scientific, USA). Thermal cycling was carried out on the Veriti™ 96-Well Thermal Cycler (Thermo Fisher Scientific, USA), beginning with 30 s at 98°C, followed by 30 cycles of 5 s at 98°C, 10 s at 55°C, and 30 s at 72°C, and the final extension performed at 72°C for 5 minutes. All PCR products were analyzed by electrophoresis on a 1.5% agarose gel and visualized by GelRed™ Nucleic Acid Stain (Thermo Fisher Scientific, USA) staining under a UV transilluminator (Wealtec, USA).

Statistical analysis: Median, Wilcoxon Signed Rank test and Fisher test were performed with STATA 16 software. Three parameters were analyzed: extracted DNA yield, the number of established a-STR loci and successful amplification of the mitochondrial D-Loop region.

Results

The DNA concentration obtained from the two extraction methods.

The DNA concentration recovered from 20 samples using both extraction technologies was illustrated in Table 1. There was a statistically significant difference ($p<0.01$) in DNA recovery between the two extraction methods. In particular, the median DNA concentration obtained from samples using the Bone DNA Extraction kit (521 ng/ml) was higher compared to the QIAamp DNA Investigator kit (96.5 ng/ml).

Sample code	Sample type	QIAamp DNA Investigator kit				Bone DNA Extraction kit			
		DNA (ng/ml)	18D loci*	Full a-STR profile	D-loop amplification	DNA (ng/ml)	18D loci*	Full a-STR profile	D-loop amplification
3.274-T3	tooth	380	1	No	Yes	686	17	No	Yes
3.242-T1	tooth	100	15	No	No	2640	18	Yes	Yes
3.243-T1	tooth	107	2	No	No	397	5	No	Yes
3.244-T1	tooth	104	8	No	No	1857	18	Yes	Yes
3.279-T2	tooth	92	11	No	Yes	564	18	Yes	Yes
3.096-T3	tooth	71	0	No	Yes	105	18	Yes	Yes
3.125-T1	tooth	370	16	No	No	1650	18	Yes	Yes
3.068-T1	tooth	81	0	No	Yes	277	0	No	Yes
2.042A-T2	tooth	39	0	No	No	62	1	No	No
3.037-T1	tooth	30	0	No	No	224	0	No	No
3.274-B4	bone	299	6	No	Yes	3500	18	Yes	Yes
3.244-B2	bone	138	17	No	Yes	3131	18	Yes	Yes
3.250-B1	bone	59	18	No	Yes	372	18	Yes	Yes
S034-B1	bone	51	0	No	Yes	1555	18	Yes	Yes
3.243-B2	bone	93	0	No	Yes	275	15	No	Yes
3.254-B	bone	35	5	No	Yes	112	7	No	Yes
2.042A-B3	bone	107	0	No	No	1777	0	No	Yes
3.342-B	bone	237	0	No	No	2428	2	No	No
3.341-B	bone	50	0	No	Yes	171	0	No	Yes
3.052-B1	bone	339	0	No	No	478	0	No	No

18D: PowerPlex 18D System kit, *: Number of amplified loci

Table 1: Quantification and amplification results for DNA extracted from 20 tooth and bone powders extracted using QIAamp DNA Investigator kit and Bone DNA Extraction kit.

The ability to establish an a-STR profile

The number of amplified a-STR loci obtained from each of the 20 samples using both extraction techniques was illustrated in Table 1. There was a statistically significant difference ($p<0.01$) in the completeness level of a-STR profiles between the two DNA extraction technologies. Among the 20 bone/tooth samples, the number of amplified loci from samples extracted using the Bone DNA Extraction kit was either equal to or higher than the number of amplified loci from samples extracted using the QIAamp DNA Investigator kit (Figure 2). Specifically, the magnetic bead technology resulted in 9 complete profiles (18/18 loci), while the silica-based column technology failed to produce any complete genetic profiles. However, there was one sample amplified 18 loci but had missing alleles. Furthermore, the magnetic bead technology successfully amplified ≥ 10/18 loci in 55% of the number of samples, while the silica column technology achieved only 20% (Table 2).

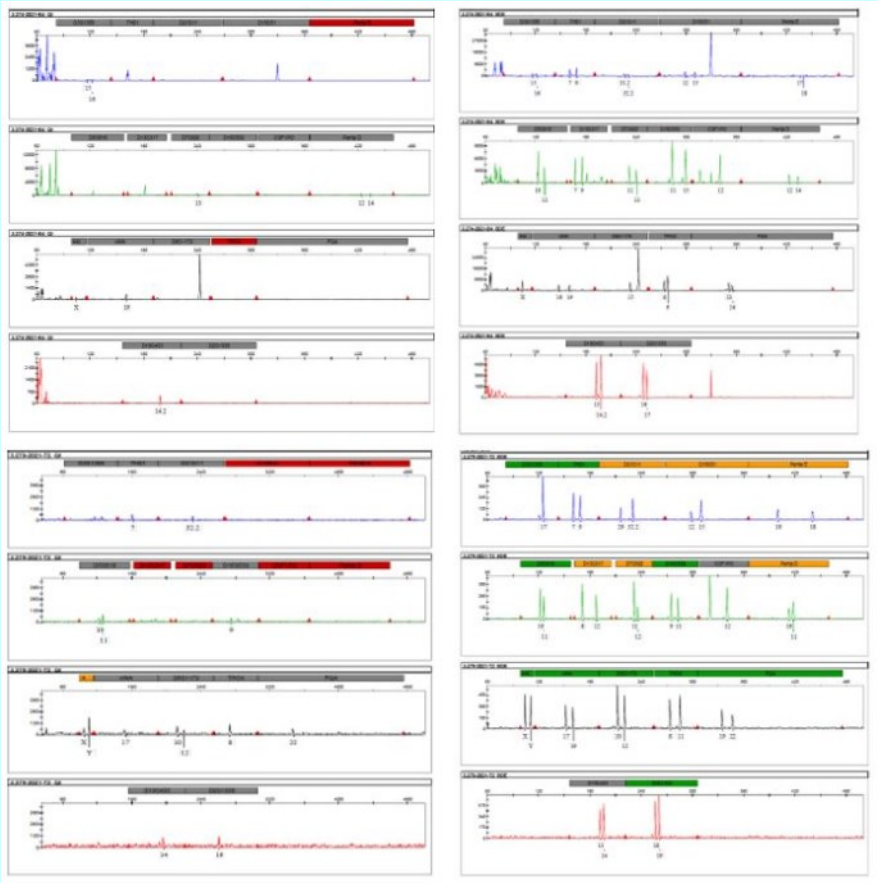

Figure 2:
Comparison between short tandem repeat (STR) profiles obtained by two different DNA extraction kits (QIAamp DNA Investigator kit (left) and Bone DNA Extraction kit (right)) from bone sample. (above) and tooth sample (under).

Assessment standards	QIAamp DNA Investigator	Bone DNA Extraction	P value
Median DNA concentration (ng/ml)	96.50	521.00	**< 0.01**[a]
The successful amplification of the D-loop region	11	16	> 0.1[b]
The completeness level of a-STR profile			**< 0.01**[b]
Full profile	0	9	
Large profile (>= 10 locus)	4	2	
Small profile (< 10 locus)	4	4	
No profile	12	5	

a: Wilcoxon Signed Rank test, b: Fisher test

Table 2:
Summary of DNA profiling results from two extraction methods (n=20).

The ability to amplify the D-Loop region

Table 2 illustrated the number of samples which successfully amplified the D-loop region between the two extraction methods. The Bone DNA Extraction kit showed a higher success rate in amplifying the D-Loop region compared to the QIAamp DNA Investigator kit. However, this difference was not statistically significant (p>0.1). On the other hand, the brightness of most samples extracted using the Bone DNA Extraction kit was higher than those extracted with the QIAamp DNA Investigator kit (Figure 3), which aligned with the obtained DNA concentration described in Table 1. However, there were still some cases where the D-Loop region could not be amplified despite using both extraction technologies.

The time range for the extraction process was limited from the incubation, lysis buffer addition, to the final DNA extraction step. The magnetic bead technology took approximately 4 hours to extract DNA, while the silica column technology required 16 hours to extract DNA from the same sample volume.

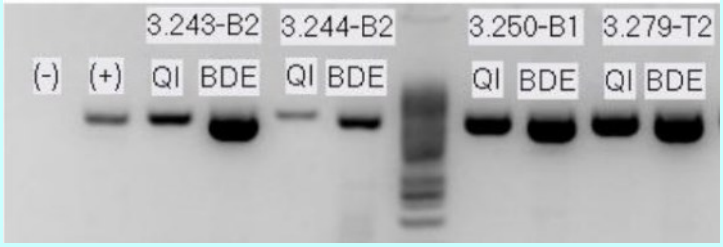

Figure 3:
The agarose gel electrophoresis results of the D-Loop region. (-): negative control, no band, (+): positive control, one band with 1.3kp in size. The main bands equivalent to positive control indicate successful amplification of D-loop region.

Discussion

The recovered DNA concentrations exhibit significant variability across extraction technology, highlighting the considerable differences in DNA concentrations obtained from bone and tooth samples. This may be attributed to the lack of initial assessment about sample quality as well as degradation levels.

Although the DNA amount obtained from bone samples was higher than that of tooth samples (Table 1), when performing a PCR reaction, with the same amount of input DNA, the tooth samples resulted in amplifying more STR alleles and generating the D-loop band with higher brightness than bone samples. This proved that DNA extracted from teeth was higher quality and less prone to contamination than DNA extracted from bones [6, 8-11]. In essence, both bones and teeth are mineralized, but teeth possess a distinct dental structure characterized by higher mineralization. Because of the special composition (teeth are among the hardest parts in the human body) and location within the jawbones, teeth are largely protected from the environmental and physical conditions that act to speed up the process of DNA degradation [12, 13].

Furthermore, employing a smaller sample volume during extraction can reduce the co-extraction of inhibitory substances, thereby enhancing the chances of generating a more informative DNA profile. Both extraction kits utilize a small sample volume (100 mg), which offers the advantage of minimizing inhibitory substances and optimizing reagent usage during sample processing. However, the poor performance of the QIAamp DNA Investigator in extracting DNA from bones and teeth has been observed in studies conducted by Kus et al. (2016) and Harrel et al. (2019) [14, 15]. In 2020, Francisca and Titia compared the efficiency of DNA extraction between an semi-automated process using the Bone DNA Extraction kit on the Maxwell automated extraction system and a manual process including demineralization process combined with the modified QIAamp DNA Investigator kit. When comparing the DNA profiles, all profiles showed more information when extracted using the Maxwell process compared to the demineralization process [16].

Harrel et al. (2019) conducted a study on increasing the number of PCR cycles as a method to improve DNA profiling from bone and tooth samples with limited DNA quantity [15]. This approach provided a suitable solution for future research to assess whether the amplified locus count can be improved by optimizing PCR reaction conditions.

Conclusions

Two different DNA extraction technologies had been compared to select the appropriate DNA extraction kit for bone and tooth samples, ensuring compliance with the rigorous requirements and standards of forensic DNA profiling. Overall, the magnetic bead technology is more effective than silica-based column technology. This finding can help improve the success rate of analyzing human remains.

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Technical Article

Recent Developments and Trends in Trace Evidence Analysis in Sri Lanka

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Introduction

Trace evidence can be viewed as a very small amount of substance left behind at a scene after a criminal activity. Conceptually, it can be defined as a surviving mark, sign, or indication of the former existence, influence, or action of some agent or event [1]. Certainly, trace evidence encompasses a wide range of materials that could be found at crime scenes. However, the evidential value of trace materials often depends on their frequency of occurrence in the local environment. This means that while they can be valuable in providing source associations based on their pertinent class characteristics, they may not always lead to definitive identifications. In premeditated crimes, offenders often adapt to evade conventional forensic identifiers such as fingerprints and DNA; In other cases, these pieces of evidence are sometimes absent or have inconclusive findings. Hence, finding traces of material or marks at the scene can be crucial to shedding light on the crime. Today, with technological breakthroughs, techniques for analyzing and interpreting trace evidence other than DNA evidence extracted from biological sources like blood or saliva continue to improve, enhancing the usefulness of trace evidence in forensic investigations.

The purpose of this article is to emphasize the importance of trace evidence in the field of forensic science and how selected trace evidence can be analyzed to maximize its value to the investigation of crimes in Sri Lanka. The Government Analyst's Department of Sri Lanka (GAD) is the sole laboratory to analyze forensic trace evidence in Sri Lanka. These evidence are collected by the trained police officers and handed over to the GAD through the court of law. Most of these trace evidence analyses are conducted at the Forensic Miscellaneous Section, a separate laboratory for the analysis of trace evidence at GAD.

Recent Developments in the analysis of trace evidence in Sri Lanka

By emphasizing the importance of all types of evidence and employing thorough procedures for their recognition, handling, collection, and preservation in the scene and the forensic laboratory, forensic examiners can maximize their chances of obtaining crucial information to a crime. Therefore, giving equal attention to all evidence is an integral need in the field of forensic science. Adopting a multi-disciplinary approach allows experts from various fields to collaborate and analyze different types of evidence in a complete way. Smooth and effective resolution of forensic cases involves careful examination of all trace evidence available and the integration of insights from diverse disciplines.

In recent years, GAD has achieved some remarkable milestones in analyzing trace evidence. Those improvements are attained by addressing several key areas of interest, including enhancing training programme for the forensic analysts in GAD, educating crime scene managers and crime scene investigators (CSI) on the value of trace evidence, acquiring sophisticated instruments for trace evidence analysis, and adopting ISO 17025:2017 international standards.

Practically, it is not an easy task to reform all the above aspects at one time. GAD has taken a step-by-step approach to improve the quality standard of trace evidence analysis over the past few years. In this article, selected types of trace evidence frequently encountered in forensic investigations in Sri Lanka and how their analyses have progressed over the years are highlighted.

Vehicle Serial Number Restoration

Vehicles alleged to have tampered serial numbers are frequently submitted to the GAD to investigate their chassis numbers and engine numbers. GAD receives about 350 such cases annually. Stealing vehicles and changing their serial numbers is a commonly encountered crime in Sri Lanka. High vehicle prices may be the main driving force behind this kind of crime.

Presently, the procedures used to analyze vehicle serial numbers are accredited under the ISO 17025:2017 laboratory standard. Adhering to internationally recognized standards like ISO 17025:2017 helps standardize processes and procedures in forensic laboratories, ensuring consistency and reliability in the results produced in trace evidence analysis. The implementation of a quality management system (QMS) within laboratories also emphasizes the proper and transparent processes of document control, personnel training, equipment calibration, maintenance, interlaboratory testing, and proficiency testing [2]. ISO 17025 accreditation is globally recognized and accepted. Forensic laboratories accredited to this standard can demonstrate their competence and credibility not only within their own country but also internationally. This can be particularly important in cases involving cross-border investigations or when benchmarking against forensic agencies in other countries.

Fibers

Fibers from garments, carpets, upholstery, and other textiles can transfer between individuals and objects, providing valuable links for establishing contact between them. Most of the fibers received by GAD are connected to cases such as burglaries and hit-and-run accidents. In recent years, the number of cases related to fiber analysis has increased drastically, and GAD receives about 100 cases of this type annually.

Recently, GAD has taken steps to improve fibre analysis by introducing new equipment such as polarized light microscopes and Fourier Transform Infrared (FTIR) Microscopy as well as improving the existing fibre analysis procedures. These instruments are designed and equipped with specialized parts or modules to visualize and enhance small details of the fibre material for the purpose of identification and comparison. With the use of advanced equipment and enhanced analytical procedures, the analysis of fibre evidence in the laboratory has proven to be effective and efficient.

Soil

Soil is part of the top layer of Earth's crust, where most plants grow. Soil composition can vary widely between locations, and traces of soil found on clothes, footwear, or objects can help link individuals to specific places or environments. The presence of soil unique to a certain area can show that a suspect or victim must have been in the relevant area. [3]

In Sri Lanka, sand minerals are widely utilized as trace evidence in cases such as illegal transportation and mining. To control the environmental damage due to sand mining in areas close to freshwater, seawater, and brackish water bodies, rules and regulations are imposed on transportation and mining. If someone allegedly transports sand without a valid license and claims to be not guilty, samples will be seized from the load and the suspected mining area for comparison. Frequent requests to GAD on such cases include the source of the questioned samples seized from the suspect's load and whether the seized samples and the samples from the suspected mining area could come from the same origin.

With about 100 such cases submitted to the laboratory, GAD has established robust procedures to analyze these samples, including texture analysis, total ion conductivity, chloride ion concentration, and pH determination (alkalinity or acidity).

Analysis of Metal Alloys

The number of cases received by GAD involving metal alloys has increased rapidly in recent years. These samples are analyzed to identify the metals that have been used to prepare these alloys. The majority of them are fake gold submitted for laboratory analysis. It has been noticed that conventional methods are not enough to deal with these samples. Therefore, GAD has recently introduced micro-X-ray Fluorescence Spectroscopy (micro-XRF) in the analysis workflow to analyze these types of samples. The use of micro-XRF in this regard leads to a more specific and confirmed identification of elements compared to conventional methods and helps to differentiate fake gold more confidently.

Education and Training

The education and training are essential components in ensuring that all relevant parties involved in forensic investigations have a thorough understanding of trace evidence. GAD ensures all individuals entering the field of forensic science go through a comprehensive orientation programs. In-service training sessions are also conducted regularly to update their knowledge and skills.

In 2023, GAD conducted a training progrmme for selected police officers who work as Crime Scene Managers and Crime Scene Investigators (CSI) in all the provinces of the island. The Crime Scene Managers oversee the overall management of crime scenes, including coordinating the activities of various parties involved. Possessing good knowledge about trace evidence helps them effectively allocate resources at scenes to ensure proper collection and preservation of all kinds of trace evidence. The CSI personnel are directly involved in collecting evidence at crime scenes. Training them about different types of trace evidence, as well as proper collection and preservation techniques specific to each and every type of evidence, enhances their ability to gather valuable information that could be crucial to solving cases.

Conclusion

Emphasizing the importance of understanding the value of all types of trace evidence and their effective preservation through education and training ensures that investigators and all crime scene personnel are attentive to potentially vital clues. By comprehensively gathering all available evidence at a crime scene, a more complete picture of what has occurred can be reconstructed. In essence, recognizing the value of trace evidence and integrating it into forensic investigations is essential to achieve comprehensive and successful outcomes in criminal investigations.

For forensic agencies such as GAD, adopting internationally recognized standards to enhance quality assurance in the laboratory, investing in the necessary equipment for more effective analysis of forensic samples, and providing training and support to the forensic analysts to enable them to conduct thorough and reliable analyses of trace evidence are crucial for maintaining the quality and integrity of forensic analysis, thus upholding the standards of forensic science.

Ultimately, a holistic and inclusive approach to forensic investigation, which prioritizes the examination of all available trace evidence, leads to more robust and reliable outcomes. By employing thorough methodologies and techniques, forensic scientists can provide credible and compelling evidence that is acceptable to all parties involved in the criminal justice system, including law enforcement, prosecutors, defense attorneys, judges, and juries.

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Case Study

Success Rates of Trace DNA-based STR Profiling in Relation to Murder and Burglary offenses in Sri Lanka

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Abstract

In the criminal justice system, DNA analysis has become a prominent intelligence tool in identifying violent criminals. Touch DNA is a type of trace evidence found at crime scenes. The donor causes the transfer of epithelial skin cells by physical contact with other objects containing trace amounts of DNA on surfaces. Targeting the relevant location is the initial step in gathering touch DNA. Even under forensic illumination sources, touch DNA in garments is usually not visible. Typically, sampling should be limited to the area of interest. Selecting a suitable sampling method is also important. In this study, both cutting out and swabbing methods were applied for the sampling of trace DNA evidence. This study was carried out to determine the success of obtaining STR profiles of different types of touch DNA evidence. In this research, 84 samples of trace and saliva-based evidence materials were compiled from murder and robbery cases received by the Serology and DNA laboratory of the Government Analyst's Department in 2019, 2020, and 2021. The samples were grouped into six different categories: bitten food items, bidi and cigarette butts, garment items, shoes and slippers, handles of tools, and other items (such as boxes of matches, door mats, pairs of gloves and mops). The percentage of success in obtaining positive DNA profiles was 40%, 89%, 40%, 15%, 8 %, and 33 % respectively. Cigarette butts represented the highest percentage of success in obtaining DNA profiles.

Introduction

DNA technology is increasingly recognized as one of the most effective forensic tools for investigating a wide range of offenses in Sri Lanka including theft, homicide, armed robbery, assaults, sex offenses, and various volume crimes. Trace DNA analysis has become a crucial tool in these investigations. The growing number of trace DNA samples requires careful handling and has broadened the scope of DNA collection from different sources such as tools, clothing, knives, vehicles, firearms, food, bedding, condoms, glass, skin, windows, doors, and more. [1] Other than such trace exhibits, bidi and cigarette butts are frequently discovered at diverse crime scenes around Sri Lanka. When analyzing these exhibits, it is important to note that conventional forensically significant biological substances such as blood and semen are not frequently found. Genetic material, including epithelial cells, fragmented cells/nuclei, and cell-free DNA, may be retained on touched surfaces in various forms. [2,3]

Trace evidence refers to small, often microscopic materials left at a crime scene that can provide crucial information in forensic investigations. These traces can originate from various sources, including bodily fluids like saliva, sweat, and blood, as well as fibers, hairs, soil, glass fragments, paint chips, and more. [4] It is important to note that the amount transferred per exhibit item is usually very small, due to the limited volume of body fluid conveying the DNA-bearing cells and the relatively small contact area. In the absence of some preliminary testing, such as the identification of blood or semen, the cellular origin of these DNA-bearing cells can only be speculated upon. When analyzing trace DNA, the cellular origin of cells is not identified as these processes may consume valuable samples. [5]

The Success of trace DNA analysis depends on several critical factors, including careful collection, sampling and preservation techniques as well as removal of contaminations through the extraction. Subsequent steps such as quantification, amplification, and detection, are integral to the overall process. [6] Overtime, various methods have been developed for the effective collection of trace DNA. [7,8] The utilization of sophisticated kits and instruments coupled with well-equipped forensic laboratories, plays a pivotal role in ensuring the generation of concrete DNA profiles. This in turn, significantly contributes to the efficacy of the criminal justice system. [9].

To collect trace samples, the first step is to figure out where to look. However, identifying these samples on surfaces is often challenging. Various methods for DNA collection, such as swabbing and cutting out specific areas are employed for DNA collection. Some laboratories utilize scraping and tape lifting. FTA cards are also used to collect touch DNA. The second preferred method employed in DNA collection is the direct PCR method. This method is advantages because the entire collected sample of touch DNA undergoes amplification without the loss incurred during extraction and purification steps. Several Studies have been conducted to optimize touch DNA profiling success. Different collection methods, handling times and contact times have been compared, along with different swab types and brands. Sometimes, double swabbing is used, where the first swab is moistened with water and a dry swab is used to collect any liquid residues and cells left behind. [10,11].Collection processes are often guided by assumptions regarding the presence of DNA-containing material. Swabbing an area smaller than the actual deposition zone may result in missing some of the sample while swabbing a larger area could spread the sample thinly. Both approaches risk providing an inaccurate understanding of the sample's actual location. Therefore, it's crucial not only to know the exact location of the targeted material but also to collect from that area appropriately.

Case History

The Serology and DNA section of the Government Analyst's Department in Sri Lanka receives various types of trace evidence from the courts related to different criminal offences. As observed in casework, obtaining usable DNA profiles from trace exhibits is not always guaranteed. Several factors influence the recovery rate of complete profiles, including an individual's cell-shedding ability, the surface of the substrates, the time of contact, the delay until analysis, and the environmental conditions.

For this research project, we gathered 84 samples of trace and saliva-based evidence materials from various murder and robbery cases that were reported between 2019 and 2021. The samples were classified into six distinct categories, and the food items were analyzed within one month of receiving them, while the other items were analyzed within six months. On average, the samples were around three months old.

1. Bitten Food Items (05 samples - 03 bite apples, 01 bite Mango, 01 sample of cashew shells)
2. Bidi and Cigarette Butts (27 samples -12 bidi butts and 15 cigarette butts)
3. Garment Items (10 samples – 04 T-shirts, 03 caps, 02 bottoms, a pair of socks)
4. Shoes and Slippers (20 samples – 12 Pair of shoes, 01 pair of slippers)
5. Handles of Tools (13 samples – 08 handles of knives, 03- handles of axes, 02 handles of scissors)
6. Other Items (box of matches, door mats, pair of gloves, mops – 09 samples)

Methods

Sampling was conducted using three techniques based on the nature of the evidence material. Swabbing was performed using sterile cotton swabs while cutting out of the area of interest and the scraping methods were employed as the other sampling techniques. Collected samples were extracted using Qiagen Investigator extraction kit. Following extraction, DNA quantification was performed using the Quantifiler real time quantification kit (Applied Biosystems, USA) according to manufacturer's protocol. Amplification was then conducted using the AmpFlSTR Identifiler Plus PCR amplification kit (Applied Biosystems, USA) accordance with the kit's instructions. This kit covers a panel of fifteen autosomal STR markers and gender identification markers. Subsequent to the amplification process, the resultant PCR products were separated by capillary electrophoresis on a Genetic Analyzer 3500 (Applied Biosystems, Life Technologies, USA) and analyzed with Gene Mapper IDX software version 3.2, from Applied Biosystems using standard procedures.

Results

Table 1 shows the minimum and maximum quantities of extracted DNA for each category of touch DNA evidence according to the RT-PCR results. In the analysis of DNA profiles for all 84 evidence sample types, profiles with 12 alleles or more were obtained for positive results, while profiles with fewer than 12 alleles or no alleles present were considered negative results. The percentage of positive results for all six categories was then calculated accordingly. Figure 1 shows the distribution of positive and negative profiles based on sample types.

Touch DNA Evidence Category	Minimum and Maximum quantities of extracted DNA amount (ng/µl)
Bitten Food Items	0.1025 - 0.0130
Bidi and Cigarette Butts	0.0178 - 4.7429
Garment Items	0.0021 - 0.0702
Shoes and Slippers	0.0019 - 0.1705
Handles of Tools	0.0041 - 0.1321
Other Items	0.0064 - 0.1211

Table 1: Minimum and maximum quantities of extracted DNA.

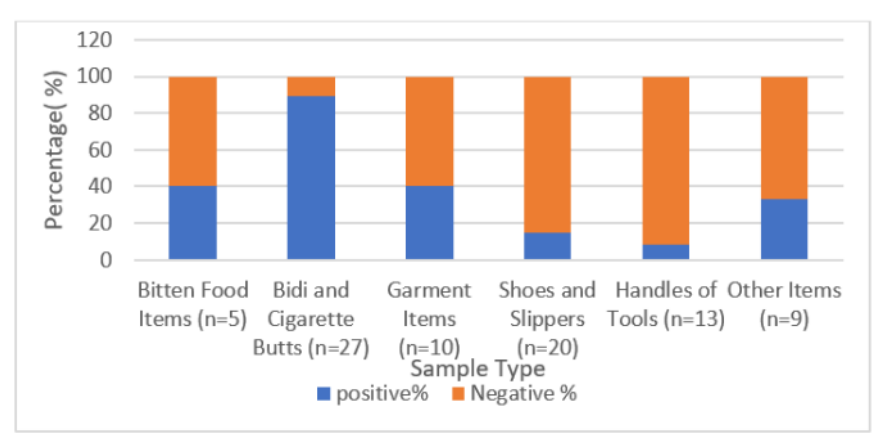

Figure 1: Percentage of DNA profiles obtained by sample type.

The highest percentage of positive results (89 %) was obtained for the bidi and cigarette butts. Out of 27 samples, a full single profile was obtained for 22, and 2 samples produced profiles with more than 12 alleles. A significant difference was observed between bidi and cigarette butts compared to other sample categories. The lowest positive results (8%) were found for the handles of tools, with only one out of 13 samples producing a profile with more than 12 alleles. For both bitten food items and garment items, 40 % of positive results were obtained.

In the case of food items, a full profile was obtained for 1 sample, and a profile with more than 12 alleles was produced from another sample. Regarding garments, full profiles were obtained from only 4 samples. In the evidence category of shoes and slippers, 15 % of positive results were obtained, showing 03 full profiles. For other items, 33 % of positive results were obtained, producing three full profiles. (two for touch DNA from 2 matches and one for saliva from bottleneck).

Allele recoveries of six categories of touch evidence are summarized in Table 2. Figure 2 displays the allele recovery details by the number of samples of each category of touch evidence.

Allele Recoveries	Number of samples					
	Bitten Food Items	Bidi/Cigarette Butts	Garment Items	Shoes and Slippers	Handles of Tools	Other Items
Neg (No allele)	03	01	02	16	06	05
Amelogenin only only	-	01	-	-	-	-
Partial (<12)	-	01	04	01	06	01
Partial (≥12)	01	02	-	-	01	-
Full (15) +Amelogenin	01	22	04	03	-	3

Table 2: Profile details observed for evidence materials of six sample categories.

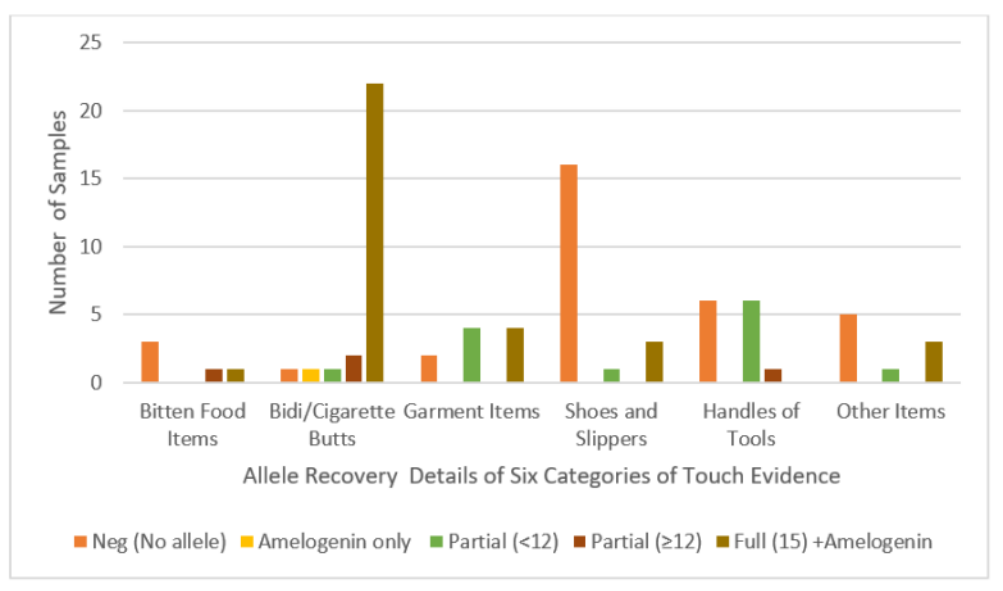

Figure 2: Allele Recovery of Six Categories of Touch Evidence by the Number of Samples.

Figure 3 displays electropherograms of positive (all alleles called) and negative results (partial profile with alleles called less than 12).

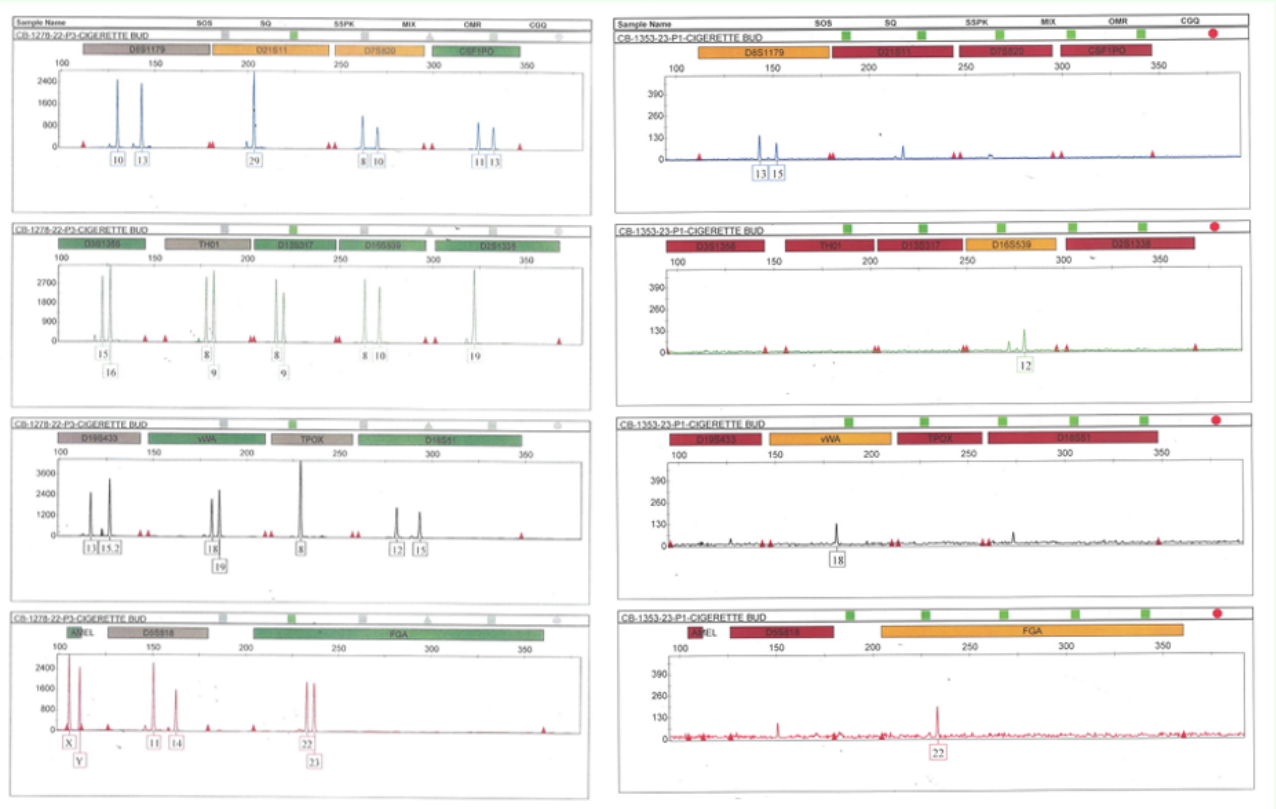

Figure 3: Electropherograms obtained for Samples – Positive (left), Negative (right).

Discussion and Conclusion

In this study, the success rates for obtaining STR profiles from bidi and cigarette butts were notably high at 89 %. However, the overall DNA recovery from other trace DNA categories was comparatively low. It is important to note that bidi/cigarette butts and bitten food evidence primarily rely on saliva DNA, while all other evidence materials in various categories are based on touch DNA.

The study revealed that across all categories of touch DNA evidence materials, the success rates for STR profiling were consistently below 40 %. Notably, garments emerged as the most successful sample type, likely due to their continuous use, facilitating the transfer of skin cells for extended periods. Conversely, the least successful profiles were obtained from the handles of tools, with a success rate of only 8 %. This could be attributed to either the limited surface area being handled or inefficiencies in the swabbing technique employed.

It is crucial to recognize that when collecting touch DNA, the choice of collection methods, such as different swab types, can significantly impact the quantity of DNA retrieved from a crime scene exhibit. Additionally, the type of surface from which samples are collected directly influences the efficiency of specific collection methods. The study highlights the need for further investigation into the influencing factors of touch DNA. Moreover, internal validation is essential to identify the most suitable collection methods for different types of surfaces.

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A Case Study: Which Concurrent Substances that Morphine-Related Deaths Autopsied at Forensic Centre of Ho Chi Minh City use from 2018 to 2020

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Abstract

Objective: The aim of this study is to find out the concurrent substances that morphine-related deaths autopsied at the Forensic Center of Ho Chi Minh City use in the series of 170 cases. **Materials and methods:** A retrospective study from 2018 to 2020 on 170 subjects committing poisoning or using morphine. **Results:** Men are still the main morphine users, mean of age 36.3 + 8.6 years. The use of morphine with other stimulants accounted for 47.6%, of which ethanol accounted for the majority, 68.2% of cases recorded blood concentrations of morphine exceeded the toxic threshold. **Conclusion:** The study shows that ethanol was the most commonly found co-administration with morphine.

Introduction

Drug overdose is the main cause of death. In Vietnam, it is estimated that every year 1,600 addict die from overdose. The majority of drug-related deaths involve the consumption of heroin in combination with other central nervous system depressants such as ethanol and benzodiazepines. This study was conducted to find out the concurrent substances that morphine-related deaths autopsied at the Forensic Center of Ho Chi Minh City use and describe some difficulties we faced.

Materials and Methods

Blood and urine samples from autopsies at Forensic Center of Ho Chi Minh City from 2018 to 2020 concluded to be caused by morphine use or poisoning. Blood and urine samples identified and quantified for morphine, while blood samples were quantified for ethanol concentration. Except for corpses which did not have completed personal records or cadaver cases had morphine concentrations in blood and/or urine below the quantitative threshold.

This retrospective study was taken place from January 1, 2018 to December 31, 2020 at Forensic Center of Ho Chi Minh City. The entire sample must meet the criteria. The obtained data are processed with Microsoft Excel 2019 and SPSS 22.0 software.

Results and Discussion

Between 2018 and 2020, we found a clear gender difference in morphine-related deaths autopsied at Forensic Center of Ho Chi Minh City, men are still the majority and similar to some other studies in other countries, there is a gradual increase in both sexes in morphine-related deaths [1, 2]. The average age of morphine-related deaths in our study was 36.3 + 8.6 years old, showing that drug addicts tend to get younger and younger, similar to Lauren A. Paul's research et al (2022), [3] mainly in working age. Many factors can impact and create favorable conditions for the increasingly popular use of addictive substances such as: the popularity of the internet, smartphones but what is worrying today is the emergence of many new and diverse types of synthetic drugs, sold in forms such as lozenges, stickers, have caused easier opportunities for many people to become addicts starting from the first try.

Substance name	Number of cases (n)	Ratio (%)
Ethanol	69	85.2
Methamphetamine	5	6.2
Codeine, Ethanol	3	3.7
Acetaminophen	2	2.5
Codeine	1	1.2
MDMA	1	1.2
Total	81	100

Table 1: Concurrent substances in morphine-related deaths.

Table 1 list the results of concurrent substances in morphine-related deaths, in 81 cases out of 170, additional substances other than morphine were detected, mainly stimulants and addictive substances. Ethanol accounts for 85.2%, possibly because accessing and using alcohol and beer in Vietnam is relatively easy, not only it is freely traded but also is not deal with heavy taxes or have any restriction for the amount of alcohol and beer consumed per person. There are many research that have establish the formula to be able to estimate the amount of alcohol a person drinks if the concentration, the drinking utility and the time from the last drink to the time of sampling are known. In reality, it is still very difficult to determine [4].

Ethanol can be of endogenous or exogenous origin. Exogenous ethanol is ethanol introduced into the body from outside when a person drinks alcohol. On the contrary, endogenous ethanol can be obtained by (1) fermentation of foods obtained from eating in the body, (2) produced by the body's own metabolites, (3) by metabolism. In the pathology of automatic distillery syndrome, the body produces endogenous ethanol to the extent that it can cause symptoms that a person may experience the same when drinking alcohol. In fact, many studies have recommended using a higher ethanol threshold, for example 5-10 mg/dl to avoid controversy that ethanol comes from food [4]. Here, we used a detection threshold of 10 mg/dl, showing that more than half of the cases had ethanol concentrations less than 10 mg/dl, the remaining group had low ethanol concentrations (10-100 mg/dl) with 28 cases, 44 cases recorded high ethanol concentrations (>100 mg/dl), of which only 1 case had ethanol concentrations above 350 mg/dl. Studies have shown that blood ethanol concentrations above 500 mg/dl cause death, but in our study, the main cause of death did not come from ethanol.

Another question is whether this morphine was used by the subject before or not? We know that morphine is used in medicine for indications such as relieving moderate to severe pain, especially in pain related to cancer, myocardial infarction and surgery. Morphine also helps reduce anxiety and insomnia that can be associated with severe pain that the patient cannot bear. Addictive substances such as heroin and codeine can also be converted into morphine, although there is strict control by the authorities, but clearly distinguishing the type of drug used by the subject is a challenge in forensic casework. Many studies have been carried out, such as testing their metabolites such as 6-monoacetylmorphine (6-MAM), which is the main metabolite of heroin but has a short lifespan, or looking for conjugates of morphine and codeine or using both the ratio of morphine/codeine in blood and codeine/morphine in urine to determine whether the subject is using codeine or morphine [5]. To do this, standard substances are needed which cost much more time and money and still remain a huge challenge for application.

Conclusion

From our research, examining a series of morphine-related deaths at the Forensic Center of Ho Chi Minh City from 2018 to 2020, we draw the following conclusions: Morphine use in men is still the main trend and is common in working age. Ethanol was the most commonly found co-administration with morphine in those cases.

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Case Study

The Profile of Mexican Methamphetamine Packaged in a Decorative Resin Frame Smuggled to Indonesia

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Introduction

In line with the total amount of drug seizures in East and Southeast Asia, methamphetamine seizures in Indonesia showed a slight decrease in 2022 compared to the previous year, from 11.74 tons in 2021 to 8.56 tons in 2022 [1-4]. Methamphetamine seized in Indonesia was dominated by smuggling cases from the Golden Triangle and countries in West Asia, where an approximately 80 percent of all drugs smuggled were trafficked by sea or maritime routes [2]. In December 2023, Center of Drugs Testing Laboratory of BNN received an ice sample linked to a Mexican Transnational Criminal Organization (TCO) for the first time, a new case that was very interesting to profile. In this paper, the unusual physical and chemical characteristics of the sample are profiled.

Case

A total amount of 5.1 kilograms of methamphetamine packed as decorative resin frame with dimension of approximately 49.5 x 64.0 cm (Figure 1a,b) were seized in a joint operation by Directorate of Interdiction of BNN and Soekarno Hatta International Airport's Customs and Excise in December 2023. Based on investigator information, the package was shipped from Guadalajara, Mexico to Indonesia using international air shipping. Two grams of white ice sample (Figure 1c) was submitted to BNN laboratory for analysis and drug profiling purpose.

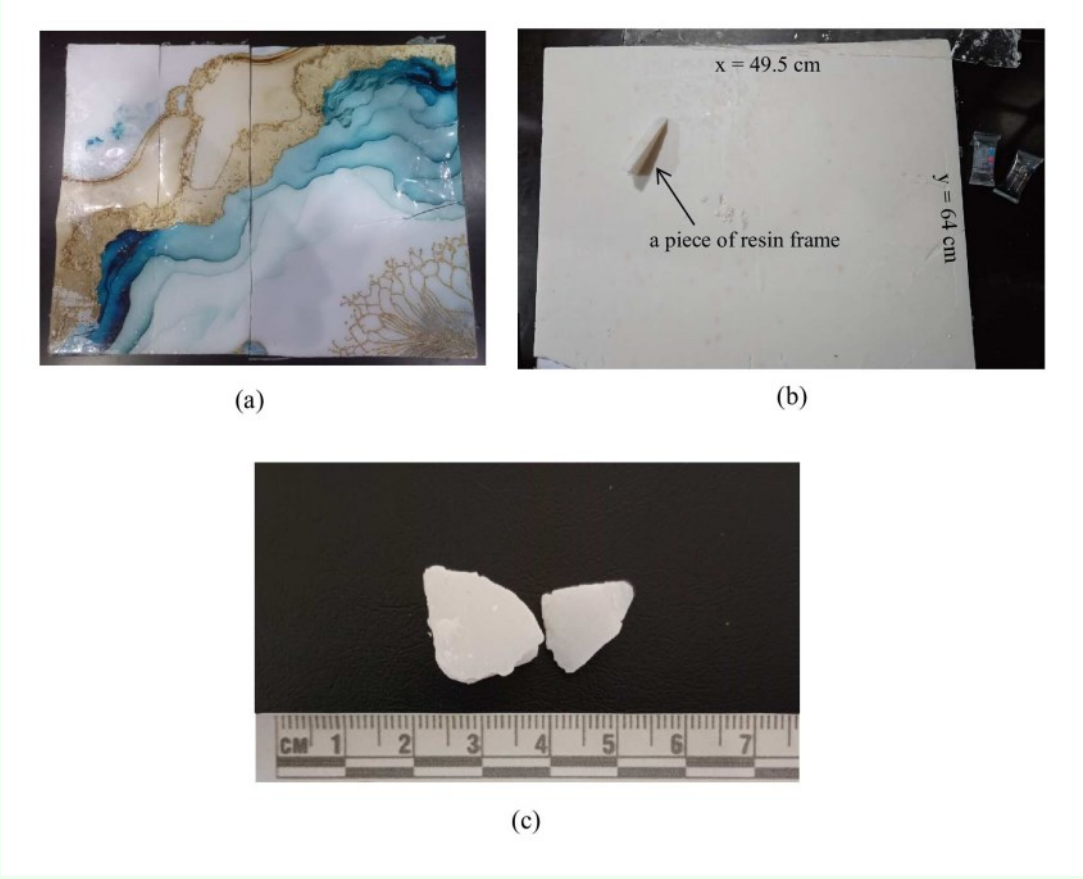


Figure 1: Appearance of (a) front side resin frame; (b) inside resin frame found to contain methamphetamine; (c) methamphetamine sample profiled.

Materials and Methods

a. Reference standards of *d,l*-methamphetamine, *d*-pseudoephedrine and *l*-ephedrine (Lipomed).
b. Water, methanol, ammonia solution 25%, nitric acid 65%, silver nitrate.
c. Nicolet Summit X FT-IR Spectrometer fitted with Everest ATR accessory (Thermo Scientific, USA).
d. Handheld Raman spectrometer Metrohm Instant Raman Analyzer (MIRA).
e. Stuart® SMP10 Melting Point Apparatus.
f. Meiji Techno Microscope HD1500M.
g. Acquity UPLC H-Class (Waters, Singapore) for chiral analysis. 100 mg sample was recrystallized with chloroform and diethyl ether prior to the analysis.
Instrumentation : The chiral column was OSAKA SODA (4.6mm x 150mm, 5µm). KH_2PO_4 20 mM/ Acetonitrile (65/35) was used as mobile phase. Flowrate was 0.7 ml/min.
h. Acquity UPLC H-Class Plus (Waters, Singapore) for trace ephedrine analysis. *l*-ephedrine and *d*-pseudoephedrine standard solution used was 100µg/ml in water.
Instrumentation: The column was CORTECS® C18 (2.1 x 100mm, 2.7µm). KH_2PO_4 50 mM/Acetonitrile (98/2) was used as mobile phase. Flow rate was 0.3 ml/min.
I. GC-MS (EI) Agilent 7890B-5977B MSD for trace impurities analysis. Sample preparation under basic and acidic conditions were adopted from [5], direct extraction was performed by diluting sample in ethyl acetate.
Instrumentation : The column was HP-5MS UI (30m x 0.250mm x 0.25µm). 1.0 µl of each extracted was injected by the splitless mode. The oven temperature was 50°C (1 min) as initial temperature with 10°C/min to 300°C (15 min). Flow rate was 1 ml/min. The injector and ion source temperature were 250°C and 300°C, respectively.
J. Shimadzu GC-2010 FID for purity measurement.
Instrumentation : 1.0 µl of 100 µg/ml samples in methanol were injected to GC-FID by the split mode (50:1), the column was DB-5 (30m x 0.250mm x 0.25µm). The oven temperature was 135°C (2 min) followed by C/min to 230°C with the flow rate of 1.2 ml/min. The injector and detector temperature were 280°C and 300° C, respectively.

Results and Discussion

Physical and chemical characterization were performed to identify the profile of the sample. Visual examination showed a white dense solid, unlike the clear crystalline form of typical methamphetamine seizures. The sample (Figure 1c) was ground to a homogeneous powder prior to further analysis. Once the ice was crushed, it was more brittle and slightly sticky than ice samples typically encountered in our laboratory (Figure 2a).

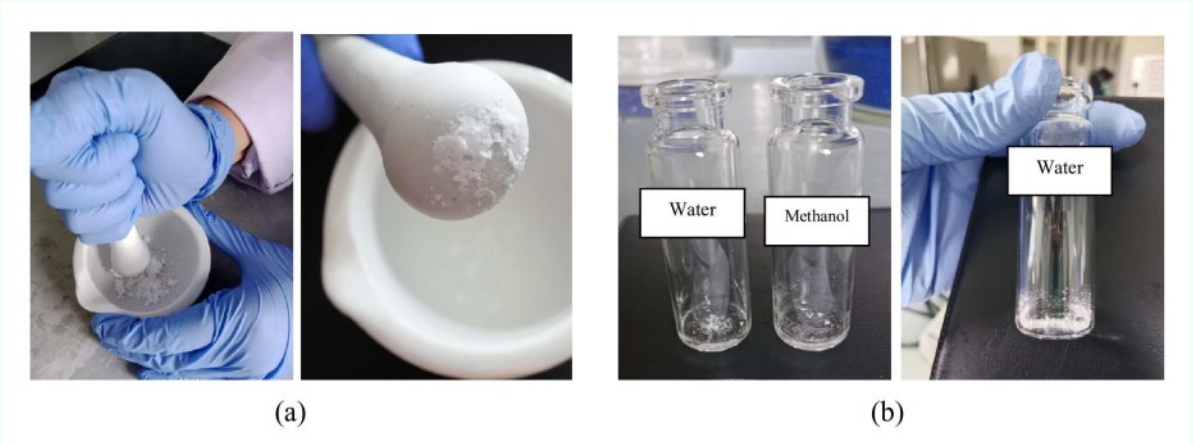


Figure 2: (a) Crushed sample; (b) Solubility test in water and methanol.

A color test using Marquis, Mandelin and Simon's reagents yielded orange that quickly turned into dark brown, light green and blue color, respectively. It indicated the presence of methamphetamine in the sample. The solubility test showed an unusual result, the sample did not dissolve completely in water and methanol, which was atypical of methamphetamine hydrochloride [6]. White powder was found floating in both solvents (Figure 2b). The homogenized sample was analysed using ATR-FTIR, and only methamphetamine was identified from the IR library. However, two unknown peaks were observed in the range of approximately 2875 - 2950 cm^{-1} and 2840 – 2860 cm^{-1}, respectively. Another test was performed using Raman spectroscopy, the sample was detected as a mixture of Methamphetamine (51%), Stearyl stearate (44%), and Benzalkonium Bromide (4%) from the Raman library. Stearyl stearate is an ester of stearyl alcohol and stearic acid that is insoluble in water and determined to be safe for use in cosmetics [7,8]. FT-IR analysis of this compound obtained a highest peak at 2920 cm^{-1} followed by a peak at 2850 cm^{-1} [9], it may correlate to the two unknown peaks in Figure 3a. From the analysis, the

undissolved substance in water and methanol could be stearyl stearate, that was mixed with methamphetamine to dilute the sample's purity. However, further analysis are required to confirm the presence of stearyl stearate in the sample.

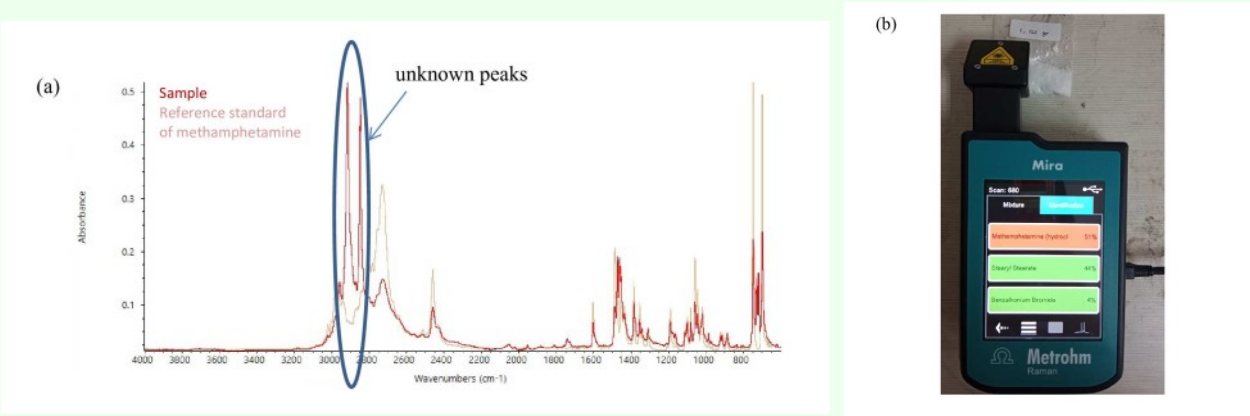
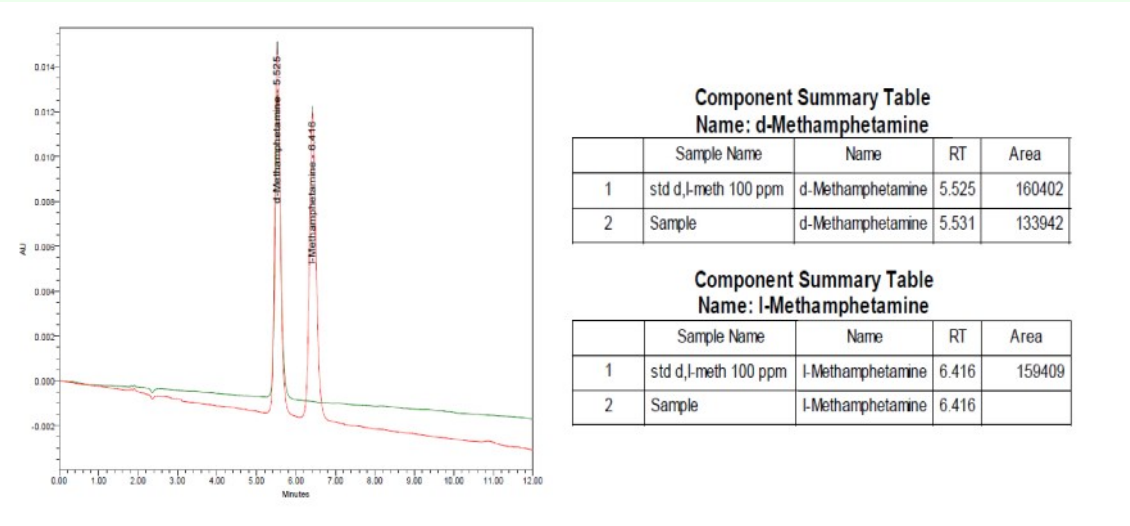


Figure 3: (a) Overlayed IR spectra of methamphetamine hydrochloride standard and sample; (b) Library identification of the profiled sample by Raman spectroscopy.

In the melting point test, the sample partially melted at approximately 60ºC and completely melted at 173-177 ºC, which corresponded with the reported melting points of stearyl stearate and *d*-methamphetamine, respectively [6,7]. The chiral analysis using LC indicated *d*-methamphetamine with 100% enantiopurity (Figure 4). Meanwhile, the sample predominantly showed the rod-shaped crystals than long-needle crystals under the microscope.



Component Summary Table
Name: d-Methamphetamine

	Sample Name	Name	RT	Area
1	std d,l-meth 100 ppm	d-Methamphetamine	5.525	160402
2	Sample	d-Methamphetamine	5.531	133942

Component Summary Table
Name: l-Methamphetamine

	Sample Name	Name	RT	Area
1	std d,l-meth 100 ppm	l-Methamphetamine	6.416	159409
2	Sample	l-Methamphetamine	6.416	

Figure 4: Overlayed chromatogram of LC-PDA chiral analysis between sample (green line) and reference standard of racemic methamphetamine (red line).

The sample was dissolved in water, then filtered using nylon filter prior to anion analysis. A white curdy precipitate was formed right after the addition of $AgNO_3$, which dissolved in ammonia solution and re-precipitated by the addition of nitric acid. It indicated the sample contained a chloride salt.

Chemical characterization to identify the synthetic route and the precursor used in the manufacturing process was performed. The *l*-ephedrine and *d*-pseudoephedrine were not detected by UPLC-PDA, leading to the assumption that P2P was used as the precursor. It was supported by the result of impurity analysis using GC-MS through direct, acidic and basic extractions. The impurity α-benzyl-N-methyl-β-phenethylamine (synonym: *α*-benzyl-N-methylphenethylamine (BNMPA)) was a new methamphetamine impurity detected in our laboratory. The identification was consistent in all extractions of the sample and were confirmed by comparison against the SWGDRUG mass spectral library (Figure 5). It was reported by Onoka, et al. (2020) that *α*-benzyl-N-methylphenethylamine is a characteristic impurity of reductive amination of P2P, where the P2P used originates from the pre-precursor of phenylacetic acid (PAA) [10].

Diphenethylamine, α-methyl (synonym : N-(α-Methylphenethyl)phenethylamine), a P2P route-specific impurity [11] was detected in trace level in the acidic and basic extractions. In this case, the impurity 1-phenyl-2-propanol (P2P-ol) was confirmed in acidic extraction. P2P-ol was reported as route specific for reductive amination by Kunalan, et al. (2009). The extracted ion chromatogram of GC with the characteristic mass fragments of P2P-ol which was m/z 92, 91, 45, and 65 is shown in Figure 6. The presence of those impurities together implies that methamphetamine was synthesized from P2P using reductive amination route, where P2P used possibly derived from phenylacetic acid (PAA). Reductive amination is an occasionally detected method in our laboratory since the common synthetic route profiled in Indonesia were Emde and Leuckart. Racemic methamphetamine resulting from P2P-based method was probably treated using tartaric acid to obtain the desired *d*-methamphetamine [12, 13]. The postulated synthetic route used for the sample is shown in Figure 7.

Other impurity detected was a cutting agent, Dimethyl sulfone. Its retention time under basic extraction was shifted compared to direct and acidic extractions. However, the peak was confirmed as Dimethyl sulfone by its mass fragmentation (m/z 79, 94, 81) and comparison to mass spectral libraries. The presence of Dimethyl sulfone and Stearyl stearate decreased the purity level of methamphetamine hydrochloride to only 33.95%, a very low purity compared to samples typically encountered in our laboratory. The average purity of methamphetamine HCl that we profiled in 2023 was 96.13%.

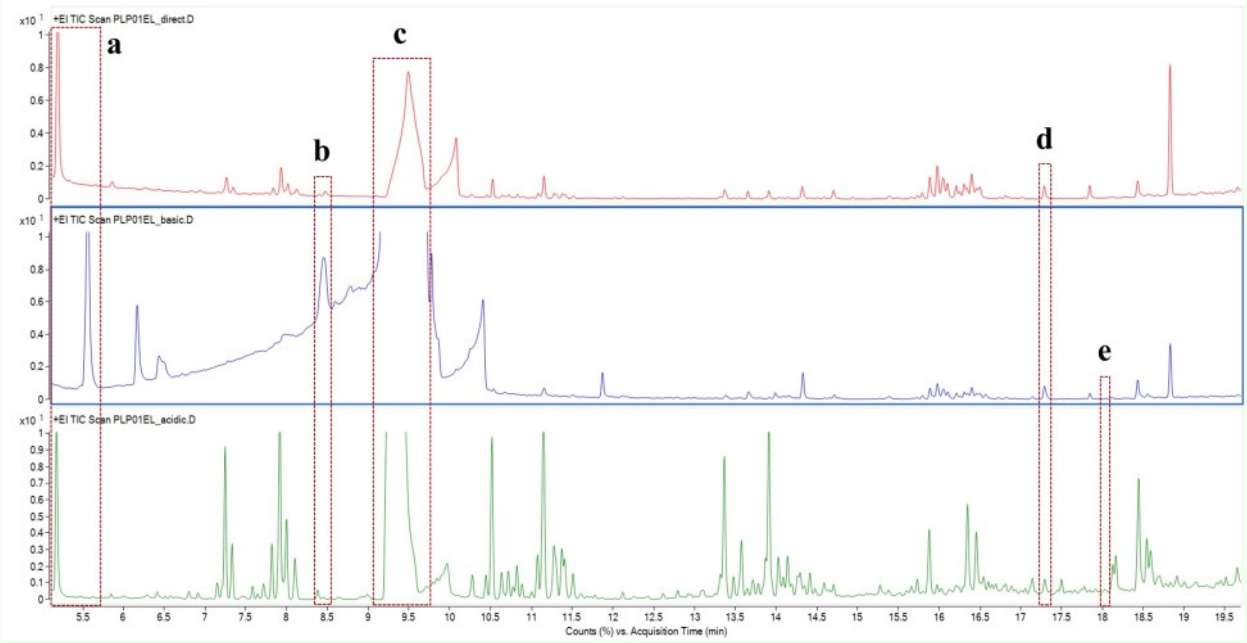


Figure 5: TIC overlayed of GC-MS under direct extraction (red line), basic extraction (blue line) and acidic extraction (green line) : (a) Dimethyl sulfone; (b) P2P; (c) Methamphetamine; (d) α-benzyl-N-methyl-β-phenethylamine; (e) N-(α-Methylphenethyl)phenethylamine.

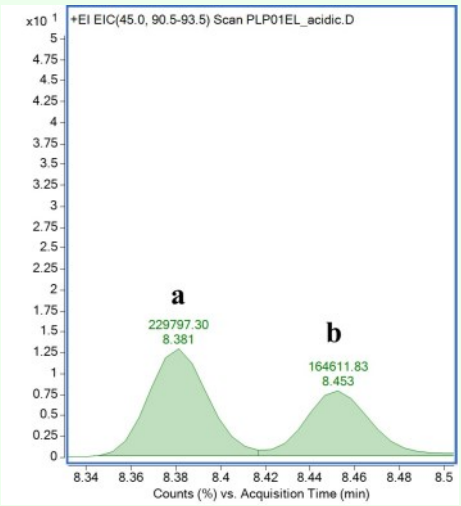


Figure 6: Peak detected by extracted ion chromatogram : (a) P2P; (b) P2P-ol.

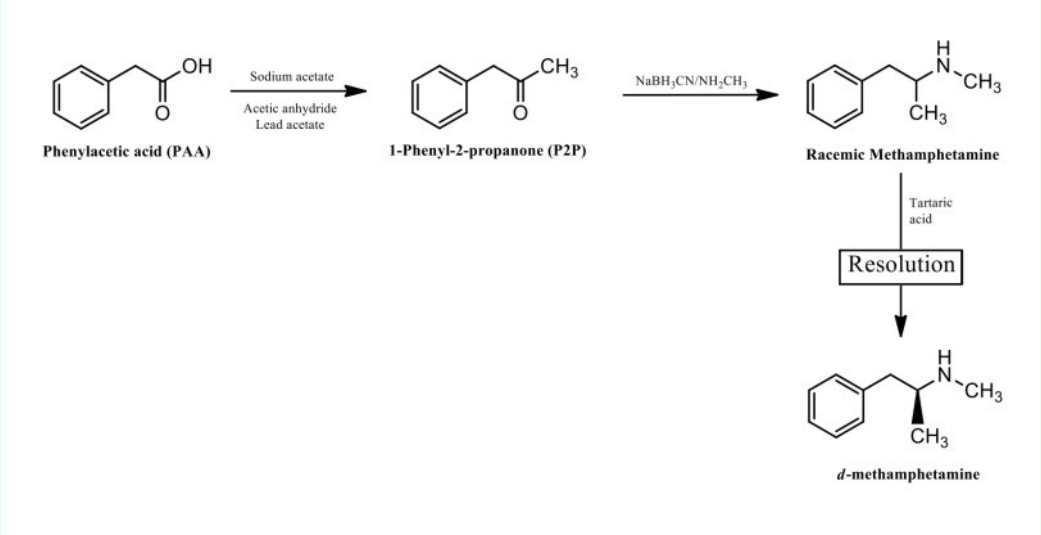


Figure 7: The postulated synthetic route used in the profiled sample.

Conclusion

A white dense solid sample containing methamphetamine smuggled from Mexican TCO was profiled for the first time in the Center of Drugs Testing Laboratory of BNN. The profile was unique as the sample was found to contain cutting agents that were Dimethyl sulfone and compound which was suspected as Stearyl stearate, with low purity of methamphetamine hydrochloride. Methamphetamine crystal was presumably pressed with these cutting agents into a rectangular board and packaged in decorative resin frame. The impurities α-benzyl-N-methyl-β-phenethylamine, N-(α-Methylphenethyl)phenethylamine and P2P-ol indicated that the sample was probably synthesized by reductive amination of the precursor P2P, which originated from the pre-precursor phenylacetic acid (PAA).

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4. Drugs profiling team of Center of Drugs Testing Laboratory of National Narcotics Board of the Republic of Indonesia

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Case Study

A Descriptive Study on Fabricated Documents

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Abstract

Fabricated documents have become a major issue as they are created with the intention to deceive people. Forgers use different methods to recreate documents that mimic authenticity. Security features within documents act as barriers that prevent counterfeiting and unauthorized duplication. Therefore, the most valuable documents, such as currency notes, passports, visas, identity cards, and driving licenses, contain high-level security features and combinations of expensive printing techniques that are very difficult to copy. Consequently, attempting to reproduce documents containing security features will result in imperfections and irregularities that facilitate detection. Experts utilize modern forensic tools and technologies to examine the physical and digital attributes of documents and decipher inconsistencies in fabricated documents.

Introduction

Even though the world has entered the digital era, paper-based documents still play a vital role in the day-to-day work of people. Fabricated documents, created to mislead individuals, organizations, and even entire societies, have emerged as a growing issue. Scientifically, a fabricated document can be defined as one that has been intentionally altered, falsified, or entirely created with the intent to deceive.

People employ various methods to create fabricated documents, including alterations (additions, physical and chemical erasures, substitutions) and counterfeit printing techniques. With the advancement of technology, forgers are experimenting with more powerful ways to create documents. Consequently, scientific examinations should be more competent in revealing inconsistencies, irregularities, or indications of tampering hidden in fabricated documents. Forensic experts employ scientific methods to examine frauds and gather evidence for legal proceedings. Techniques such as microscopic examination, as well as advanced forensic instruments like Video Spectral Comparator (VSC) and Electrostatic Detection Apparatus (ESDA) can be employed for the identification of fabricated documents.

Fabricated documents exhibit significant diversity. A document can be manipulated in various ways to mimic the authenticity. This article will discuss about the various types of fabricated documents, identification of their imperfections, detection methods, and the measures that can be taken to combat this issue.

Methodology

Video Spectral Comparator (VSC) 8000, Foster & Freeman Company, UK and Stereo microscope LEICA M205C, Foster & Freeman Company, UK were used for examination of fabricated document. Examinations were carried out under suitable magnification using different light conditions such as flood light, UV, Infra-red radiation, spotlight, side-light and transmittance light.

Case No: 1

A title deed dated 25.09.1996, submitted for examination, was found to have a stamp printed in 1987. It is not possible to use a stamp printed in 1987 for a deed written 9 years later as stamps typically have expiration dates or validity periods. This time discrepancy observed between the deed and the stamp is evidence of counterfeiting. Further scrutiny revealed re-writings on the stamp, done with different ink over previous writings (Figure 1).

Figure 1: A stamp printed in 1987, displays various rewrites and the date '25.9.1996'.

The presence of additional layers of a strange paper on the reverse side of the stamp serves as evidence that the stamp was removed from another document (Figure 2).

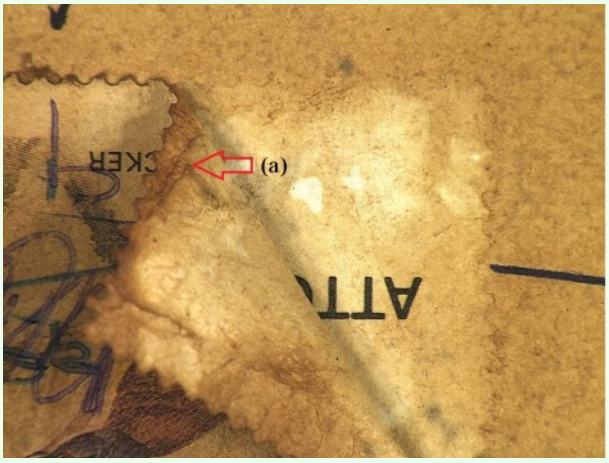

Figure 2: (a) The reverse side of the stamp features additional layers of paper.

In order to pretend the deed was very old, a color was artificially applied on the deed using a particular tool, as indicated by the unusual colored patches (Figure 3).

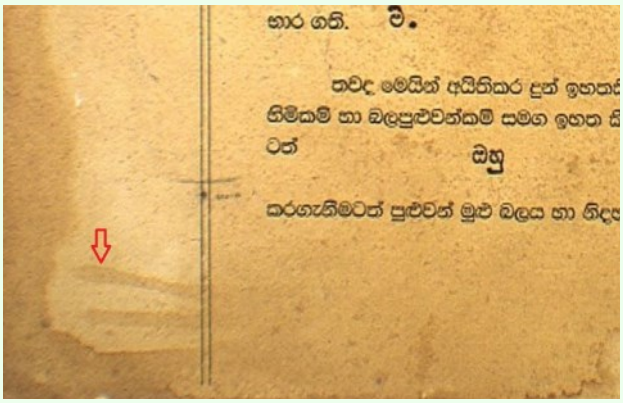

Figure 3: The artificially aged deed. The arrow indicates the presence of unusual coloured patches which resulted from colour application using a specific tool.

The above-mentioned questioned deed was compared with the specimen deed. The pages of the questioned and specimen deeds exhibit different fluorescence under UV (Figure 4). Both deeds display different fiber distribution and different colors under sidelight and normal visible light too.

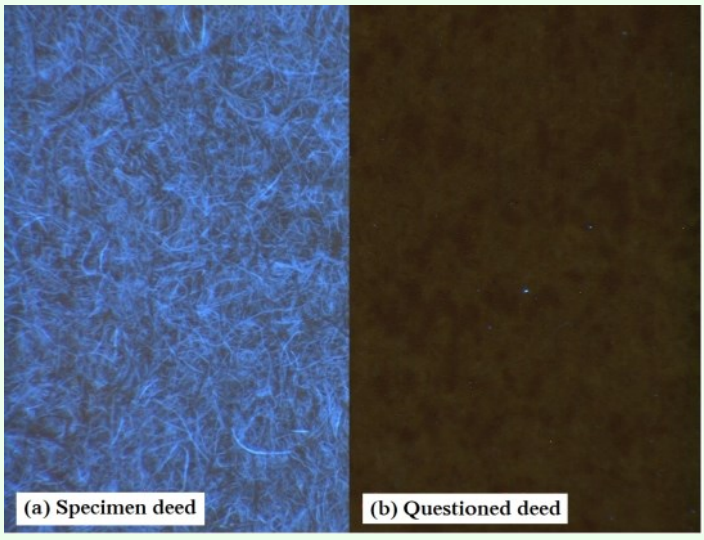

Figure 4: Fluorescence shown under UV.

Case No: 2

This case involves a motor car that was rented from the owner and then fraudulently sold. The questioned certificate of registration of motor vehicles which was used in the transaction was submitted for examination. When comparing the questioned certificate with the specimen certificate, differences became evident.

The specimen certificate possesses original fibers that fluoresce under UV light, while the questioned certificate lacks any original fibers (Figure 5). Additionally, the watermark observed in the specimen certificate differs from that in the questioned certificate (Figure 6). The presence of fluorescent fibres and watermarks serve as strong security features that cannot be imitated by forgers because they are incorporated into the paper during the paper manufacturing process [1].

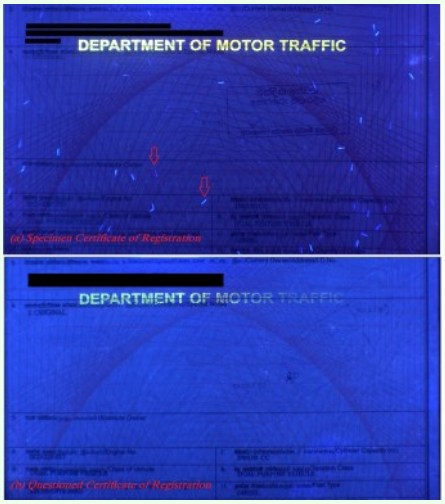

Figure 5: Fluorescence shown under UV (a) Specimen Certificate (b) Questioned Certificate. The arrows indicate the fluorescent fibres on the specimen certificate.

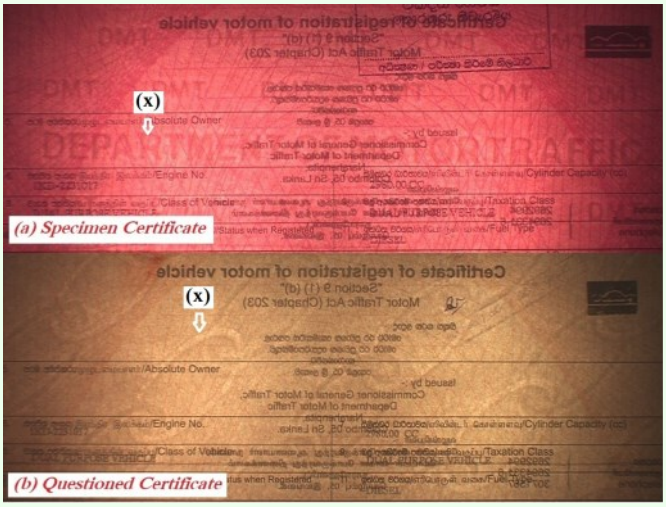

Figure 6: (x) Water marks observed under transmittance light. (a) Specimen Certificate (b) Questioned Certificate

Case No: 3

A Sri Lankan passport, along with a Schengen visa, was submitted for examination to determine the authenticity. The bio-data page of the specific Sri Lankan passport in question was examined under appropriate lighting conditions and suitable magnification. It was then compared with the specimen bio-data page. Discrepancies were observed between the questioned bio-data page and the specimen, particularly in printing techniques, security features, and fluorescence under UV light. Security documents such as passports, visas, and currency notes are manufactured using UV-dull papers, which are not readily available in the market [1]. The questioned and specimen bio-data pages, consisting of paper and laminate, were examined under UV light. The questioned bio-data page fluoresced under UV radiation, while the specimen bio-data page did not. (Figure 7). Furthermore, the photo of the questioned bio-data page did not display Invisible Personal Information (IPI), whereas the IPI of the image of the specimen bio-data page could be deciphered. (Figure 8).

Figure 7: Fluorescence shown under UV (a) Specimen bio-data page. (b) Questioned bio-data page.

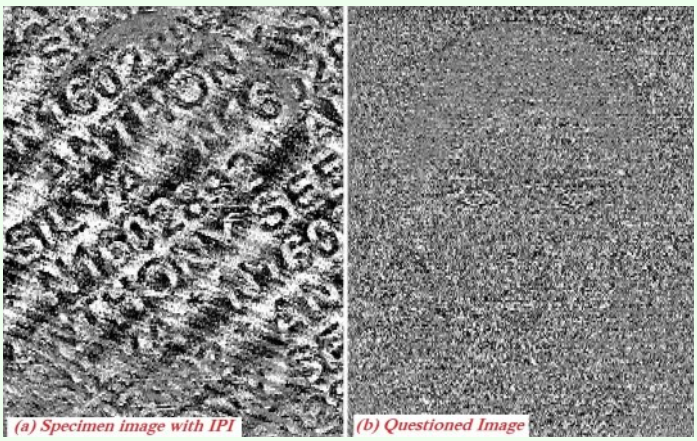

Figure 8: (a) Specimen image with IPI. (b) Questioned image without IPI.

Similarly, the questioned Schengen visa was examined under appropriate lighting conditions and suitable magnification, utilizing the VSC 8000, and compared to the relevant specimen visa. Several printing methods were observed in the specimen visa. Micro-printing on the background of the specimen visa was accomplished using 'Intaglio Printing', where the ink of the printed letters raised above the surface. In the questioned visa, micro -printings are embossed to mimic the raised effect of intaglio printing found in genuine visa (Figure 9). The visa number on the specimen is printed using letterpress printing, resulting in very sharp edges around the image. In contrast, the visa number on the questioned visa is printed using a different printing technology , resulting in messy edges (Figure 10). Additionally, the background of the specimen visa is printed using the offset method/ rainbow printing, in which micro-printings are very clear. However, forgers cannot afford these expensive printing techniques, so that they simply print the entire questioned visa using inkjet printing method, resulting in poor quality visa.

Figure 9:
Micro printings observed under sideling
(a) Raised ink of micro-printing of specimen visa
(b) Embossed micro-printing of questioned visa.

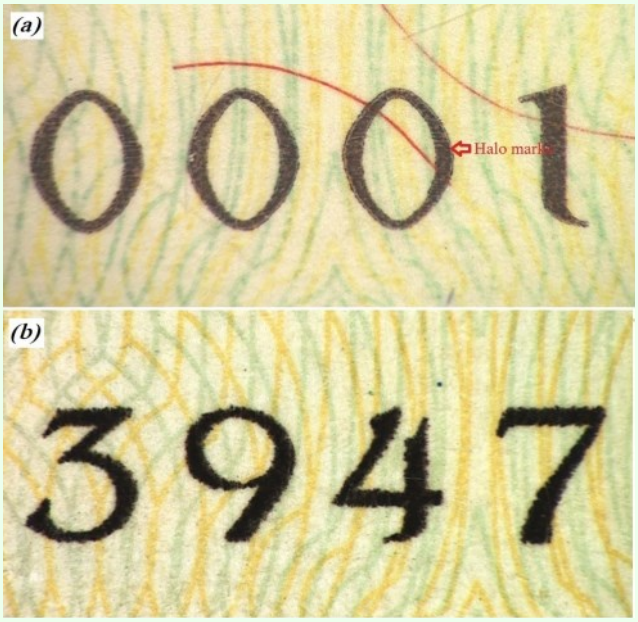

Figure 10:
(a) Visa number of specimen visa printed with Letter Press Printing. (b) Visa number of questioned printed with different printing technique.

Furthermore, the specimen visa contains an original kinegram, considered to be a high-level security feature containing intricate visual effects, holography and microstructures that produce different colors when tilts. Counterfeiting and duplicating the kinegram are not possible for forgers so that they cut the kinegram from a genuine visa and introduce it to the fabricated visa. When the visa is observed under sidelight, the cutting edges of the kinegram of the fabricated visa can be observed. (Figure 11)

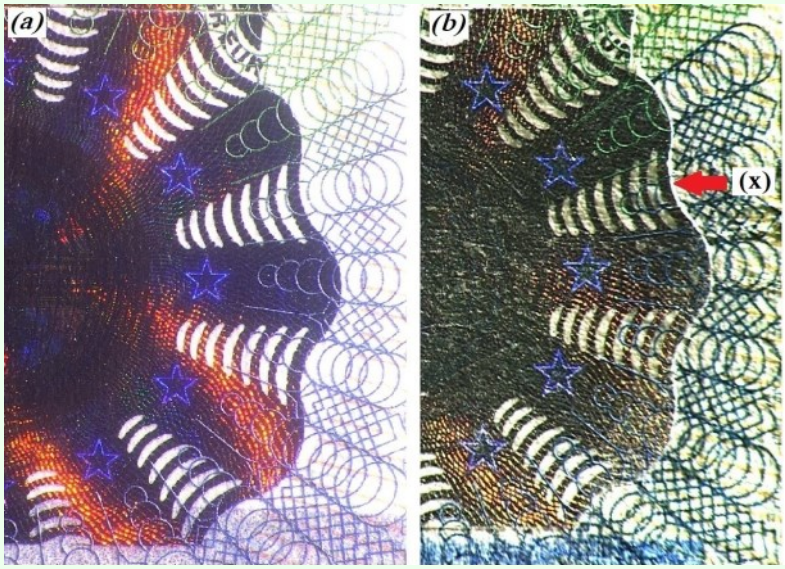

Figure 11:
a) Kinegram of specimen visa.
b) Kinegram of Questioned visa.
x) The cut mark observed under sidelight on the questioned kinegram serves as evidence of forgery.

Discussion

As discussed above, security features such as water mark, fluorescent paper fibers , Kinegram,Intaglio printing and letter press printing etc. provide high level of security by enhancing authenticity of documents and make it difficult for counterfeiters to reproduce fabricated documents. Even though, the forgers are taking effort to replicate them, detection of forgery is easier for experts as they are utilizing advanced forensic techniques and tools. In addition to forensic techniques, document authentication centers utilizing databases [2] (Example: Databases of Passport/visa, identity Cards) and machine learning algorithms such as artificial intelligence, machine readable devices (Example: micro-chips in e-passports [1], facial recognition systems, biometrics) can play a vital role in identification of fabricated documents. Formation and circulation of fabricated documents can be prevented by introducing advanced security features to valuable documents, conducting people awareness programs and strengthening rules and regulations.

Conclusion

The prevalence of fabricated documents causes bad impacts on individuals, organizations and the country as a hole. Strategic measures should be taken to prevent the formation and the use of fabricated documents. Altered or inconsistent text/printing, discrepancies in information such as dates, anomalies in security features, including watermarks and kinegrams, evidence of tampering are crucial in the identification of fabricated documents. By understanding the characteristics found in fabricated documents, experts can enhance their skills in the detection of document frauds effectively.

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Case Study

A Study of Rubber Stamp Impressions and their Significance in Forensic Document Examination in Sri Lanka

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Abstract

The identification of rubber stamp impressions is equally important as signature and handwriting analysis in document examination. There are three main types of rubber stamp namely traditional rubber stamp, self-ink stamp, and pre-inked stamp. The questioned and specimen stamp impression analysis was carried out by using Video Spectral Comparator (VSC) 8000 and microscope under different light conditions and appropriate magnification levels. The authenticity of the stamp impressions is determined based on factors such as size, arrangement, design and most importantly the defects. The seal is printed with electrography using toners which can be identified based on the observations showed under different wavelengths of light and their granular-like appearance displayed under high magnification.

Introduction

The identification of rubber stamp impression is crucial in document authentication. The forensic document laboratory generally receives three main types of rubber stamp including traditional rubber stamp, self-inked stamp, and pre-inked stamp. [1] Traditional rubber stamps have a wooden handle and need a separate ink pad. It needs to be inked on the ink pad to create an impression on the document. [2] These stamps are customizable in different sizes and can be made with specific text or designs. They are commonly utilized for document authentication by imprinting official seals or signatures.

Self-inking rubber stamps have an integrated ink pad, eliminating the need for a separate ink pad. When you press the stamp onto the document, the ink pad automatically re-inks itself. [2] These stamps are convenient and efficient for repetitive stamping tasks. Pre-inked rubber stamps contain ink within the stamp itself that is evenly distributed throughout the stamp. This results in clear and consistent impressions without the need for an additional ink pad. [2] They are commonly used for document authenticity, as they provide sharp and long-lasting impressions. They are ideal for detailed designs, logos, or intricate text.

All three types of rubber stamp can enhance document authenticity by imprinting official seals, signatures, dates, or other relevant information. It is important to choose the appropriate type based on specific needs, considering factors such as convenience, durability, and the level of detail required for the impression.

The Content, font size, type face , design or whole stamp can be forged in rubber stamp forgeries. There are two main types of defects namely manufacturing defects and wear and tear defects. Certain defects cannot be forged as originals. Some individual characteristics / defects are occurrence of air bubbles, distortion or misalignment, poor quality control, damaged caused by cutting the stamp, etc. [1] This paper will discuss different types of stamp impression forgeries and identification of forgery types using different optical and analytical devices/methods.

Instruments and Methods

Video Spectral Comparator (VSC) 8000 (Foster & Freeman Company, UK) and Leica Stereo microscope (M205C, Foster & Freeman Company, UK) were used to examine the questioned and specimen stamp impressions under different light conditions such as flood light, side light, IR (Infrared Radiation) light etc. and appropriate magnification levels. [3]

Case 01

A questioned stamp impression on a document was forwarded to examine with the relevant specimen stamp impressions. The Examinations revealed that, the questioned stamp impression was mechanically generated using a scanner with high resolution and printed from toner technology as shown in the Figure 1. As a result, this questioned impression did not undergo reactions such as absorption, reflection, or transmission when exposed to different wavelengths of light. Moreover it's granular like appearance was evident under high magnification. In contrast, the ink of the impression blotted onto adjacent paper fibers and appeared smudged because the specimen stamp impressions were inked. (Figure 2) The date of the questioned stamp impression was not the part of it and was found to be a separate inked stamp impression when subjected to various wavelengths of light. (Figure 3). This distinction highlights the differences in the authenticity and characteristics of the questioned stamp impression compared to the specimen stamp impressions.

Figure 1: (a) Questioned stamp impression. (b) Specimen stamp impression.

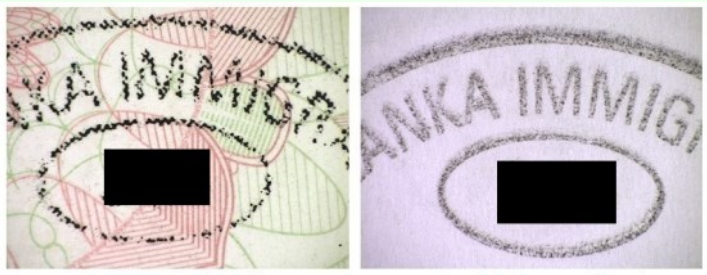

Figure 2: (a) granular-like appearance under high magnification. (b) specimen stamp impression looks smudged and ink of the contents blotted to adjacent paper fibers.

Figure 3:
The questioned (a) and specimen (b) stamp impressions were observed under 485 nm and 610 nm wavelengths, (a) does not undergo any reactions (except date) as (b) does.

(a) The questioned stamp exhibits no reactions apart from changes in the 'date' element, while the (b) specimen stamp impression undergoes reactions in the presence of different wavelengths.

Case 02

A stamp impression of a Sri Lankan passport was observed and compared with the respective specimen stamp impressions under various light conditions and magnification levels. Upon closer inspection, fiber disturbance was noted on the seal number of the stamp impression when viewed under side light, indicating potential tampering. (Figure 4) Additionally, it was discovered that the seal number had been altered using black ball-point writing ink. Furthermore, significant differences were observed in the size, design, and contents of the questioned seal impression compared to the specimen stamp impressions, further raising suspicions regarding the authenticity of the stamp impression on the passport. (Figure 5)

Figure 4: Fiber disturbance could be observed under side light.

Figure 5: Questioned stamp impression differs from the specimen stamp impression in content, design of the characters and size.

Case 03

The questioned Malaysian visa stamp impression on the passport was compared with the corresponding specimen stamp impressions under different light conditions and magnification levels. It was observed that the questioned visa impression was similar in size to the specimen stamp impressions, but differed in terms of the design of the characters and the presence of defects. (Figure 6) The observed differences in the questioned seal provide evidence to conclude that it is indeed a forged seal.

Figure 6: (a) Questioned stamp (b) Specimen stamp The arrows indicate the differences in the typefaces of both the questioned and specimen seals, as well as the gap in the specimen seal, which serves as a security feature.

Discussion

As mentioned above, determination of the authenticity of any stamp impression on a document can be performed under suitable experimental conditions. However, by looking at the stamp impression, it is difficult to determine which stamp was used to create the particular impression.

Conclusion

Forgers can form fraudulent stamp impressions using mechanical devices or making alterations to genuine stamp impression s or reproducing entirely different stamp impressions . The authenticity of the stamp impressions is determined based on the size, arrangement, design and most importantly the defects such as manufacturing defects and wear and tear defects which are unique to that particular type of stamp impression. The stamp impression is created by printing process or mechanically which can be identified based on the reactions at different wavelengths and their appearance showed under high magnification.

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	4	Directorate of Forensic Science, Himachal Pradesh
Indonesia	5	Centre of Indonesian Automated Fingerprint Identification System of the Indonesian National Police
	6	Department of Police Medicine of the Indonesian National Police
	7	Eijkman Institute for Molecular Biology
	8	Forensic Laboratory Centre of Indonesian National Police Headquarters
	9	Indonesian Association of Forensic Pathologist
	10	Laboratory of National Narcotics Board
	11	Master Program of Forensic Science, Postgraduate School, Universitas Airlangga
Lao PDR	12	Food and Drug Quality Control Center
Malaysia	13	CyberSecurity Malaysia
	14	Department of Chemistry
	15	Malaysian Communications and Multimedia Commission
	16	National Institute of Forensic Medicine, Hospital Kuala Lumpur
	17	Royal Malaysia Police Forensic Laboratory
Mongolia	18	Mongolian National Institute of Forensic Science
People's Republic of China	19	Beijing Forensic Science Institute
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	21	Forensic Science Center of Guangdong Provincial Public Security Department
	22	Forensic Science Division, Department of Fujian Provincial Public Security
	23	Gansu University of Political Science and Law, Key Laboratory of Evidence Science Techniques Research and Application
	24	Guangzhou Forensic Science Institute
	25	Institute of Forensic Science, Ministry of Public Security
	26	Institute of Forensic Science, Dezhou Public Security Bureau
	27	Institute of Forensic Science, Hangzhou Public Security Department
	28	Institute of Forensic Science, Shandong Public Security Department
	29	Institute of Forensic Science, Suzhou Public Security Bureau
	30	Institute of Forensic Science, Tianjin Public Security Bureau
	31	The Institute of Evidence Law and Forensic Science, China University of Political Science and Law
	32	Forensic Science Division of the Government Laboratory, Hong Kong Special Administrative Region
	33	Forensic Science Department of Judiciary Police, Macau Special Administrative Region
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	35	National Bureau of Investigation
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	41	Department of Forensic Sciences, Sungkyunkwan University
	42	Graduate School of Forensic Science, Soon Chun Hyang University
	43	Institute of Forensic and Anthropological Science
	44	Korea Coast Guard Research Institute
	45	National Digital Forensic Center of Supreme Prosecutors' Office
	46	National Forensic Service
	47	Scientific Investigation Center of Korean National Police Agency
	48	Scientific Investigation Laboratory, Ministry of National Defense
	49	SJS Institute of Forensic Science & Medicine
Republic of Uzbekistan	50	Republican Scientific and Practical Center of Forensic Medical Examination, Ministry of Health, Republic of Uzbekistan
	51	Republican Center for Forensic Examination under the Ministry of Justice, Republic of Uzbekistan
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	53	Health Sciences Authority
	54	Ministry of Home Affairs
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Thailand	57	Central Institute of Forensic Science
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	59	Department of Forensic Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University
	60	Division of Forensic Medicine, Thammasat University Hospital
	61	Department of Medical Sciences
	62	Faculty of Medicine, Chiang Mai University
	63	Human Genetics Unit, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital
	64	Institute of Forensic Medicine, Police General Hospital, The Royal Thai Police
	65	Narcotics Analysis and Technical Service Institute Office of Narcotics Control Board
The Republic of the Union of Myanmar	66	Defence Services Medical Research Centre
Timor-Leste	67	Polícia Científica de Investigação Criminal - Laboratório de Polícia Científica
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