ForensicAsia

THE ASIAN FORENSIC SCIENCES NETWORK NEWSLETTER | ISSUE 3 | 2011

AFSN President's Address

Congratulations to Dr Chung Heesun of the National Forensic Services, Korea, Chairperson of the organising committee of the AFSN 3rd Annual General Meeting & Symposium and her team for successfully organising this interesting scientific symposium on 25 – 27 May 2011 in Seoul, Korea. 392 participants from 23 countries participated in the symposium which comprised 3 plenary lectures and a total of 43 oral presentations by the five different workgroups / committee. In addition, there were 2 workshops and 43 poster presentations during the symposium. This AFSN 3rd Annual General Meeting was attended by all member institutes except the Food and Drug Quality Control Center from Lao PDR.

Currently, the Department of Chemistry, Malaysia, being the Vice-President of AFSN for the term 2009 – 2011, has been given the responsibility to lead AFSN as the President, replacing Dr Paul Chui of the Health Sciences Authority, Singapore, as stipulated in the constitution for the next 2 years term from 2011 – 2013. It is an honour for me to represent the Department of Chemistry, Malaysia, to lead this dynamic scientific network in Asia. Dr Paul Chui was the 1st President of AFSN and on behalf of all the members, I will like to take this opportunity to thank and congratulate the previous board members under the leadership of Dr Paul Chui for actively promoting AFSN as an internationally recognised forensic science network. Through their commitment and dedication, AFSN is an official member of International Forensic Strategic Alliance (IFSA) since 5 October 2010. Recent IFSA activities include a teleconference which was held among all IFSA members on 8 June 2011 and a face-to-face IFSA annual meeting which was held on 16 September 2011 in Funchal, Maderia, Portugal, in conjunction with the 19th Triennial Meeting of the International Association of Forensic Sciences (IAFS).

At the 3rd AFSN AGM on 26 May 2011, the election of new board members was carried out and I will like to congratulate all the newly elected board members and they are Dr Chung Heesun, National Forensic Services, Korea; Dr Lam Kian Ming, Health Sciences Authority, Singapore; Dr Khunying Porntip Rojanasunan, Central Institute of Forensic Science, Thailand; and Dr Zhou Yunbiao, Institute of Forensic Science, People's Republic of China. The new board members will continue to work together and play an active role to achieve the objectives of the network and in addition with the strong support, commitment and cooperation from all member institutes, we will continue to seek and create opportunity for collaborations among Asian Forensic Science community as well as among global forensic science community. During the AGM in Seoul, the board has approved 9 new member institutes from 10 applicants, thus our current membership strength has grown from 18 to 27 member institutes from 11 countries. A very warm welcome to all the new member institutes, your interest and active participations and support in all AFSN activities/programmes are much appreciated.

Thank you to the Central Institute of Forensic Science, Thailand, for volunteering to take up the challenge in hosting the next AFSN 4th Annual General Meeting & Symposium in Bangkok, Thailand, to be held from 26 to 28 November 2012. Strong support and active participations by all member institutes are welcome to ensure the success of the next AGM and symposium in this beautiful and exciting city of Bangkok, Thailand.

Mr Lim Kong Boon
AFSN President

International Scene

Report on the International Forensic Strategic Alliance Annual Meeting

Dr Lam Kian Ming
ILO, AFSN

IFSA Vision

To create opportunities for strategic collaboration across the global forensic science community

IFSA Goals and objectives

- to represent the operational forensic science community
- to develop and execute a rolling agenda for strategic issues related to forensic science
- to be a strategic partner to other relevant international organisations and partnerships
- to encourage the exchange of information related to experience, knowledge and skills between the member networks

From left to right: Mr Üllar Lanno (ENFSI), Mr Lim Kong Boon (AFSN), Prof Jose Lorente (AICEF), Ms Susan Johns (ASCLD), Mr Edward Ngokha (SARFS), and Prof Stephen Cordner (SMANZFL).

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Editor's Address

Dear readers,

A very warm greeting to all! It has been a very exciting time to work with many new members in the Editorial Committee: Dr Lam Kian Ming, Chief Advisor; Dr Justice Tettey, International Guest Editor; Dr Lee Sangki, Dr Quan Yangke, Dr Vanitha Kunalan and Dr Worawee Waiyawuth, Guest Editors; and Ms Hozanna Ngoh, Editorial Assistant. Thank you to the whole Editorial Committee for so unreservedly giving your time, knowledge and expertise for this newsletter.

Starting from this issue, we have also introduced a Reviewer's Form to assist all Guest Editors in reviewing the submitted articles. We would like to thank all Guest Editors who have done excellent reviews and given many comments and suggestions. It is our hope that the quality of articles published in this newsletter will be raised through critical reviews.

This issue has also seen an increase of the original 16 pages to the current 24 pages. This dramatic increase of 50% is credited to superb response from all AFSN members in submitting articles. We regret that we were not able to accept additional articles submitted after the deadline due to space constraint.

We hope that you will enjoy reading the articles in this issue and please do give us your comments and feedback at hsa_asg@hsa.gov.sg.

Dr Angeline Yap
Editor

AFSN 4th Annual Meeting and Symposium, Bangkok, Thailand, 26 - 28 November 2012

For more details, please log on to **www.asianforensic.net**

Editorial Committee

For enquiries, feedback or contribution of articles, please email to hsa_asg@hsa.gov.sg. For contribution of articles, please read the guidelines at www.asianforensic.net.

AFSN 3rd Annual Meeting and Symposium in Seoul, Korea, 2011

Dr Heesun Chung
National Forensic Service, Korea

AFSN Board together with invited speakers and organising committee.

AFSN 3rd Annual General Meeting.

Dr Chung Heesun, Chairperson of Organising Committee, giving a welcome address.

The Welcome Dinner of 3rd AFSN meeting.

Samulnori performance at the Welcome Dinner.

NFS Lab tour.

The Asian Forensic Sciences Network (AFSN) 3rd Annual Meeting & Symposium was successfully held in Seoul, Korea on 25 – 27 May 2011. This meeting was held in conjunction with the 23rd Conference of Korean Society of Forensic Science, as a three-day program at the Lotte Hotel Seoul (25 – 26 May) and the National Museum of Korea (27 May). In this AFSN meeting, we had a great number of participants, totaling 392 forensic scientists from 23 countries.

On 25 May, the Meeting was opened with congratulatory speeches by Dr Paul Chui, the President of AFSN and Mr Namseok Kim, the Vice Minister of the Ministry of Public Administration and Security, Korea. After the opening ceremony, Prof Pierre Margot (University of Lausanne, Switzerland), Dr Justice Tettey (UNODC, Austria), and Ms Anja Einseln (ASCLD/LAB, USA) each delivered their keynote speech.

For two days (25 – 26 May), several workshops and scientific sessions organised by the five Workgroups (DNA, Toxicology, Illicit Drugs, Trace Evidence and Quality Assurance & Standards Committee) and business meetings were held concurrently from 9 a.m. to 6 p.m. A total of 43 oral presentations and 43 posters were presented. The AFSN Board meeting was held on 26 May.

Distinguished guests were also invited to speak to the various Workgroups. Dr Alain Verstraete (Ghent University Hospital & Ghent University, Belgium) was invited to the Toxicology Workgroup, Mr Christian C Matchett (Georgia Bureau of Investigation, USA) to the Illicit Drugs Workgroup, Dr Edward M Suzuki (Washington State Crime Laboratory, Washington State Patrol, USA) to the Trace Evidence Workgroup and Dr Ate D Kloosterman (Netherlands Forensic Institute, Netherlands) to the DNA Workgroup.

The welcome dinner, held on the first evening of the meeting, was graced by the Chief of the National Police Agency, Korea, who delivered a congratulatory address to the AFSN participants. The dinner also showcased a Samulnori performance, a traditional Korean percussion quartet. The participants had an enjoyable time networking with fellow participants, sharing ideas of forensics, and experiencing the Korean culture.

On 27 May, the 23rd Conference of Korean Society of Forensic Science was opened at the National Museum of Korea. In the morning, 3 presentations in the field of digital forensics were given. In the afternoon, 3 optional tours were offered to the AFSN participants: a visit to the National Forensic Service laboratories, a city tour and a cultural tour. About 70 participants took the tour to NFS labs (forensic medicine, forensic DNA, criminal psychology, document & image, drug & toxicology, narcotic analysis, chemical analysis, physical analysis and traffic engineering). For city tour, participants took a tour of Seoul Tower and Gyengbokgung palace (a palace of Joseon Dynasty). Seoul Tower is located at Mt Namsan, so participants could get a beautiful view of Seoul city. The last group of participants visited Insa-dong and Namsan Hanok Village (Korean's original style of houses) to know more about the traditions of Korea. At Insa-dong, the participants could stroll and shop along the alleys which are lined with traditional teahouses, antique galleries and Korean restaurants.

After two previous successful meetings, the AFSN 3rd Annual Meeting and Symposium gave an opportunity for members to share their experiences with each other again, through networking, scientific sessions and tours. The friendship among members was built or further strengthened.

It was a great honour for me to hold the AFSN 3rd Annual Meeting and Symposium in Seoul, Korea. I would like to express my appreciation to all of you AFSN members who participated in this meeting.

(continued from page 1)

The annual meeting of the International Forensic Strategic Alliance (IFSA) was held on 16 September 2011 in Funchal, Madeira, Portugal, in conjunction with the 19th International Association of Forensic Sciences (IAFS) meeting. This meeting was attended by representatives from the following member organisations:

- Academia Iberoamericana De Criminalistica Y Estudios Forenses (AICEF)
- American Society of Crime Laboratory Directors (ASCLD)
- Asian Forensic Sciences Network (AFSN)
- European Network of Forensic Science Institutes (ENFSI) and
- Senior Managers of Australian and New Zealand Forensic Laboratories (SMANZFL)

In addition, the United Nations Office on Drugs and Crime (UNODC) also participated in the meeting.

At the start of the meeting, AICEF which was represented by Prof Jose Lorente officially took over from ENFSI as the Chair of IFSA for the coming year. One exciting development during this meeting was the addition of a new member, the Southern Africa Regional Forensic Science (SARFS) network, bringing the total number of members to six.

Mr Paul Ludik gave an introduction to SARFS. This organisation covers 15 countries in the South Africa region, with a population of approximately 200 million. Forensics has been established under the umbrella of the Southern African Regional Police Chiefs Cooperation Organisation (SARPCCO) since 1998 and its growth has been rapid. SARFS was established as an executive committee to deal with forensic issues and with the fundings, meetings are held regularly whereby resolutions on harmonisation and standardisation for forensic science in the region are approved.

All IFSA members also gave an update on their respective organisations. AFSN was represented by its President Mr Lim Kong Boon, Vice-President Dr Heesun Chung and International Liaison Officer Dr Lam Kian Ming. Dr Lam gave an update on some of AFSN's activities in the last year. AFSN held its 3rd Annual Meeting and Symposium in May 2011 in Seoul, Korea, which was attended by 392 participants from 23 countries. Currently, AFSN has 27 members from Brunei, China, Indonesia, Korea, Lao PDR, Malaysia, Mongolia, Philippines, Singapore, Thailand and Vietnam. AFSN has an annual newsletter, *ForensicAsia*, which will be publishing its 3rd issue at the end of 2011. Dr Lam also shared the result of an AFSN survey which was presented at the annual meeting in May. This survey is useful for formulating strategy for laboratory twinning. Lastly, Dr Lam presented the strategic plan of AFSN, which includes creating a database of all forensic scientists from member institutes to enhance collaboration and networking, increasing the number of member institutes and seeking recognition from respective governments to recognise AFSN as the official reference on matters relating to forensic science.

There were many issues presented and discussed at the meeting. UNODC presented the results of a survey on international collaboration. The survey which was conducted via the online survey tool SurveyMonkey® was an important means to gather information on coordinating technical assistance: to determine the type of assistance provided and by which organisation. The survey found that laboratories seeking assistance do have multiple sources of donors and better coordination could help to avoid duplication. The IFSA members were also encouraged to help market this survey in future to increase participation even further. There was also a discussion on promoting accreditation and how IFSA members might assist each other. Both ENFSI and ASCLD shared about mentorship programmes in their regions.

The next IFSA meeting will be held in conjunction with the 21st International Symposium on the Forensic Sciences of the Australian and New Zealand Forensic Science Society (ANZFSS) on 23 – 27 September 2012 in Hobart, Tasmania, Australia. Dr Justice Tettey of UNODC has further kindly offered to explore assistance from the UNODC to host a forensic issues expert group in Vienna to do some planning for IFSA in early 2012.

This meeting will look deeper into the vision and mission of IFSA and how we could best work towards that goal collectively as an alliance.

This IFSA meeting in Madeira has been an exciting time where there were lots of sharing of current happenings, projects, and collaborations in each network. It has also presented all networks with opportunities to collaborate and exchange information. There is certainly a lot more that IFSA hopes to achieve and we look forward to strengthening forensic science together with all networks in this strategic alliance in the coming year!

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All results correspond to analysis by Cason and Maheux [1, 2] except that the proton NMR spectra in our experiment provided slightly different chemical shifts compared with Cason's experiment, perhaps, due to differences in the basic and salt forms of the substance and solvents used.

FTIR

Infrared spectra were obtained with a Bruker IR Spectrometer. The resolution was set at 4.000 cm^{-1}, with 32 scans between 400 cm^{-1} and 4000 cm^{-1}.

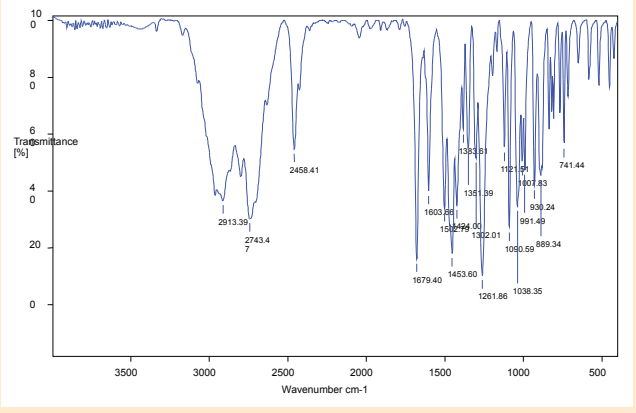


Figure 4: The FTIR of N-methyl-3,4-methylenedioxycathinone from white crystal.

Conjugation of the carbonyl group with the methylenedioxyphenyl group results in absorption (1679.4 cm^{-1}) within the theoretical 1685-1666 cm^{-1} range[2] as shown in Figure 4.

Discussion and Conclusion

This is the first time for our laboratory to detect N-methyl-3,4-methylenedioxycathinone (methylone) in a case study. GC-MS, NMR and FTIR were used for identification of N-methyl-3,4-methylenedioxycathinone hydrochloride salt in form of white crystalline powder. Combining this finding with analysis of 2C-I, 2C-E, 2C-B (we will give more reports of these substances later) in our laboratory, we suggest that more attention should be paid to these designer drugs in China and Asia.

Acknowledgement

The authors would like to thank Ms Xu Meifeng for providing excellent NMR spectra.

References

1. T. A. D. Cason, "The characterization of some 3,4-methylenedioxycathinone (MDCATH) homologs," *Forensic Science International*, vol. 87, no. 1, pp. 9-53, 1997.
2. C. R. Maheux, et al., "Characterization of Three Methcathinone Analogs: 4-Methylmethcathinone, Methylone, and bk-MBDB," *Microgram Journal*, vol. 7, no. 2, pp. 42-49, 2010.
3. N. V. Cozzi, et al., "Inhibition of plasma membrane monoamine transporters by b-ketoamphetamines," *European Journal of Pharmacology*, vol. 381, no. 1, pp. 63–69, 1999.
4. United Nations, *Recommended methods for the identification and analysis of amphetamine, methamphetamine and their ring-substituted analogues in seized materials.* New York: United Nations, 2006.

Introduction to the Scientific Working Group for the Analysis of Seized Drugs

Mr Scott Oulton
Chair, SWGDRUG

SWGDRUG members at the Boston Meeting in July 2011.

It is my honour to contribute to the AFSN *ForensicAsia* Newsletter and introduce the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG).

History

Abuse and trafficking of controlled substances are global problems, and in recent years law enforcement has looked to international solutions for these problems. In 1997, the U.S. Drug Enforcement Administration (DEA) and the Office of National Drug Control Policy (ONDCP) co-sponsored the formation of the Technical Working Group for the Analysis of Seized Drugs (TWGDRUG).

Forensic scientists from the United States, England, Canada, Australia, Japan, Germany and the Netherlands, as well as representatives of the United Nations, several international forensic organisations and academia were invited to meet in Washington, DC. In 1999, this group, with input from the international forensic science community, finalised recommendations for the education and professional development of forensic drug practitioners. Two years later, recommendations to enhance quality assurance protocols and define methods for the analysis and identification of seized drugs in forensic laboratories around the world were adopted. The SWGDRUG name was adopted in 1999.

Mission

SWGDRUG is comprised of a core committee of more than 20 forensic scientists from around the world. The mission of SWGDRUG is to recommend minimum standards for the forensic examination of seized drugs and to seek their international acceptance. SWGDRUG Core Committee members have received thousands of responses to international surveys and requests for comments from forensic drug analysts. Methods of communication have included our website (www.swgdrug.org), MICROGRAM, presentations at numerous local, national and international meetings, and personal contacts.

All SWGDRUG recommendations and supplemental documents reside on our website and are available for download at any time. The main recommendations cover a wide aspect of seized drug aspects, including a code of professional practice, education and training, methods of analysis, and quality assurance practices. The supplemental documents are intended to be a resource for those individuals responsible for implementing SWGDRUG recommendations. Our supplemental documents cover implementation of a code of professional practice, quality assurance and validation examples for numerous techniques along with an example validation plan, and examples for estimating the measurement uncertainty associated with seized drug weight determinations.

Resources and Tools

The SWGDRUG website contains, or links to, numerous tools and resources to help seized drug analysts and laboratory management meet the challenges of their work. Seized drug chemistry is becoming a more demanding field every year, both analytically with dozens of new synthetic drugs coming into the marketplace and from a regulatory perspective with increasingly rigorous accreditation standards being implemented throughout the world. The amount of information and expertise needed to meet these demands constant vigilance and professional development.

SWGDRUG has compiled a mass spectral library to be used for investigative purposes only. We ask that the library be used as a tool to obtain preliminary information about their unknown substances. Final confirmation and identification should always be done using traceable reference materials.

Also on the website are numerous links to external resources which SWGDRUG considers as excellent references for all labs. Examples include links to resources from the European Network of Forensic Science Institute (ENFSI), A Focus for Analytical Chemistry in Europe (Eurochem), United Nations Office on Drugs and Crime (UNODC), and numerous measurement uncertainty resources. SWGDRUG would like our website to be viewed as a portal to the larger world of seized drug chemistry.

The website also contains links to numerous forensic science organisations including AFSN and scientific journals. We encourage all seized drug analysts and managers to be aware of, and involved with, the larger seized drug community.

Current and Future Direction

SWGDRUG is currently working on multiple projects to further assist the seized drug community. These include recommendations for the analysis of evidence from suspected clandestine laboratories and recommendations on uniform reporting language and formatting. We are also working with ENFSI in developing training guidelines for new seized drug analysts.

Expertise in our field is not limited to a single country or organisation. We have worked consistently over the past 14 years to help provide the seized drug community with universal standards and recommendations to assist all analysts in the performance of their duties. The SWGDRUG committee has been internationally focused from the beginning and our strength lies in our diversity of views and experience.

I look forward to further developing a relationship between SWGDRUG and the AFSN. It is only through international cooperation and openness that we can all ensure that the best forensic science standards are being utilised in all laboratories.

Quality Management – Its Evolving Role in the Forensic Sciences?

Mr Alastair Ross AM
Director, Australia New Zealand Policing Advisory Agency National Institute of Forensic Science (ANZPAA NIFS)

"Quality is not an act. It is a habit."
- Aristotle 384BC-322BC

Obviously, people were thinking about matters related to quality long before the advent of the forensic sciences as we know them. As forensic practitioners, we may be relative late-comers to the concept and practice of quality management but it should be an integral part of our working philosophy. A properly developed and embedded quality system provides a basis for confidence in the end product for both the practitioner and the end user. Such a system includes training and competency testing for individuals, documentation and validation of test methods, calibration of instruments, quality control of procedures and peer review of outputs. In a quality-based organisation, these are the shared responsibilities of both managers and practitioners.

Indeed, quality management is defined by the International Organisation for Standardisation (ISO) 9000 program as:

"…that aspect of the overall management function that determines and implements the quality policy."

From a forensic science perspective, it is essential that quality is built in at every step of the forensic process; inclusive of the crime scene, all tests and examinations, the interpretation and qualification of results and the presentation of expert evidence. It can not be added in at the end [1].

Those aspects of the forensic sciences that warrant focus with respect to quality management can be summarised as the provider (laboratory), the practitioner, the process and the product. In 2009, The National Research Council of the National Academies in the USA produced a report titled "Strengthening Forensic Science in the United States: A Path Forward". The report is critical of the level of quality management in the forensic sciences in the USA:

"There is no uniformity in the certification of forensic practitioners or in the accreditation of crime laboratories. Moreover, accreditation of crime laboratories is not required in most jurisdictions."

In 2008, the British Government created the Office of the Forensic Science Regulator and the appointed Regulator was critical of the registered forensic practitioner's scheme operating in the UK at the time. He advocates a 'one-stop-shop' for both accreditation and certification and there is considerable merit in that approach.

There is a balance in all of this, though. As the forensic sciences community, we need to be careful not to overburden ourselves with unnecessary processes under the guise of quality management. We should not countenance quality management programs that are so prescriptive that they take away incentive and stifle initiative, such that ideas and opportunities for enhancement of the forensic sciences are missed. A key element of any quality system is continuous improvement and there must be room in any programme to allow for this to flourish.

In Australia, many of the forensic science service providers are reviewing their quality systems because over time, they have layered additional requirements over those expected or mandated. The programmes have become cumbersome and difficult to manage. A key aspect of an accreditation programme is that an organisation must do what its policies, procedures and protocols say it will do. The more detail that is added, the more difficult it is to meet and assess the documented requirements. Prescriptive and detailed procedures and protocols also limit the flexibility required in the non-routine fields of the forensic sciences.

Increasingly, accreditation is seen as a risk mitigation strategy both for the accreditation agencies and for laboratories. Obviously, there are stipulated criteria which must be met. However, consideration needs to be given to levels of acceptable risk so that additional requirements, often self-imposed by the forensic science organisation, are not continuously built up as layers over the original criteria. Any accreditation or certification programme should be 'fit for purpose'.

An example of this is the emergence of a preference for the use of the international standard ISO 17020 for crime scene accreditation, particularly in Europe. In brief, ISO 17020 is focused more on inspection activities than on analytical processes which are more the focus of ISO 17025. Most established forensic science accreditation programmes are based on the latter standard (ISO 17025) and where crime scene investigation is included in these programs it is also covered by this standard. Increasingly, volume crime scenes such as house burglary and theft of and from motor vehicles are being attended for the purposes of collecting forensic evidence; in particular DNA, fingerprints and shoe impressions. Data from these scenes in addition to increasing crime clearance rates are enhancing national databases and the capability of forensic science to contribute to criminal intelligence.

Often, the officers attending volume crime scenes have a lower level of training (but fit for purpose) than those who attend major crime scenes such as homicides and sexual assaults. Also, they do not conduct any analysis of the samples that they collect; these being passed on to those with a more appropriate skill set to conduct the analysis. It could well be argued that accreditation to ISO 17020 is more appropriate than ISO 17025 for this particular situation as it adequately mitigates the risks associated with producing a quality product and is less onerous to apply.

For those new to the concept of accreditation, a lot can be learned from those facilities already accredited and from the accreditation agencies. This includes how documentation (manuals, protocols and procedures) is best structured, for example, with the use of flow charts and other aids. It could also include clarification of the role of accreditation criteria, how to establish effective audit programmes and how to manage proficiency testing.

However, having taken the opportunity to learn from others, it is vital that any facility seeking a quality management system develops its own capacity through the engagement of all its people. There has to be a collective understanding of what the implications and benefits of quality management are and how it will impact the organisation and the individual. Acceptance of and commitment to quality management by everyone in an organisation is difficult and perhaps impossible to achieve particularly in the short term. However, optimising opportunity for engagement and comment will encourage most people to 'come on the journey'.

In the forensic sciences, our end product often plays a key role in effective justice outcomes. To that end, a key aim in the evolution of quality management in the forensic sciences should be that it becomes a habit and not an act.

Reference

1. A. Ross and A. Davey, "Quality Management and Expert Evidence," in *Expert Evidence* (Freckelton and Selby), Law Book Company Limited, 2011.

The Challenges of Drug Precursor Control

Dr Barbara Remberg[1]
International Narcotics Control Board (INCB), Austria

Introduction

Chemicals[2] are critical for illicit drug manufacture. At the same time, many of them have a wide range of legitimate uses in various branches of industry and only a fraction of the volumes used legitimately is sufficient to manufacture the drugs required to meet the global illicit demand.

The international precursor control regime, governed by Article 12 of the United Nations Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances of 1988 (1988 Convention), currently applies to 23 substances.[3]

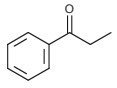
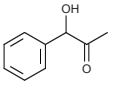
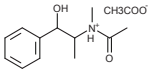
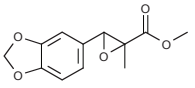
The International Narcotics Control Board (INCB) plays a central role in the international precursor control system by assisting Governments in preventing the diversion of chemicals. Specifically, INCB provides platforms for: (1) the exchange of information on legitimate trade, and (2) operational cooperation to investigate suspicious orders, stopped shipments and seizures of precursors. INCB is also responsible for assessing chemicals to determine whether they should be placed under international control.

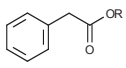
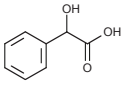
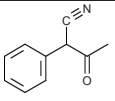
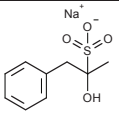
Latest Developments and Challenges

In response to better global controls of the primary precursors, major developments have included the use of:

- **pre-precursors**, i.e. non-scheduled precursors of the controlled primary precursor;
- different **products** containing the controlled primary precursor, for example, pharmaceutical preparations and natural products containing ephedrine and pseudoephedrine (e.g. *Ephedra* extracts);
- **derivatives** of the controlled primary precursor, such as esters, amides, or derivatives made purposely to circumvent existing controls; and
- **new or altered methods of processing or manufacture and the illicit manufacture of "designer" drugs** that require non-scheduled precursors as starting material.

Specifically, the following non-scheduled substances have emerged:

Precursors for ephedrine and pseudoephedrine			**Precursor for 3,4-MDP-2-P (PMK)**
			
P-1-P (syn.: propiophenone)	*l*-Phenylacetylcarbinol (*l*-PAC)	*N*-Acetylpseudoephedrine acetate	Methyl 3-[3',4'-(methylenedioxy)-phenyl]-2-methyl glycidate ('3,4-MDP-2-P-methyl glycidate')
CAS# [93-55-0]	CAS# [90-63-1]	CAS#	CAS# [13605-46-6]

Precursors for phenylacetic acid and P-2-P (BMK)			
			
Esters of phenylacetic acid	Mandelic acid	*Alpha*-phenylaceto-acetonitrile (APAAN)	P-2-P bisulphite
CAS# (various)	CAS# [90-64-2]	CAS# [4468-48-8]	CAS#

Many of these derivatives can be easily converted to the controlled primary precursor by hydrolysis. Others are common intermediates. What is new is that there are now attempts by illicit operators to source these chemicals in large scale. Examples include APAAN, an intermediate in the manufacture of P-2-P from benzyl cyanide, and *l*-PAC, an intermediate in the manufacture of ephedrine by the fermentation of yeast. P-2-P bisulphite is a common product in the purification of P-2-P. Being a white to off-white crystalline substance, it allows traffickers to disguise the identity of P-2-P (a yellowish liquid).

In terms of volume, phenylacetic acid derivatives, specifically esters, dominate amphetamine-type stimulant (ATS) precursor seizures, with almost 550 tons having been reported seized since the beginning of 2011. Individual seizures involved 10 to 60 tons; with one seizure of phenylacetamide amounting to almost 900 tons. That these volumes of precursors are indeed destined for illicit ATS manufacture is reflected in recent seizures of illicit laboratories and chemical warehouses in Mexico, which correspond in size to the multi-ton precursor seizures (Figure 1). Illicit laboratory seizures also identified a number of chemicals necessary for the conversion of phenylacetic acid to P-2-P, such as acetic anhydride, as well as chemicals required for reductive amination methods, thus indicating a shift from pseudo/ephedrine towards P-2-P-based methods.

Moreover, available forensic laboratory data suggest that illicit operators have the technical know-how and equipment to produce very high-purity *d*-methamphetamine despite employing an achiral syntheses (starting from P-2-P). This is evidenced by seizures of the required chemicals (such as tartaric acid) and from drug characterisation and profiling studies.

Figure 1: Illicit methamphetamine laboratory in Querétaro, Mexico, June 2011. (Photo: Procuraduría General de la República, México)

In terms of overall character, the illicit ATS market appears to become increasingly more global and organised, with the sourcing of precursors becoming a separate criminal industry from illicit drug manufacture. While diversion from legitimate trade still supplies the major share of ATS precursors, there are indications of organised crime groups specialising in the illicit manufacture of primary precursors, with subsequent smuggling to destination countries.

Responses and Outlook

In response to the observed changes in ATS precursor seizures, especially the shift to P-2-P-based methods, INCB has begun to look into the international trade in phenylacetic acid esters. This activity, carried out in close cooperation with national regulatory authorities, has drawn the attention of those authorities and industry to the potential of these esters being used in illicit ATS manufacture. It has also resulted in the aforementioned seizures and revealed that Central American countries have quickly been targeted in response to more stringent controls introduced in Mexico.

More generally, in response to the use of substitute chemicals, INCB established already in 1998 a Limited International Special Surveillance List of non-scheduled substances (ISSL). ISSL is aimed at aiding Governments in targeting those non-scheduled substances most likely to be diverted from legitimate

(continued on page 10)

1 The views expressed herein are those of the author and do not necessarily reflect the views of the United Nations or the International Narcotics Control Board.
2 This includes essential chemicals, reagents, catalysts and other substances used in illicit drug processing (extraction, synthesis, purification, refining, etc.).
3 A list of the 23 substances as well as an overview of the functions of the INCB and major trends in licit trade and illicit trafficking in precursors is provided in the annual INCB Precursor Report, accessible at: http://www.incb/precursors_reports.html). Information about identification and analysis is available at: http://www.inodc.org/unodc/en/scientists/substances-for-the-manufacture-of-narcotic-drugs-and-psychotropic-substances.html

Scientific Investigation Laboratory, Korea

Dr Jeun Chung-hyun
Scientific Investigation Laboratory, Korea

The Scientific Investigation Laboratory (SIL) was established in 1953 under the Ministry of National Defense (MND). Its key mission is to provide investigators with scientific information to solve incidents within the military through scientific analysis of physical evidence or on-site investigation. The SIL also provides education on scientific investigation to investigators in the Army, Navy, and Air Force.

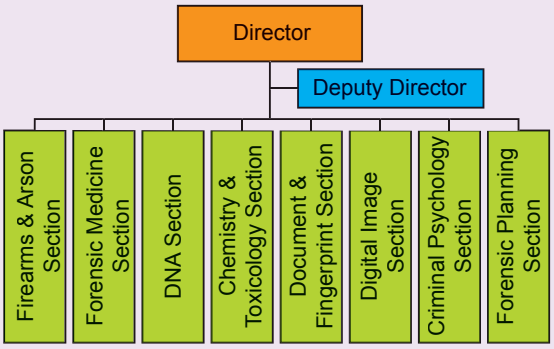
History

In 1953, the SIL was established under the MND. In 2006, the SIL was merged with the Criminal Investigation Command (CIC), MND. The SIL was accredited under the Korea Laboratory Accreditation Scheme (KOLAS) in 2009 and awarded the grand prize of forensic science from the National Police Agency, Korea, in 2010.

Organisation

The SIL has 8 sections dedicated to carrying out rapid and precise examinations to solve incidents in the military.

The key missions of each section are as follows:



- The Firearms and Arson Section performs experiments involving explosives, fires, restoration of obliterated numbers, and carries out identification of firearms.
- The Forensic Medicine Section conducts examinations related to postmortem autopsy, pathological study of tissues, detection of plankton, and forensic inquiry.
- The DNA Section examines DNA in samples retrieved from crime scenes. Also, a unique service offered by the SIL is human identification via a database which was established with the remains of soldiers who died during the Korean War (1950-1953) and the blood of their bereaved family members.
- The Chemistry and Toxicology Section performs the analysis of trace evidence of fibre, paint, soil, rubber and accelerants, alcohol, drugs and poisons in postmortem and related samples.
- The Document and Fingerprint Section compares handwriting and other traces left on paper. It examines ink, paper, printer, and toner to confirm the possibilities of forgery. Also, this section develops and examines fingerprints and handprints on firearms and documents to identify individuals, and examines footprints as well.
- The Image Analysis Section enhances the quality of images or video footage and compares diverse images and video clips. This section reconstructs crime scenes suited to each case through special shootings such as microscopic, three-dimensional and ultraviolet shootings.
- The Criminal Psychology Section has been conducting polygraph examinations on military criminals since 1960, and psychological autopsy since 2007. The section aims to verify the truthfulness of statements with polygraphs, computerised voice stress analyzer, observation of behavioral symptoms and statement-analysing. It also uses brainwave analysis to detect criminal-related information.
- The Forensic Planning Section is in charge of administrative tasks, such as receiving and returning physical evidence, and maintaining the KOLAS system.

Research and Quality Assurance Activities

The SIL consistently makes an effort to provide speedy and precise results and meet the requirements of the investigators. The SIL has been performing a variety of studies, such as those on estimation of hand grenade explosion pattern, mtDNA extraction from old bones, brainwave analysis, 3D image construction, fibre analysis, soil discrimination and Military Intelligence Analysis Support in times of war. The SIL received an accreditation from KOLAS in 2009, and is incessantly working towards the goal of providing more reliable output.

Department of Medical Sciences, Thailand

Ms Sooksri Ungboriboonpisal
Department of Medical Sciences, Thailand

The Department of Medical Sciences (DMSc) was founded on 10 March 1942 under the auspice of the Ministry of Public Health, Thailand. Since then, the department structure has been reorganised occasionally to improve its efficiency and responsibilities to serve the public nationwide.

The DMSc consists of 26 Sectors:

- 12 Center Agencies located at the Department headquarters at Nonthaburi province near the suburb of Bangkok.
- 14 Regional Medical Sciences Centers (RMSc) located at different provinces around the country; which are, Trang, Udon Thani, Chon Buri, Samut Songkharm, Nakhon Ratchasima, Khon Kaen, Ubon Ratchathani, Nakhon Sawan, Phitsanulok, Chiang Mai, Surat Thani, Songkhla, Chiang Rai and Phuket.

Every sector in the DMSc carries out work on research and development, analysis of health products for consumer protection purposes, and provides analytical testing services for the nation. One of the DMSc's main responsibilities is to provide testing services for illicit drugs, narcotics, and abused substances in Thailand.

The DMSc is designated as the national quality control laboratory for testing the quality of medicines and testing of narcotics, and assigns the Bureau of Drug and Narcotic (BDN) to act as the department's focal point to handle the strategic plans and budgets for drug and narcotics testing. The BDN works in cooperation with the other 14 Regional Centers to provide analytical testing services including testing of seized materials, drug precursors, psychotropic substance, and urine drug testing. Tens of thousands of samples have been analysed annually. The analysts also serve as expert witnesses in the court for reported cases issued by them.

The DMSc also acts as a National Proficiency Testing (PT) Provider for pharmaceutical and narcotics testing. Different PT programmes provided include the PT programme for Narcotics

Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University, Thailand

Dr Nat Tansrisawad, Dr Kornkiat Vongpaisarnsin
Chulalongkorn University, Thailand

History and Background

The Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University, was established in 1968. When the department was first established, its purposes were to educate undergraduate medical students and to provide medico-legal service in King Chulalongkorn Memorial hospital. Afterward, a training programme for the certification of proficiency in Forensic Medicine was set up. Since the amendment on the code of criminal procedure on postmortem investigation in 1999, the department was authorised to investigate crime scenes and conduct autopsy in the center and eastern area of Bangkok metropolitan. In 2010, our mission was to encourage excellence in academia, service and research. Practices were performed under international guidelines and/or standards, and we sought accreditation in all laboratory services, developed international curricula, and conducted basic and applied research in collaboration with international institutes.

Organisation

The Department of Forensic Medicine has 4 divisions: Forensic Pathology, Forensic Clinic, Forensic Serology and Genetics, and Forensic Toxicology. We also offer many educational programmes appropriate for a student's requirement. The curriculum for undergraduate medical students includes forensic medicine, professional laws, and medical ethics. We run two postgraduate curricula: Certificate of Proficiency in Forensic Medicine and Master of Medical Science in Forensic Medicine.

Since 2010, the department's focus has been on research. We have many materials and resources which can be used in teaching and conducting research. We seek international collaboration and are pleased to invite all researchers to work with us.

Forensic Pathology Division

The work of forensic pathology covers all aspects of medico-legal postmortem investigation: scene investigation, autopsy, and being an expert witness in the court. About 1,500 scene investigations and 1,000 autopsies are performed each year. The Division has handled many interesting cases, including 2,002 cases of hidden fetal remains in the morgue of a temple in November 2010.

Forensic Clinic Division

The duty of the Forensic Clinic Division is to take care of the injured patient who possibly has medico-legal problems, for example, sexual abuse, domestic violence, and traffic accident.

Forensic Serology and Genetics Division

The Forensic Serology and Genetics Division has competent staff and sophisticated instruments for areas such as STR, insertion/deletion study, mitochondrial DNA, and SNPs. The services include paternity testing, criminal DNA matching, and identification. Many research projects with international collaboration are undertaken in this division.

Forensic Toxicology Division

The Forensic Toxicology Division serves clinical patients and carries out postmortem toxicological investigation.

Our Philosophy

"We are committed to achieving academic excellence and serving the public with virtue."

Major Milestones of Department of Forensic Medicine

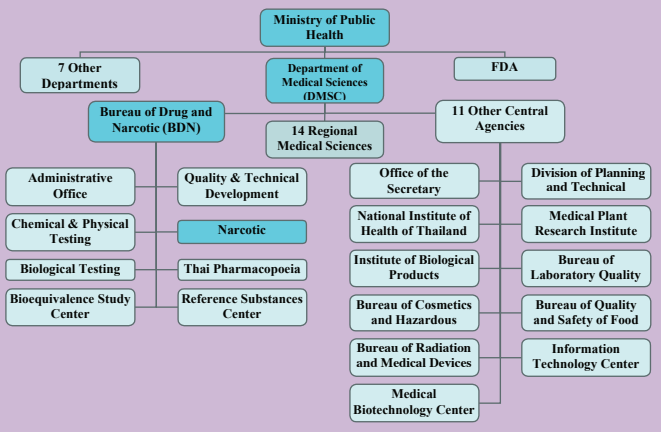
2006 - Working in Tsunami disaster of the southern part of Thailand
2007 - Establishment of the Forensic Medicine Journal (3 prints/year, in Thai)
2011 - Start the medical student exchange programme
- Support a visiting professor grant (Professor Bruce Budowle)
- Accomplishment in research collaboration with the University of North Texas Health Science Center and the Yale School of Medicine USA
- Official member of AFSN

testing and the PT programme for urine drug testing. In order to suppress and control drug abuse, one of the department's activities is to provide test kits for the government organisations nationwide.

Department of Medical Sciences.

The DMSc shares its responsibility with the Thai Food and Drug Administration (FDA) in drug regulation. Senior staff are members of subcommittees and advisory bodies to provide their expertise in specialised areas. It is responsible for monitoring the quality of medicines available in the market. This is conducted via the annual surveillance programme, jointly exercised by Thai FDA and provincial health offices, where reports are published and quality alerts are issued to all parties concerned.

The DMSc enhances the quality of laboratories' quality services by strengthening the implementation of the international quality system ISO/IEC 17025 and participating in the International Quality Assurance Programme (IQAP) provided by UNODC, both in Seized Material (SM) group and Biological Specimens (BS) group. The DMSc develops reliable and practical analytical methods for medicines, narcotics and recombinant pharmaceutical products and provides training, workshops and seminars in drug abuse testing to government officials, pharmaceutical industries and officers of the other countries in the region, in collaboration with international agencies, e.g., WHO and SIDA, etc.



Institute of Forensic Medicine, Police General Hospital, The Royal Thai Police, Thailand

Police Colonel Pornchai Suteerakune
The Royal Thai Police, Thailand

The Beginning

Medico-legal activities in Thailand began way back in 1898 when the Royal Police Force in Bangkok built a small hospital to take care of ill police officers as well as to examine the injured and those who died of unnatural death. In 1916, the Royal Police Force was upgraded to the Royal Police Department that was responsible for the whole country.

Formation of the Institute of Forensic Medicine

In 1952, The Police Hospital was built with facilities that included a Forensic Unit, which later expanded to **The Institute of Forensic Medicine** (IFM) in 1979. Thailand Medico-legal investigation system is based on the **Police system**, and each forensic organisation is responsible for its respective geographical area. On average, IFM handles more than 40,000 cases annually for Bangkok city and surrounding provinces.

Organisation Structure

The Institute comprises 4 forensic subdivisions and 2 supporting units:

- **Subdivision of Forensic Pathology** provides forensic medicine service in the examination of unnatural death cases. Our forensic pathologists attend crime scenes and carry out examination in the Mortuary to determine the cause of death. At the crime scene, our own **Police Special Unit** provides support for the forensic pathologist working on the case and helps to maintain the scene.

- **Subdivision of Toxicology** performs analysis of drugs of abuse, toxic substances and blood alcohol content in biological specimens of dead persons, suspects and patients. It also identifies the toxin that may be a cause of death or illness, monitors drug level for treatment, and supports law enforcement.

  Our Laboratory participates in various forensic networks in Thailand, such as the Drug Abuse Network to share data of suspects in drug trafficking, and the Forensic Toxicology Network to standardise standard protocols and forensic laboratory testing in Thailand.

- **Subdivision of Biochemistry** provides Forensic Biology/ DNA services. This subdivision performs analysis and identification of biological fluids and tissues for the purpose of determining their source, species, origin, and genetic composition. Subsequently, DNA analysis produces DNA profiles that will link the material to a person. DNA analysis routinely uses nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) for the identification of a deceased. Y-STRs are used for examining sexual assault evidence and tracing paternal lineages. X-STRs are used for kinship and paternity testing.

  We also collaborate with the Police Office of Forensic Science in developing the National Databank containing DNA profiles in the country.

  In 2008, the laboratory participated in a quality assurance programme administered by AICEF. In 2011, the laboratory is working towards ISO/ IEC 17025:2005 in order to improve the quality and technical competency of staff.

The Institute of Forensic Medicine, Police General Hospital, Royal Thai Police.

One of the autopsy cleanrooms with 5 autopsy beds.

Analysis of drugs and metabolites using LC/MS (TOF) and LC/MS/MS (Ion Trap).

- **Subdivision of Identification** comprises pathologists specialised in forensic anthropology for the identification of missing persons, unidentified human remains and disaster victims by cooperating with **Subdivision of Biochemistry** for DNA analysis and **Medical Illustration Unit**, for superimposition technique.

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trade in a flexible manner, preventing their use in the illicit manufacture of drugs and, at the same time, being sensitive to the requirements of legitimate trade. The ISSL, which has grown since its establishment to now include 52 substances, is distributed to regulatory authorities for use with industry.

Moreover, there is a clear need to improve the gathering and sharing of information on precursors seized from legitimate trade and illicit laboratories. Currently, and all too often, a precursor seizure is considered the end of an investigation rather than its beginning. To support investigations into precursor cases and prevent future diversions, it is paramount to obtain and share information that will help to identify the sources of seized precursors, the methods of diversion and smuggling, and the individuals involved.

Forensic laboratories are currently under-utilised as a resource in precursor control. Their expertise does not only support precursor investigations before, during, and after an illicit laboratory raid and the identification of substitute chemicals. Systematic forensic characterization of finished drugs also helps to understand the specific manufacturing methods used, the chemicals employed and any links between seizures. Moreover, the pooling and sharing of information from these analyses between forensic networks and with bodies such as INCB, Interpol, the World Customs Organization and the United Nations Office on Drugs and Crime supports international precursor investigations.

The AFSN Illicit Drugs Workgroup is well placed to address these issues.

Inauguration of Forensic Science Center (FSC) as a National Judicial Authentication Institution, China

Mr Zhang Rui
Forensic Science Center of Guangdong Province, China

On 6 May 2011, the Public Security Department (PSD) of Guangdong Province held a ceremony for the inauguration of its Forensic Science Center (FSC) as a national judicial authentication institution. So far, the FSC of Guangdong Provincial PSD is the only provincial department level unit of China which is approved as a national judicial authentication institution.

Mr Huang Huahua, Governor of Guangdong Provincial Government, and Mr Li Dongsheng, Deputy Minister of China's Public Security Ministry, unveiled the national judicial authentication institution tablet for the FSC of Guangdong Provincial PSD in the ceremony, which was presided over by Mr Liang Weifa, General Director of Guangdong Provincial PSD and member of Provincial Party Committee.

Also in attendance at the ceremony were the chief officers of the Legal Committee of the Provincial Party Committee, the Provincial Higher People's Court, People's Procuratorate, National Security Department, Judicature Department, Development and Reform Committee, Science and Technology Department, Human Resources and Social Security Department, Metrology Institute, and all the municipal level Public Security Bureaus of the whole province.

Firmly pursuing the mission of "serving all the policing affairs and supporting the work of criminal investigation" and with the full support from the leaders of different higher levels and related departments, the FSC of Guangdong Provincial PSD has been playing more and more important roles in the security tasks of major events such as the Guangzhou 16th Asian Games, as well as dealing with urgent and serious cases, and the works of judicial authentication in recent years.

Mr Liang Weifa, General Director of Guangdong Provincial PSD, presided over the inauguration ceremony.

The FSC has also been successful in achieving a series of remarkable awards. In 2008, the FSC became the first forensic centre of provincial level to be accredited by the China National Accreditation Service (CNAS). In 2010, the subordinated forensic pathology laboratory of the FSC successfully passed the mid-term assessment as one of the key laboratories of China's Public Security Ministry. In October of the same year, the FSC was approved as the only provincial departmental level unit in China to achieve the qualification of national judicial authentication institution, which means that it is the 'pioneer' of all the FSCs of Provincial PSD level.

Mr Huang Huahua and Mr Li Dongsheng unveiled the national judicial authentication institution tablet.

Prediction of Ethnicity: A Challenge for Forensic Hair Investigation

Dr Waraporn Promwikorn[1], Ms Thaweporn Kerekoj[2]
[1] Central Institute of Forensic Science, Thailand *[2] Prince of Songkla University, Thailand*

Abstract

The microscopic characteristics of hair belonging to 2 Thai ethnic groups, Thai Malay and Thai Chinese, were compared. Nine of 16 hair characteristic data showed statistically significant differences between these 2 groups ($p < 0.05$). A discriminant function equation was next formulated from 3 variables (characteristics) which were uncorrelated and significantly different. The accuracy of the equation on ethnic prediction was 80%. This new challenge could be introduced as a new guideline for presumptive hair investigation.

Introduction

Hair is a type of evidence which is found in most crime scenes. Although recent DNA technology is efficient in identifying the owner of the hair sample, presumptive hair investigation is still required. However, up-to-date presumptive investigation of hair such as morphological comparison is still laborious, time-consuming and not fully reliable. It would be helpful if an easier and faster method which yields more accurate results is introduced and used in presumptive investigation of hair.

The relationship between hair microscopic characteristics and ethnicity have been studied among independent ethnic groups, such as comparisons among the Asian, Caucasian and African groups [1, 2]. Other studies also report that hair microscopic characteristics are different among people who have closely related ethnicity, for example, comparisons made among Hawaiians of 26-100% ethnicity [3]. This is interesting, as it could be useful in identifying suspects from other nationalities living in the same country. We hereby challenge a new method for forensic hair investigation by introducing a discriminant function equation for ethnic prediction using three data from hair microscopic characteristics, including cuticle thickness, cuticle scale count/100 µm hair length and cross section area.

Materials and Methods

Sample Collection

The volunteers were 20 Thai males, aged 20-25 years, living in the same region, and descended from Malay or Chinese parents. The two older generations of the volunteers have never been married to women from another ethnic group. A total of 100 strands of hair were taken from 5 anatomical head regions. This study was approved by the Ethics Committee of Prince of Songkla University and all volunteers gave written informed consent.

Hair Examination

Sixteen hair characteristics were examined, at the region of 13 mm away from the tip of the hair root, under a light microscope (LM), scanning electron microscope (SEM) and a transmission electron microscope (TEM). Five morphological hair characteristics, including the distribution of cortical pigment, the appearance of the medulla, and hair color data set, were examined. Hair characteristic data set included cortex color, cortical pigment color, medulla color, the distribution of cortical

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pigment, and the appearance of the medulla. The measuring data set included the cross section maximum diameter (D_1), cross section minimum diameter (D_2), hair diameter, medulla diameter, cuticle scale count/100 µm hair length (CSC), cuticle scale width and cuticle thickness (CT). The calculated data set included medulla index (a ratio of medulla diameter to hair diameter), scale index (a ratio of cuticle scale width to D_1), hair index (a ratio of D_2 to D_1) and cross section area (CSA, $(D_1 X D_2 / 4) \pi$).

Statistical Analysis

All morphological data were compared using the chi-square test. Most of the numerical hair data were compared using the student's t-test. Two of the numerical hair data including the cuticle scale width and scale index were compared using the Mann-Whitney-U test.

Results, Discussion and Conclusion

Comparison of Head Hair Microscopic Characteristics

The hair microscopic characteristics of Thai Malay and Thai Chinese, were examined using LM, SEM and TEM (Figure 1). It was found that, in total, 9 of the 16 hair microscopic characteristics were significantly different between the Thai Malay and Thai Chinese males. Five of the hair data, including scale index, cuticle scale width, CT, CSC and CSA, were highly significantly different between the 2 ethnic groups ($p < 0.01$). Four of the hair data, including medulla index, hair diameter, D_1 and D_2, showed significant differences ($p < 0.05$) (Table 1).

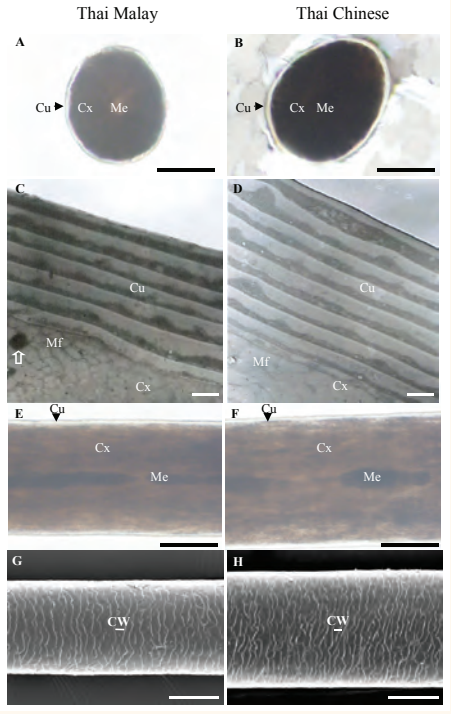

Figure 1: Images of Thai Malay and Thai Chinese hair shafts were photographed in cross section (A - D) and longitudinal (E - H) views with LM (A - B, E - F), TEM (C - D) and SEM (G – H) showing the cuticle (Cu), cortex (Cx), medulla (Me), melanin pigment granule (arrow) and macrofibril (Mf). Scale bars in A, B, E-F = 50 µm, C – D = 500 nm. CW = cuticle scale width.

Hair Characteristic Data		Ethnic Group		*P*-value
		Thai Malay (n = 50)	Thai Chinese (n = 50)	
1.	Hair diameter (µm)	111.5 ± 12.8	117.6 ± 18.2	0.027*
2.	Medulla diameter (µm)	23.3 ± 5.0	23.2 ± 7.5	0.445
3.	Cuticle thickness (µm)	3.2 ± 0.6	3.6 ± 0.6	0.001**
4.	Cuticle scale count/100 µm hair length	14.3 ± 1.3	15.0 ± 1.3	0.004**
5.	Cuticle scale width (µm)	7.2 ± 1.1	6.6 ± 0.8	0.003**
6.	D_1 (µm)	106.2 ± 11.1	111.9 ± 16.2	0.021*
7.	D_2 (µm)	80.2 ± 10.3	83.3 ± 7.8	0.049*
8.	Medulla index (%)	20.8 ± 3.9	19.1 ± 4.4	0.022*
9.	Scale index	6.8 ± 1.5	6.1 ± 1.1	0.003**
10.	Hair index	76.3 ± 11.2	75.6 ± 10.2	0.370
11.	Cross section area (µm²)	6,702.4 ± 1,152.5	7,353.6 ± 1,432.0	0.007**

* significant difference between the two ethnic groups ($p < 0.05$).
** highly significant difference between the two ethnic groups ($p < 0.01$).

Table 1: Measuring and calculated hair characteristic data obtained from 100 Thai Malay and Thai Chinese males.

The significant difference of hair characteristics may relate to the distinction of the gross appearance of the hair between the 2 ethnic groups. Thai Malay hair looks finer and weaker, thus causing wavier and fluffier hair, than the Thai Chinese hair. The hair diameter seems to correlate to other hair characteristic data as shown in Figure 2.

Figure 2: The relation of the hair diameter to other hair characteristic data.

Seven hair characteristics were similar between the Thai Malay and the Thai Chinese males. These included the medulla diameter, hair index and all the morphological hair characteristics examined.

Prediction of Ethnicity using the Discriminant Function Equation

In order to obtain the variables for formulating a discriminant function equation, the uncorrelated hair microscopic characteristic data, that showed statistically significant differences between the Thai Malays and Thai Chinese hair, were analysed. The obtained variables included the CT (mm), CSC and CSA (mm²). The formulated discrimination function equation was -12.178 + 1,062.658 CT + 0.455 CSC + 0.269 CSA. The data of CT, CSC and CSA were substituted in the equation to get a discriminant score. If the resulting discriminant score was below zero, the hair was predicted to be Thai Malay, and vice versa, if the discriminant score was above zero, the hair was predicted to be Thai Chinese. This method was 80% accurate in determining the ethnic origin of the hair samples (Table 2), which is a similar result in other studies [4], with 70% validity of the formulated equation.

Ethnic Group	Sample Number	Head Regions	CT (mm)	CSC	CSA (mm²)	DS	Ethnic Prediction	Correct/ Incorrect
Thai Malay	1	Front	2.71 x10⁻³	15.67	5.402885	- 0.7168	Thai Malay	Correct
	2	Middle	3.12 x10⁻³	14.67	5.589417	- 0.6788	Thai Malay	Correct
	3	Back	2.50 x10⁻³	12.67	5.579599	- 2.2556	Thai Malay	Correct
	4	Left temple	2.50 x10⁻³	13.33	5.821764	- 1.8902	Thai Malay	Correct
	5	Right temple	2.50 x10⁻³	15.33	4.977461	- 1.2073	Thai Malay	Correct
Thai Chinese	1	Front	3.33 x10⁻³	14.67	7.075128	- 0.0578	Thai Malay	Incorrect
	2	Middle	3.75 x10⁻³	15.00	8.469210	0.9102	Thai Chinese	Correct
	3	Back	3.75 x10⁻³	14.33	6.073746	- 0.0390	Thai Malay	Incorrect
	4	Left temple	4.58 x10⁻³	15.67	10.36398	2.6102	Thai Chinese	Correct
	5	Right temple	3.54 x10⁻³	15.33	8.937177	0.9649	Thai Chinese	Correct

CT = Cuticle thickness (mm), CSA = Cross section area (mm²), CSC = Cuticle scale count/ 100 µm hair length, DS = discriminant score.

Table 2: Prediction of ethnicity using the formulated discriminant function equation. Ten new independent hair samples were randomly selected. Two of the 10 samples were incorrectly classified.

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Identification of N-methyl-3,4-methylenedioxycathinone from the Unknown White Crystalline Substance

Dr Huang Xing, Ms Wang Weixin, Ms Xu Peng, Mr Zhang Chunshui, Dr Zhu Jun, Prof Gao Lisheng
Institute of Forensic Science, China

Abstract

N-methyl-3,4-methylenedioxycathinone (a.k.a. methylone) was detected recently by the drug laboratory of IFS (Institute of Forensic Science, Ministry of Public Security, P. R. China). The white crystalline substance concealed in an envelope was delivered by the police to our laboratory. The white crystalline substance was screened by colour tests and the presence of N-methyl-3,4-methylenedioxycathinone was confirmed by GC-MS, NMR and FTIR. This is the first report of finding this substance in China Mainland. It is suggested that more attention should be paid on cathinone family designer drugs in Asia.

N-methyl-3,4-methylenedioxycathinone

The tendency may be more common that most new designer drugs are prepared to circumvent existing legislation. Cathinone family, such as methylone, 4-methylmethcathinone, MDPV (3,4-methylenedioxypyrovalerone), etc. is becoming attractive to illicit drug "cookers" in the world. Seized methcathinone has been delivered to our lab many times. Recently, an unknown white crystalline substance (shown in Figure 1) concealed in an envelope was delivered by the police to our laboratory. After the anion test, colour test, GC-MS, NMR and FTIR were performed, the presence of N-methyl-3,4-methylenedioxycathinone (methylone) was confirmed. The structure of methylone is shown in Figure 2. It is also known as "M1", 3,4-methylenedioxy-N-methylcathinone and bk-MDMA. It is a stimulant of the phenethylamine, amphetamine, and cathinone classes. It was originally patented by Peyton Jacob and Alexander Shulgin in 1996 as an antidepressant. Methylone is a close structural analogue of MDMA, differing by the addition of a β-keto group [3]. All analytical results are shown below.

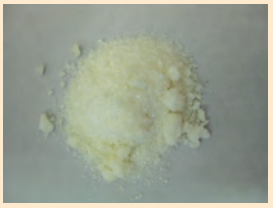
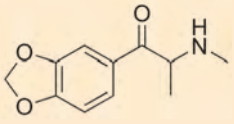


Figure 1: The unknown white crystalline substance submitted to the drug laboratory.



MW: 207.2

Figure 2: Structure and molecular weight of N-methyl-3,4-methylenedioxycathinone.

Anion Test

Approximately 1mg of the white crystalline substance was dissolved in several drops of deionized water and treated with 1-2 drops of 1.7% aqueous silver nitrate solution. A white curdy precipitate that is insoluble in concentrated nitric acid was formed. The precipitate is soluble in dilute ammonia solution, from which it can be re-precipitated by the addition of nitric acid, thus indicating the presence of the hydrochloride salt.

Screening Tests [1, 4]

The white crystalline substance produced a positive reaction to the Marquis reagent, presenting intense yellow. Simon's test was positive with slightly blue, spot precipitate, as N-methyl-3,4-methylenedioxycathinone is a secondary amine. Upon the addition of the last component of Chen's reagents, no color change was observed and it gradually turned to yellow in several minutes. It can be thus concluded that none of ephedrine, pseudoephedrine, norephedrine, phenylpropanolamine and methcathinone is contained in the crystalline substance. Gallic acid test provided intense yellow indicating the methylenedioxy-substructure in the crystalline substance.

Definitive Identification by GC-MS, NMR and FTIR

GC-MS

GC-MS (EI) analysis condition is: Agilent GC-MS 7890/ 5975 C, column DB-5 MS 30 m × 0.25 mm × 0.25 μm, temperature: column 60ºC (0 min) with 15ºC/min to 300ºC (15 min); injector: 280ºC; split ratio: 20:1; transfer line: 250ºC; ion source: 230ºC; 70 eV ionization energy; injection volume: 1μL; Helium gas with flow: 1.0 ml/min; Scan parameter: 40-500 m/z.

Approximately 1 mg white crystal was dissolved in 1 mL methanol and then subjected to GC-MS analysis. The spectrum is shown in Figure 3.

N-methyl-3,4-methylenedioxycathinone in the white crystal was identified by GC-MS with fragmentation ions of 58, 91, 121, 135, 149, 176 and 207 [2].

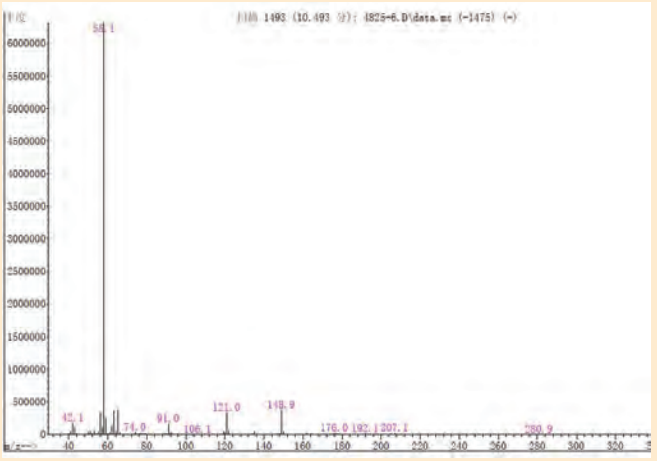


Figure 3: GC-MS spectrum of the crystalline substance.

^{1}H and ^{13}C NMR

^{1}H and ^{13}C NMR spectra were recorded in 5 mm NMR tubes on a Varian Inova 600 spectrometer on solutions in DMSO-d_6. Chemical shifts are reported in parts per million (ppm). The following abbreviations are used to designate NMR absorption patterns: s, singlet; d, doublet; q, quartet; dd, doublet of doublets. All results are illustrated in Table 1.

Position	^{13}C (ppm)	^{1}H (ppm)	Multiplicity	Structure Assignment
1	194.242	-	-	
2	57.870	5.067-5.102	q	
3	15.671	1.421-1.433	d	
1'	127.389	-	-	
2'	125.731	7.676-7.692	dd	
3'	108.467	7.099-7.113	d	
4'	152.635	-	-	
5'	148.145	-	-	
6'	107.843	7.515-7.518	d	
7'	102.398	6.173-6.174	s	
1''	30.571	2.537	s	

Table 1: Data of ^{1}H and ^{13}C NMR.

(continued on page 4)

Rapid Comparison of Same Colour Ink Marks and Paints by Spectral Imaging

Dr Quan Yangke, Mr Sun Zhenwen, Mr Tao Keming
Institute of Forensic Science, China

Abstract

Pairs of same colour paint, printed ink and written ink, which cannot be identified with the naked eye, were compared with spectral imaging. The results showed that spectral imaging is an effective and rapid method for classification of metamerism materials.

Introduction

Spectral imaging employs software and three-dimension dataset to classify the pixel of an image in terms of spectral signatures [1]. The dataset was obtained by collecting the spectra at each point across the sample surface, so it is a 3D data cube of X-Y-λ. This data can be represented and analysed in different ways. For each position of the sample (X and Y), we can get a single spectrum from this cube; we can also get all points of sample which have the same spectrum and map these positions. Since the different molecules have their characteristic spectrum, this selective map represents the distribution of that molecule across the whole sample. In this paper, we investigate the potential of visible reflection spectral imaging in the comparison of paints and inks.

Materials and Methods

Six automobile paints collected from different automobile makes were divided into three pairs based on colour similarity: one pair of browns, one pair of reds and one pair of greens. Each pair of paint chips was put side by side on a platform and data cubes were collected using a Nuance Multispectral Imaging System (Cambridge Research & Instrumentation, Inc.).

Two groups of black ink characters were collected, one printed by ink jet printer, the other from a Xeroxed document and data cubes were collected with the above-mentioned method.

A spectral dataset of a bill date altered by using the same colour but different ink pens was collected using the system mentioned above.

All spectra were in visible-wavelength (400 nm to 720 nm), the Bandwidth is 10 nm, and image mode is reflection.

Results and Discussion

Automobile Paint Chips

Spectral imaging data can be visualised in different ways. In one mode, single-point spectra can be extracted from a pixel in any position of the paint sample. Since a particular spectrum corresponds to the chemical composition contained in that pixel, so the special distribution of chemical components can be displayed using a "false" color.

Figure 1 to Figure 3 show the visible light images, classified images ("false" color images) and reflective spectra of brown paint pair, red paint pair and green paint pair respectively.

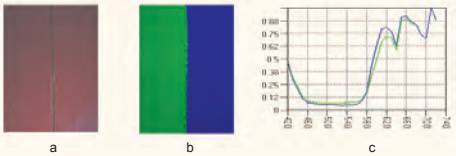


Figure 1: Images of a brown colour automobile paint pair. (a) visible light image displayed in true colour; (b) classified imaging displayed in "false" colour; (c) reflective spectra of two brown paint samples.

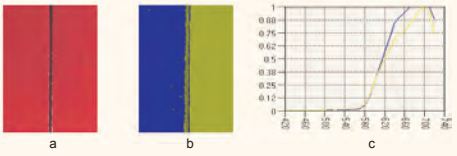


Figure 2: Images of a bright red colour automobile paint pair. (a) visible light image displayed in true colour; (b) classified imaging displayed in "false" colour; (c) reflective spectra of two red paint samples.

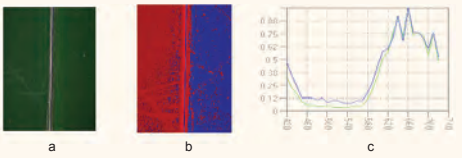


Figure 3: Images of a green colour automobile paint pair. (a) visible light image displayed in true color; (b) classified imaging displayed in "false" colour; (c) reflective spectral of two green paint samples.

Altered Bank Check

Figures 4(a) and 4(b) show the visible light images and classified images ("false" colour image) of an altered bank check. The date on the check was altered by the same colour ballpoint pen, so it is very difficult to find the difference between original characters and altered and new characters, but Figure 4(b) shows that the date was written by two different types of ink. By comparing the two images, the added number and added strokes could be found out very easily.

Figure 4: Altered bank check. (a) visible light image; (b) classified image displayed in "false" colour.

Printed Inks

Figures 5(a) and 5(b) show the visible light images and classified images ("false" colour image) of two groups of printed characters. The number "2008" was printed by an ink jet printer, and the number "2014" was formed by Xerox. They have similar colour, but the inks used in printer and copier machine are different, so they can be discriminated in classified images.

Figure 5: Images of printed characters and Xerox characters. (a) visible light image; (b) classified image displayed in "false" colour.

Conclusions

In this paper, we have only presented the results of a small number of paint and ink samples, but the results demonstrated some of the main advantages of chemical imaging technology. Firstly, spectral data from the entire field of view is collected at one time, so the analysis time is greatly reduced compared with traditional techniques, such as microspectrophotometer [2], which require multiple spectra to obtain an average profile of the sample. Secondly, this detection is non-destructive, non-contact and does not require sample preparation. Thirdly, spectral imaging can give the map of spatial distribution of different chemicals across the sample. This is very useful for finding specific trace evidence in a multi-component or inhomogeneous sample or localising the target materials in a complex background. As spectral imaging is an effective and rapid method for classifying metamerism materials, the potential in forensic science will be more apparent when further and deeper studies are carried out.

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Vietnamese Kinh Population Data on 15 STR Loci using Identifiler® Kit

Dr Le Thi Thu Thuy, Dr Nguyen Van Ha, Mr Trinh Tuan Toan
Forensic Science Institute, Vietnam

Abstract

Allele frequencies for 15 short tandem repeats (STRs) autosomal loci D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA (AmpFISTR® Identifiler®, Applied Biosystems) were studied in 170 unrelated individuals from the Vietnamese Kinh population. Population and forensic parameters were estimated.

Population

Samples were collected from 170 unrelated healthy individuals from the Vietnamese Kinh population living in Vietnam.

DNA extraction

DNA was extracted from peripheral blood samples by Chelex Extraction, using Chelex 100 (Bio-Rad, USA) [1-3].

DNA Quantitation

Quantitation of DNA was carried out using Quantiblot® Human DNA Quantitation kit, Applied Biosystems, USA.

Polymerase Chain Reaction (PCR)

According to the manufacturer's instruction, 0.5-1.25ng of template DNA was used to amplify the 15 STRs loci (AmpFISTR® Identifiler®, Applied Biosystems, USA) on a ABI-9700 PCR machine [4].

STR Typing of DNA

PCR-amplified products were separated and detected using the AB 3130 DNA Genetic Analyzer. Alleles were determined using GeneMapper® ID (Applied Biosystems, USA).

Data Analysis

Statistical parameters were performed with the EasyDNA_ PopuData software [7].

Results

The distributions of the observed allele frequencies and statistical parameters for the 15 STR loci are shown in Table 1.

Discussion and Conclusion

The STRs with the highest power of loci discrimination were FGA, D8S1179, D2S1338, D18S51 (in a Malay population, these were D2S1338, D18S51 [5]; in a Guatemala Mestizo population, these were FGA and D18S51 [6]), and the least discriminating loci were TPOX and D3S1358 (similar to a Guatemala Mestizo population [6]; in a Malay population, these were TPOX, CSF1PO and D3S1358 [5]). There were no detectable departures from Hardy-Weinberg equilibrium (HWE) for the 15 loci (in a Malay population, three of the loci showed a detectable departure from HWE [5]). The combined probability of identity (PI) and combined power of exclusion for the 15 STR loci were $2.38.10^{-18}$ and 0.999999 respectively.

In conclusion, we obtained potentially useful data using the AmpFISTR® Identifiler® kit for forensic analyses and paternity testing in the Vietnamese Kinh population.

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	THO1	D13S317	D16S539	D2S1338	D19S433	VWA	TPOX	D18S51	D5S818	FGA
5															
6						0.141									
7			0.009	0.006		0.362	0.003							0.026	
8	0.006		0.147			0.068	0.341	0.006				0.591			
9			0.056	0.047		0.326	0.132	0.221		0.015		0.103	0.003	0.059	
9.1			0.003												
9.3						0.038									
10	0.171		0.206	0.247		0.059	0.124	0.112				0.026		0.188	
11	0.150		0.344	0.259		0.006	0.224	0.297		0.003		0.259	0.009	0.318	
11.1												0.021			
12	0.147		0.203	0.347			0.135	0.232		0.044			0.094	0.203	
12.2										0.012					
13	0.147		0.024	0.068			0.029	0.112		0.238			0.109	0.185	
13.2										0.050					
14	0.156		0.009	0.021	0.03824		0.012	0.021		0.253	0.259		0.176	0.018	
14.2										0.115					
15	0.118			0.006	0.31471					0.059	0.018		0.194		
15.2										0.150					
16	0.071				0.35				0.012	0.015	0.168		0.215	0.003	0.003
16.2										0.041					
17	0.029				0.20294				0.115		0.235		0.068		
17.2										0.003					
18	0.006				0.08235				0.074		0.191		0.035		0.009
18.2										0.003					
19					0.00882				0.235		0.106		0.038		0.106
20					0.00294				0.118		0.024		0.018		0.056
20.2															
21									0.047				0.021		0.141
21.1															0.003
21.2															0.003
22									0.050				0.009		0.212
22.2															0.021
23									0.174				0.009		0.138
23.2															0.012
24									0.132						0.132
24.2															0.018
25									0.038				0.003		0.068
25.2															0.009
26		0.0029							0.006						0.041
26.2															0.006
27		0.0059													0.018
28		0.0559													0.003
28.2		0.0029													
29		0.2588													
29.2		0.0029													
30		0.25													
30.2		0.0206													0.003
31		0.0824													
31.2		0.0559													
32		0.0235													
32.2		0.1824													
33		0.0029													
33.2		0.0471													
34.2		0.0059													
OH	0.877	0.895	0.858	0.726	0.779	0.743	0.814	0.805	0.85	0.858	0.770	0.593	0.858	0.805	0.929
PI	0.034	0.048	0.083	0.104	0.119	0.111	0.073	0.086	0.035	0.051	0.071	0.218	0.039	0.073	0.026
PD	0.966	0.952	0.917	0.896	0.881	0.889	0.927	0.914	0.965	0.949	0.929	0.782	0.961	0.927	0.974
PE	0.729	0.673	0.572	0.52	0.487	0.502	0.597	0.565	0.724	0.663	0.607	0.301	0.708	0.6	0.759
P	0.4785	0.303	0.9905	0.994	1	0.409	0.176	0.814	0.4965	0.9995	0.171	0.893	0.4415	0.337	0.144

OH (observed heterozygosity), PI (probability of identity), PD (power of discrimination), PE (power of exclusion of paternity), P (Exact test for Hardy-Weinberg equilibrium)

Table 1: Allele frequencies and statistical parameters for 15 STR loci in Vietnamese Kinh population (n=170).

Acknowledgements

This work was supported by Grant-in Aid for Scientific Research (BC-08-C21-03) from Ministry of Public Security of Vietnam.

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Sibship Analysis: An Alternative to Paternity Analysis

Ms Nongnuch Boonderm, Asst Prof Worawee Waiyawuth, Dr Khunying Porntip Rojanasunan
Central Institute of Forensic Science, Thailand

Abstract

Presently, the lack of parentage references poses as problems for identification of missing persons and proof of kinship for immigration purpose. As such, sibship analysis may potentially be used to overcome this problem. The evaluation of the probability of sibship between individuals using short tandem repeat markers employs a Combined Sibship Index (CSI). The CSI is the likelihood ratio of observing the genetic results if the two individuals are full siblings against the same observations under the alternative hypothesis that no kinship exists between the two individuals. This study focuses on full siblings analysis performed by evaluating 200 pairs of full siblings against 200 pairs of non-siblings. The results of this study will be used for statistical assessment in the identification of missing persons as well as proof of sibship for immigration in Thailand.

Introduction

DNA profiling has proven to be a powerful technique for human identification including paternity testing [1], which is commonly used to verify parent-child relationships. This test is applicable in situations such as the settlement of inheritance, immigration application as well as to identify missing persons. Short tandem repeat, otherwise known as STR markers, have been widely used for these purposes for more than a decade. Paternity testing requires the DNA profiles of both parents and the child to generate a paternity index (PI) which is indicative of whether the alleged male fathered the child in question. However, there are many cases where parentage references are lacking. In these cases, the alternative sibship analysis, which calculates the probability of two individuals being full siblings, half siblings, or avuncular, was considered. This study focuses on the analysis of full siblings for the purpose of setting a statistical threshold to establish if two individuals are true siblings.

Materials and Methods

Two hundred pairs of full siblings were selected from families within the Thai population which underwent paternity testing and two hundred pairs of non-siblings were randomly selected from unrelated individuals. The DNA profiles were obtained using AmpFISTR® Identifiler® PCR Amplification consisting of 15 STR markers: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA.

The evaluation of the probability of sibship between individuals with STR markers uses the Combined Sibship Index (CSI) which is indicative of the probability of observing a pair of true siblings against the probability that the pair of individuals is not true siblings [2]. The CSI were computed using the Starfruit IdentiTrack® software under the guideline of the National Research Council; NRCII [3]. The allele frequencies of Thai population were employed [4, 5] without the use of a sub-population model.

Results and Discussion

The combined sibship index (CSI) of full siblings and non-siblings were calculated and shown in Table 1. The statistical values were categorized relating to a verbal scale [6] consisting of 'extremely strong', 'very strong', 'strong', 'moderately strong', 'moderate', 'limited' and 'inconclusive' respectively.

As shown in Table 1, the CSI of full siblings were categorised from 'moderate' up to 'extremely strong', whereas the CSI of non-siblings were found to be classified under 'inconclusive' or non-related. For paternity testing, most laboratories use either the posterior probability with a value of at least 99.99% or the paternity index with a minimum value of 10,000 as the threshold that an individual is the father of a child [7]. From our data, 126 out of 200 pairs of full siblings met this criterion. To be consistent with paternity testing, the CSI value cut-off for sibship testing should also be equal or greater than 10,000 before two individuals are confirmed as full siblings. Therefore, this study can be used as a reference in setting a threshold for sibship analysis of full siblings.

Combined Sibship Indice	Full Siblings	Non Siblings	A Verbal Scale*
1,000,000 +	45	-	Extremely strong
100,000 – 1,000,000	35	-	Very strong
10,000 – 100,000	46	-	Strong
1,000 – 10,000	42	-	Moderately strong
100 – 1,000	32	-	Moderate
10 – 100	-	-	Limited
1 – 10	-	24	Inconclusive
less than 1	-	176	

* Referred to J. Buckleton, C.M. Triggs, and S. Walsh. *Forensic DNA Evidence Interpretation.*

Table 1: The combined sibship index (CSI) of full siblings and non-siblings.

Although the CSI clearly differentiated between full siblings and non-siblings, 74 pairs of full siblings had CSI values of less than 10,000. It might be worthwhile to investigate why these values could be generated from the DNA profiles of true siblings. Alternatively, more STR markers could be examined for these cases [8]. Besides Y-STR and X-STR, mitochondrial DNA may also be considered to enhance accuracy of the test result. If the DNA profiles of two individuals give a CSI value of less than ten, they can be excluded as being full siblings.

An agreement should be made between various scientific working groups to establish and adopt a common threshold value for establishing whether two individuals are full siblings. Besides its application in missing person identification and proof of kinship, sibship analysis could be used for familial searches in DNA databases [9, 10] as well as the investigation of criminal cases. In summary, the benefits of this study could potentially be used in various aspects within the legal system of Thailand.

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AccuTOF-DART as a Rapid Screening Method for the Analysis of DDNP

Ms Liu Zhanfang, Mr Xu Jianzhong, Ms Zhou Hong, Mr Sun Yuyou
Institute of Forensic Science, China

Abstract

DDNP is an initiator of blasting caps. It is highly sensitive and easily undergoes thermal decomposition. Current analytical methods used for the confirmation of DDNP include TLC and GC/MS, but these techniques have low sensitivity, and they are time-consuming. A new technique for rapid screening of DDNP was conducted using the DART source coupled to an accurate mass time-of-flight mass spectrometer. This technique does not require sample preparation and yields accurate mass spectra usually in few minutes.

Introduction

Diazodinitrophenol (DDNP) is an organic explosive, it has been used as an initiator in industrial blasting caps [1]. Currently, the TLC and GC/MS methods are employed to identify DDNP. However, GC/MS techniques could be problematic due to thermal instability of DDNP in the analytical system and the low sensitivity of the TLC method. Presented herein is a rapid confirmatory instrumental method using direct analysis in real time (DART) – time of flight (TOF) mass spectrometry which makes this task significantly more efficient than traditional analytical methods, while still producing high quality data suitable for confirmation.

The DART ion source is coupled with an accurate mass time-of-flight (TOF) mass spectrometer (AccuTOF-DART). DART is a unique, open-air ionization source that requires little-to-no preparation as the sample can be introduced in their native form, whether solid, liquid, or gas [2]. It employs a gas, such as helium, to produce metastable species that in turn ionize water (positive mode) or oxygen (negative mode) molecules [3]. Due to the exact mass capabilities of the AccuTOF, there is little chance of two unrelated compounds overlapping, chromatography is not required prior to ionization [4]. Because of the speed of analysis and its sensitivity, the AccuTOF-DART is an ideal technique for screening forensic samples [5-8].

In this paper, we report the validation of a new and reliable screening method for DDNP analysis using the AccuTOF-DART which offers potential benefits because of the analysis speed and sensitivity.

Materials and Methods

AccuTOF-DART Parameters

Analysis was carried out by using the DART ion source coupled with a JEOL AccuTOF mass spectrometer (JMS-T100LP) operated in negative-ion mode.

The mass spectrometer operated at a constant resolving power of approximately 6000(FWHM definition). All measurements were taken with the ion guide peak voltage at 500V, reflection voltage of -950V, orifice 1 voltage of -30V, orifice 2 voltage of -10V and ring lens voltage of -10V. Orifice 1 temperature was typically kept warm at 80°C.

The DART ion source was operated with helium as the ionizing medium at a flow rate of 2.5L/min. The gas beam was heated to 250°C, discharge electrode needle at 3001V, and perforated and grid electrode voltages were -100V and -250V respectively.

A mass spectrum of poly(ethylene glycol) (average molecular weight 600) was included in each data file, measured separately from the analyte, as an external reference standard for exact mass measurements.

The specimen of DDNP was dissolved in acetone. Sampling was done by dipping the closed end of cleaned glass melting point tubes into the specimen vials and holding the tube in the DART gas stream. Analysis of data was accomplished by creating averaged, background subtracted, centroided spectra that were subsequently calibrated to a PEG-H_2O+O_2-H mass reference table.

GC/MS Parameters

Analysis was carried out with a GC/MS (SHIMADZU QP2010) operated in negative-NCI ion mode. The stationary phase consisted of a DB-5MS column (8m × 0.25mm × 0.25µm) and the carrier gas used was helium. The GC oven was programmed at 80°C for 1 min followed by a 20°C/min ramp to 250°C with a final hold time of 5min. The mass detector was set to scan from 40amu to 450amu.

Results and Discussion

Figure 1 shows the AccuTOF-DART mass spectrum of DDNP. Since there is no prior chromatography, the AccuTOF-DART spectrum shows peaks that represent DDNP and ionized diluents present in the specimen. The measured ions at 183.00452Da and 167.00999Da represent $[M+H-N-N]^+$ and $[M+H-N-N-O]^+$, respectively. They are all highly characteristic ions resulting from DDNP in the heated gas stream. It is important to note that there are no other combinations of atoms that will give peaks at these masses.

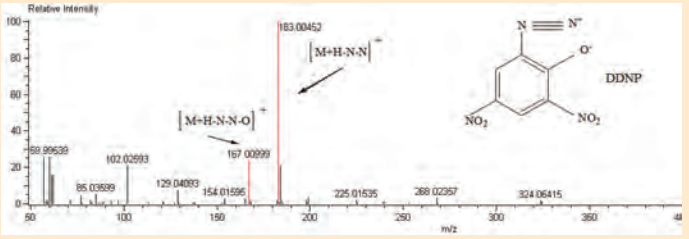

Figure 1. AccuTOF-DART mass spectrum of DDNP.

Compared with the method of AccuTOF-DART, it is difficult to analyse DDNP by GC/MS because DDNP is thermally unstable and decomposes during the analysis. The peaks in Figure 2 (A) consisted of mainly decomposition products while the TIC chromatogram of DDNP is relatively weak and difficult to recognise.

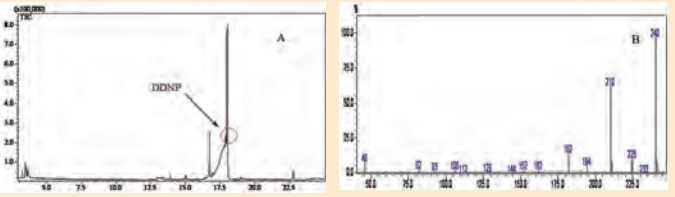

Figure 2. (A) TIC chromatogram and (B) mass spectrum of DDNP by GC/MS.

The spectra generated with the AccuTOF-DART are much simpler and clearer than the information typically obtained from GC/MS. This instrument produces protonated molecules using the soft ionization mode and direct injection, hence eliminating the problem of sample decomposition. In general, AccuTOF-DART is an appealing tool in forensic chemistry as it does not require sample preparation and reduces analysis time. The use of this technique can improve case turn-around time and solve the problem of thermal decomposition of an organic explosive such as DDNP.

References

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8. L. Ojanpera, A. Pelander, S. Laks, M. Gergov, E. Vuori, and M. Witt, "Application of accurate mass measurement to urine drug screening," *Journal of Analytical Toxicology*, vol. 29, no. 1, pp. 34-40, 2005.

Ecstasy Tablets Containing Legal Highs Encountered in Singapore

Ms Wendy Lim Jong Lee, Dr Angeline Yap
Health Sciences Authority, Singapore

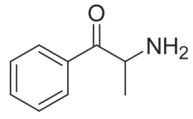
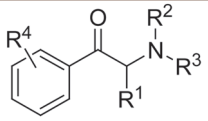
Introduction

Legal highs are designer drugs that mimic the effects of certain desired controlled drugs. These drugs are synthesised specifically to produce desirable pharmacological effects but at the same time being able to circumvent existing legislation and hence are legal to possess. These legal highs are often disguised and sold as research chemicals, plant food or bath salts and some of these had been suspected to cause a number of deaths in several countries. Recent examples of legal highs include synthetic cathinones and synthetic cannabinoids.

Synthetic Cathinones

Cathinone occurs naturally in the leaves of khat *(catha edulis)*. It has the structure 2-amino-1-phenylpropanone (Structure 1) and is similar in structure to amphetamine apart from the beta-ketone. Cathinone hence has effects similar to amphetamine. Starting from this cathinone structure, various modifications can be made at the α-carbon (R^1), amine nitrogen (R^2 and R^3) and/or aromatic ring (R^4) (Structure 2) to obtain derivatives of cathinone [1]:

- R^1 = single alkyl group
- R^2 / R^3 = H, alkyl or ring structure such as pyrrolidino / phthalimido
- R^4 = alkyl, alkoxy, alkylenedioxy, halide or other ring structure

Structure 1	Structure 2
	
Cathinone (2-Amino-1-phenylpropanone)	Derivatives of Cathinones

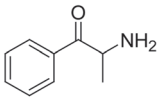
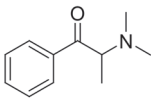
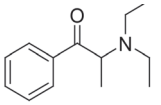
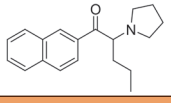
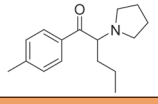
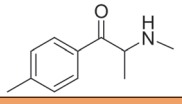
		
Cathinone	N,N-Dimethylcathinone	Diethylcathinone
		
Naphyrone	Pyrovalerone	Mephedrone

Table 1: Structures of some common cathinone derivatives.

Synthetic Cannabinoids

Synthetic cannabinoids refer to substances with structural features that allow the drug to bind to one of the known cannabinoid receptors, i.e. CB_1 or CB_2 which are present in the human body. The synthetic cannabinoids thus mimic the effects of the psychoactive tetrahydrocannabinol (THC) present in cannabis plants. Many of these substances do not have a structure that is chemically similar to THC.

These synthetic cannabinoids fall into seven major groups as classified by the Advisory Council on the Misuse of Drugs (ACMD) [2]:

(1) Naphthoylindoles
(2) Naphthylmethylindoles
(3) Naphthoylpyrroles
(4) Naphthylmethylindenes
(5) Phenylacetylindoles
(6) Cyclohexylphenols
(7) Classical Cannabinoids

The general structures are tabulated in Table 2. R^1 to R^4 can be substituted with alkyl, alkenyl, cycloalkylmethyl, cycloalkylethyl, 2-(4-morpholinyl)ethyl or halide.

Ecstasy Tablets containing Legal Highs

From April to July 2011, our laboratory received 23 cases of Ecstasy tablets which were analysed and found to contain ketamine and a variety of legal highs (refer to Table 3). This paper presents the analytical results of the legal highs found in Ecstasy tablets.

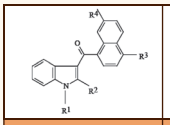
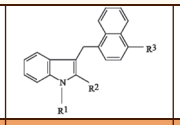
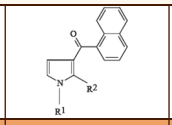
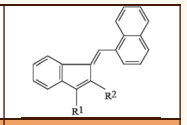
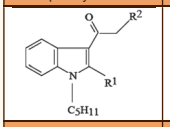
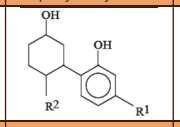
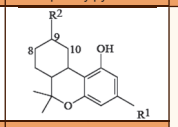
			
Naphthoylindoles	Naphthylmethylindoles	Naphthoylpyrroles	Naphthylmethylindenes
			
Phenylacetylindoles (benzoylindoles)	Cyclohexylphenols	Classical Cannabinoids (dibenzopyrans)	

Table 2: General structures of synthetic cannabinoids.

Experimental Conditions

Gas Chromatography/Mass Spectrometry (GC/MS)

The Ecstasy tablet was pulverised and a suitable amount (about 50 mg) was dissolved in about 1 ml of water, basified with 1N NaOH and extracted with about 2 ml of ethyl acetate. The ethyl acetate extract was analysed by GC/MS.

An Agilent 6890N GC equipped with an Agilent 5973 quadrupole mass-selective detector was used with parameters set as follows:

- Oven Temperature: 80°C (3 min) → 300°C (25 min) at 40°C / min
- Solvent Delay: 3 min
- Inlet Temperature / Pressure: 280°C / 8 psi
- Run Time: 33.5 min
- Scan Range: 35 – 480 amu
- Injection volume / Split Ratio: 1 µl / 40:1

High Performance Liquid Chromatography (HPLC)

The quantification of the amount of ketamine present in these Ecstasy tablets was performed using an Agilent 1100 Series HPLC equipped with a LiChrospher 60 RP-Select B column (25 cm x 4.0 mm i.d., 5µm). The instrument parameters were set as follows:

- Mobile Phase: 10% MeOH: 90% H2O, 0.5% diethylamine (pH=3)
- Flow rate: 1.8 ml / min
- Column Temp: 45°C
- Injection Volume: 5 µl
- Wavelength: 215 nm
- Detector Spectrum range: 205 – 350 nm
- Run time: 25 min

Results and Discussion

The compounds detected in these Ecstasy tablets via GC/MS are tabulated in Table 3. GC Chromatograms of two types of Ecstasy tablets are given in Figures 1 and 2. The structures and the mass spectra of these legal highs are given in Table 4 and Figures 3 to 7 respectively.

The compounds identified by GC/MS through simultaneous analysis of drug reference standards included ketamine, 4-methylmethcathinone (4-MMC), fluoromethcathinone (FMC), 3,4-methylenedioxymethcathinone (Methylone or βk-MDMA), and 1-pentyl-3-(1-naphthoyl) indole (JWH-018). For fluoromethcathinone, the exact position of fluorine atom in the aromatic ring could not be determined by using GC/MS but could be differentiated through FTIR (for a pure sample or extract), ^{19}F-NMR [3] or by CI GC/MS [4]. The exact isomer of fluoromethcathinone was not determined in the current study.

In one of the Ecstasy tablets, tadalafil, a sexual enhancement drug was also detected. The presence of 3,4-methylenedioxypyrovalerone (MDPV) in these Ecstasy tablets was suspected as a simultaneous analysis of drug reference standard was not possible due to lack of reference standard. However, the MS of the samples were matched successfully to MDPV in the SWGDRUG MS library (http://www.swgdrug.org) as well as that from literature [5].

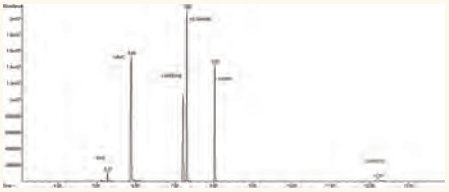


Figure 1: Expanded GC Chromatogram of the red ecstasy tablet marked "S".

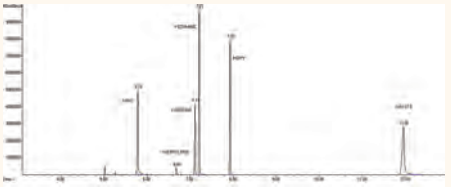


Figure 2: Expanded GC Chromatogram of the beige ecstasy tablet stamped with an imprint of a crown.

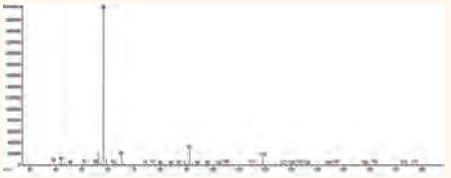


Figure 3: Mass Spectrum of 4-methylmethcathinone (4-MMC).

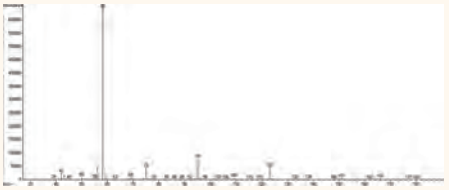


Figure 4: Mass Spectrum of fluoromethcathinone (FMC).

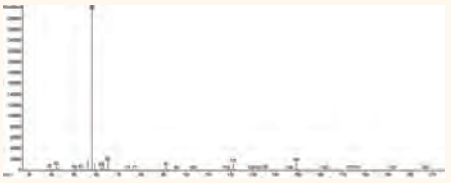


Figure 5: Mass Spectrum of Methylone (3,4-methylenedioxymethcathinone).

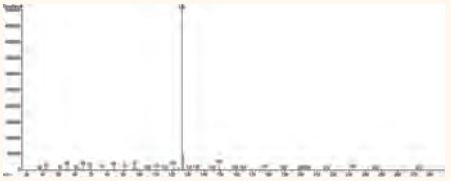


Figure 6: Mass Spectrum of suspected 3,4-methylenedioxypyrovalerone (MDPV).

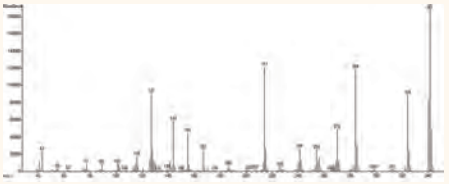
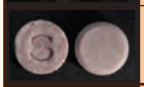
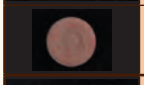
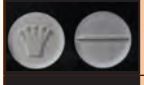


Figure 7: Mass Spectrum of 1-pentyl-3-(1-naphthoyl) indole (JWH-018).

The purity of ketamine present in these Ecstasy tablets was determined to be between 8% – 19% (refer to Table 3). This is equivalent to a dosage of 22 to 53 mg of ketamine per tablet. As compared to the amount of ketamine normally encountered in Ecstasy tablets not containing legal highs (between 50 to 105 mg per tablet), this amount is only about half of the usual dose. The usual ketamine-containing Ecstasy tablets are frequently found to contain substances like methamphetamine, parcetamol, caffeine and chloroquine.

Ecstasy Image	Colour	Imprint	GC/MS Results	Purity of Ketamine	No. of cases
	Green	MITSUBISHI	K, MMC, FMC, *MDPV, JWH-018, Caffeine	9%	11
	Red	S		11%	
	Beige	MITSUBISHI	K, MMC, *MDPV, Caffeine	19%	7
	Orange-coloured	Ω		8%	
	Beige	Crown	K, MMC, *MDPV, JWH-018, Methylone, Caffeine	10%	2
Photograph of tablet not taken	Beige	Crown	K, MMC, *MDPV, JWH-018, Methylone, Tadalafil, Caffeine	10%	2
	Green	Bat	K, MMC, *MDPV, Methylone, Caffeine	16%	1

*suspected

Table 3: Ecstasy Tablets containing Legal Highs.

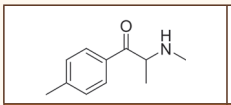
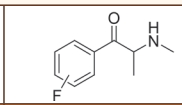
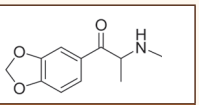
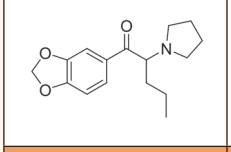
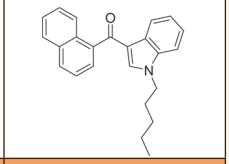
		
4-MMC (Cathinone derivative)	FMC (Cathinone derivative)	Methylone (Cathinone derivative)
		
MDPV (Cathinone derivative)	JWH-018 (Synthetic cannabinoid, Naphthoylindoles)	

Table 4: Structures of Legal Highs detected in Ecstasy Tablets.

Legislation in Singapore

Ketamine and 4-methylmethcathinone are Class A Controlled Drugs under The Misuse of Drugs Act, Singapore. 1-Pentyl-3-(1-naphthoyl)indole (JWH-018), 3,4-methylenedioxypyrovalerone and fluoromethcathinone are not Controlled Drugs in Singapore.

Challenges

There are various new challenges encountered in the analysis of legal highs as compared to traditional drugs of abuse:

1. Reference materials are often not available particularly for newly emerging legal highs. When available for purchase, these reference materials could often be quite costly. Commercial drug companies that offer some of these reference materials include LGC Standards, Cayman Chemical Company, Research Triangle Institute, Tocris Bioscience, Chiron AS and Toronto Research Chemicals.
2. Literature and studies containing detailed analytical data of such legal highs are often limited.
3. Due to the above two challenges, there is a constant need to identify alternative techniques and to develop capabilities to fully characterise these new legal highs. Some examples of alternative techniques include IR, NMR, GC-TOF MS, LC-TOF MS and LC-MS/MS. Some of these equipment can be very costly to acquire. In addition, techniques such as IR and NMR analyses require pure samples. Hence the drug of interest in mixture solutions needs to be isolated and purified using either preparative HPLC or column chromatography. NMR is a very powerful technique that could provide structural elucidation to an unknown compound. This technique however is not routinely available in most forensic laboratories.

Conclusion

Our analysis shows that this group of Ecstasy tablets was found to contain between 8 to 19% of ketamine, and the following: 4-methylmethcathinone, fluoromethcathinone, 1-pentyl-3-(1-naphthoyl) indole (JWH-018), taladafil and caffeine (refer to Table 3). The specific positional isomer of fluoromethcathinone was not determined in this study. The presence of 3,4-methylenedioxypyrovalerone was also indicated by the mass spectrometric data acquired. To analyse legal highs, it is essential to develop and validate other suitable analytical methods in order to identify the numerous analogues that are being produced globally at an alarming rate. The sharing of forensic data such as mass spectrometric library and chromatographic data would be very useful for all drug-testing laboratories.

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The Elemental Analysis of Black Electrical Tapes by SEM/EDS

Mr Sun Zhenwen, Dr Quan Yangke, Mr Tao Keming
Institute of Forensic Science, China

Abstract

The backings of 27 brands of black electrical tape were analysed using scanning electron microscopy/energy dispersive spectroscopy (SEM/EDS). The relative amounts of chlorine to calcium varied greatly between brands. Quantitative differentiation of electrical tapes was achieved through multiple comparison statistical technique, and resulted in a discrimination of 95.2%.

The forensic importance of electrical tapes has been well recognised. Studies have been undertaken in western countries to establish an optimal instrumental methodology for electrical tape comparisons as well as develop statistical tests to quantitatively assess similarity or dissimilarity [1-3]. These studies have also established that SEM/EDS is able to discriminate tape samples based on the surface microtexture and elemental composition of the tape backing, despite relatively small sampling areas. In addition, multivariate statistical techniques such as agglomerative hierarchical clustering and discriminate analysis were able to discern subtle differences between samples [1-3].

An electrical tape consists of two basic components: the backing and the adhesive. In China, electrical tapes are available in a wide variety of backing colours, but black is the most common colour. Polyvinyl chloride (PVC) is the main component of most backings. Stabilisers, fillers, and flame retardants may also be present in a plasticised PVC backing. The elemental compositions varied greatly between brands and could be used for distinction [1-2].

The aims of our study are to evaluate whether the comparison of electrical tapes using SEM/EDS quantitation is also applicable to Chinese samples, and to develop a quantitative statistical means for sample discrimination.

Materials and Methods

Tape Collection

Twenty-seven black electrical tape samples were collected for analysis. All of the samples were purchased from hardware stores or manufacturers in China. The tapes originated from China, Japan, Germany and the United States. The sample set represented tapes that could be easily obtained by consumers and would be comparable to casework evidence submitted to forensic laboratories. Table 1 is a summary of the products that were evaluated in this work.

Sample Roll	Brand Name	Manufacturer	Country of Origin
1	3M 1500	3M	USA
2	Tesa 4252	Tesa AG	Germany
3	Shushi	Xiaogan Shushi	China
4	Vini-Tape	Denka	Japan
5	Tian Tan	Beijing Tiantan	China
6	Yongle	Hebei Huaxia Enterprise	China
7	Cobra	Hebei Huaxia Enterprise	China
8	Haijia	Qingdao Haijia	China
9	Jingfeng	Beijing Jingfeng	China
10	Youxin	Dongguan Youxin	China
11	Deli	Delitools	USA
12	Bisheng	Beijing Bisheng	China
13	Wan.E	Tianjin Yahua	Japan
14	Masion	Full Tech Trading	China
15	Anglia	Zhejiang SAM	China
16	Elfy	Jingjiang Henghe	China
17	Seebest	Xiamen Seebest Tech	China
18	Gummed	Hongxin Tech	China
19	Wonder	Achem Technology	China
20	Jingla	Chongqing Lihe	China
21	Plyon	Chongqing Yongchao	China
22	Weifeng	Chongqing Lihe	China
23	Dian	Chongqing Shan Xiang	China
24	Sincere	Ningbo Xinshan	China
25	Shenshan	Ningbo Xinshan	China
26	3M 1600	3M	USA
27	3M 1721	3M	USA

Table 1: Product information for sample set.

Method

All samples were attached onto an aluminum plate. Analysis was performed on a FEI Quanta FEG 650 ESEM and EDAX Apollo XL EDS. The working distance of SEM was set at 10 mm, the take-off angle was 35°, and the accelerating voltage was 20 keV. The magnification was set at 200×. The energy dispersive spectrometer utilized an EDAX SUTW detector with EDAX Phoenix analyzer and EDAX Genesis software. The EDS was operated with a dead time of c. 30% and counting time of 100 sec. Each sample was analysed at 3 different sites.

Data Analysis

The standardless quantitation algorithm of the EDAX Genesis software was used to generate de-convoluted net peak areas for chlorine (Cl) and calcium (Ca). Carbon and oxygen were not included in this study as they largely reflect the organic portion of the tape and were found to be less useful for discriminating tape samples. Cl was included as a convenient measure of the amount of PVC in a tape sample versus the amount of inorganic fillers. In this study, Ca was unsurprisingly found in each sample, as calcium carbonate is the most common filler in electrical tapes. Therefore, the relative amount of Cl to Ca was selected as the parameter to discriminate different samples.

The between-sample comparisons of the relative amounts of Cl to Ca were tested by one-way ANOVA analysis. If a significant difference was found in variance analysis, *p*-values of post-hoc multiple comparisons were computed using the LSD t-test method. All analyses were two-tailed; *p*-values <0.05 were considered statistically significant.

Results and Discussion

Table 2 lists the results of Cl to Ca ratios using SEM/EDS quantitation. These ratios varied greatly between the 27 brands. The relative standard deviation (RSD) of all samples was less than 5%, indicating the good reproducibility of the conditions and resultant data. This is of paramount importance, particularly when comparing a known sample with an unknown sample.

Sample Roll	Brand Name	Ratio of Cl/Ca Peaks			Mean	SD	RSD (%)
		1st time	2nd time	3rd time			
1	3M 1500	3.90	3.88	3.84	3.85	0.026	0.68
2	Tesa 4252	8.24	8.23	8.30	8.26	0.038	0.46
3	Shushi	4.29	4.25	4.52	4.35	0.146	3.36
4	Vini-Tape	5.59	5.62	5.47	5.56	0.079	1.42
5	Tian Tan	3.27	3.46	3.52	3.42	0.131	3.83
6	Yongle	2.17	2.13	2.16	2.15	0.021	0.98
7	Cobra	1.85	1.80	1.81	1.82	0.026	1.43
8	Haijia	3.90	3.91	3.88	3.90	0.015	0.38
9	Jingfeng	2.68	2.68	2.58	2.65	0.058	2.19
10	Youxin	2.24	2.23	2.20	2.22	0.021	0.95
11	Deli	3.42	3.36	3.39	3.39	0.030	0.88
12	Bisheng	6.36	6.50	6.55	6.47	0.098	1.51
13	Wan.E	2.04	2.10	2.08	2.07	0.031	1.50
14	Masion	3.48	3.50	3.44	3.47	0.031	0.89
15	Anglia	1.93	1.93	1.93	1.93	0.00	0.00
16	Elfy	2.83	2.80	2.82	2.82	0.015	0.53
17	Seebest	4.27	4.15	4.36	4.26	0.105	2.46
18	Gummed	1.80	1.77	1.73	1.77	0.035	1.98
19	Wonder	2.92	2.79	2.86	2.86	0.065	2.27
20	Jingla	1.96	1.94	1.93	1.94	0.015	0.77
21	Plyon	3.15	3.22	3.15	3.17	0.040	1.26
22	Weifeng	3.52	3.57	3.55	3.55	0.025	0.70
23	Dian	2.89	2.83	2.81	2.84	0.042	1.48
24	Sincere	6.00	6.40	6.41	6.27	0.234	3.73
25	Shenshan	2.75	2.67	2.66	2.69	0.049	1.82
26	3M 1600	3.55	3.65	3.67	3.62	0.064	1.77
27	3M 1721	4.10	4.13	4.23	4.15	0.068	1.64

SD = standard deviation, RSD = relative standard deviation.

Table 2: Ratio of Cl to Ca peaks of each brand sample (black backing).

A total of 334 sample groups of the 351 compared samples were distinguished using the multiple comparison statistical technique, which meant a discrimination rate of 95.2%. The indistinguishable samples are listed in Table 3.

Compared Samples	*P*-Value	Compared Samples	*P*-Value	Compared Samples	*P*-Value
1 vs 8	0.448	7 vs 15	0.077	16 vs 19	0.516
3 vs 17	0.133	7 vs 18	0.387	16 vs 23	0.664
5 vs 11	0.664	9 vs 25	0.448	17 vs 27	0.087
5 vs 14	0.358	11 vs 14	0.178	19 vs 23	0.828
6 vs 10	0.257	14 vs 22	0.235	22 vs 26	0.215
6 vs 13	0.196	15 vs 20	0.828		

Table 3: Indistinguishable Samples via LSD t-test.

Previous studies detected several elements using SEM/EDS, for example Mg, Al, Si, S, Pb, Cl, Sb, Ca, Ti, Zn, etc. Multivariate statistical techniques were used for discrimination. In this study, two representative elements of electrical tape backings were chosen for discrimination. The LSD t-test was used for multiple comparisons, and this method also achieved a similar discrimination rate in comparison with multivariate statistical techniques. However, as the LSD t-test is the most sensitive multiple comparison statistical technique to identify differences between samples which may increase Type I Error Rate (false positive), SEM/EDS combined with Fourier transform infrared spectroscopy (FTIR) and/or pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) techniques are suggested to further improve the overall discrimination.

Conclusion

Comparison of Cl to Ca ratios using SEM/EDS quantitation is applicable to Chinese electrical tapes. Quantitative differentiation of electrical tapes was achieved through LSD t-test with a discrimination rate of 95.2%.

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(continued from page 24)

The tracking of the unknown source set forth a series of DNA screening for the potential perpetrator or contributor. A total of 157 individuals were tested and comprised of personnel from the forensic pathology department which carried out the post-mortem, investigation personnel, staff members at the place of the death event; and those who were last with the deceased, including cleaners, maintenance and security personnel of the building where the deceased's body was found and a few witnesses. All these 157 individuals were excluded.

Retrospective Assessment of the Mixture

The DNA profile of the deceased was easily read from the mixture as the major contributor (see Figure 1 [STR profile] and Figure 2 [Y-STR profile]). The mixture showed DNA profiles arising from DNA of sufficiently good quality (quantitation level by Quantifiler® Human DNA Quantification Kit being 1.05 ng/µl). The unknown foreign DNA profile was readily visualised despite being the minor component in the mixture, thus supporting the association with body fluid or tissue sources. Foreign DNA originating from contact or sporadic sources are usually low template DNA and would be expected to be masked by the stronger presence of the deceased's DNA.

Tracking the Source

The post-mortem examination table was subsequently considered as a possible surface which could come into contact with the back of the blazer if the deceased was laid onto it. Contamination became a strong consideration and two bodies which were prior autopsied were tracked. DNA was collected from their immediate family members and both their DNA profiles were reconstructed. It became apparent that one of the prior autopsied bodies (profile reconstructed from three sons and wife) was the unknown foreign DNA contributor in the mixture. The Y-STR profiles of the sons shared a common Y-haplotype with the foreign contributor and the inferred STR profile of the foreign contributor concords with being the biological father.

Conclusion

The possibility exists that DNA could be transferred during post-mortem examination. The following guidelines are proposed to either minimise the chance of contamination or to identify an occurrence:

1. Personnel wear disposable laboratory coats, gloves and face masks.
2. Benches and equipment are treated with bleach (or equivalent) and irradiated with UV light after completion of each post-mortem examination.
3. A database of post-mortem room personnel and investigators of crime scene to be made available and all results to be compared against the database.
4. A record of all bodies autopsied, dates of autopsy and next-of-kin contact.

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Case Study of Detection of Sexual Assaults and Missing Child by using 16 STR Autosomal and 17 Y- Chromosomal DNA Markers

Dr Ganbold Suren, Ms Purevdulam Sharavdorj, Ms Bayarmaa Rentsendorj
National Institute of Forensic Science, Mongolia

Introduction

Forensic DNA analysis method and technology has been employed in the forensic field in Mongolia since 2004. Back then, the method and technology used was DNA typing with 9 loci using GenePrint STRIII, FFv and CTT MultiPlex systems kit.

Since 2008, we started using PowerPlex-16 Systems, PowerPlex-Y system, AmpFlSTR® Identifiler®, and AmpFlSTR® Yfiler kits in our DNA laboratory. DNA analysis has been used to confirm genetic relationship between a child and the father as well as with paternal relatives, distinguish biological evidence found at the crime scene and in cases of finding missing persons.

Between 2009 to 2010, DNA analysis has been successfully carried out on approximately 1300 samples from 350 cases.

In this case study, we present the results of DNA analysis carried out in two cases and describe some difficulties we faced.

Materials and Methods

Sample Collection

In Case 1, blood samples were collected from suspects using EDTA Vacutainer.

In Case 2, an autopsy was carried out on the victim by a forensic expert under the supervision of investigators and prosecutors. The autopsy was performed in accordance to proper procedures for forensic examination and autopsy. Five vaginal swabs and a liquid blood sample were collected from the victim and dried at room temperature according to proper procedures. Then, each sample was placed into a paper bag, sealed with stamp, and transported to our DNA laboratory under special protection. We received the samples 24 hours later.

Blood samples and buccal swabs were also obtained from suspects, dried and sent to our laboratory for forensic identification.

DNA Extraction

Genomic DNA was extracted from whole blood using the Wizard Genomic DNA Purification kit (Promega Corporation, Madion, WI, USA) and from buccal swab and dried blood using the standard method of phenol-chloroform-isoamyl alcohol extraction [3].

In Case 2, the vaginal swabs had a lot of blood on them. We prepared microscopic slides from these samples and observed microscopically for presence of sperm cells.

DNA was extracted from the specimens in Case 2, where stains were mixed with the sperm and vaginal cells, by using the phenol chloroform method and Differex systems Promega (Promega Corporation, Madion, WI, USA).

Microscopic examination and DNA extraction were conducted within five days following the date of the homicide.

PCR Amplification

DNA samples were directly amplified with PowerPlex-16 Systems, AmpFlSTR® Identifiler® or Yfiler PCR Amplification kit by using an Applied biosystems 2720 thermal cycler.

The PCR conditions for PowerPlex-16 Systems were as follows: 95°C for 11 min, 10 cycles of 94°C for 30s, 60°C for 30s, 70°C for 45s, 20 cycles of 90°C for 30s, 60°C for 30s, 70°C for 45s and final extension of 60°C for 30 min.

The PCR conditions for AmpFlSTR® Identifiler® were as follow: 95°C for 11 min, 28 cycles of 94°C for 1 min, 59°C for 1 min, 72°C for 1 min and final extension of 60°C for 60 min.

The PCR conditions for AmpFlSTR® Yfiler were as follows: 95°C for 11 min, 30 cycles of 94°C for 1 min, 61°C for 1 min, 72°C for 1 min and final extension of 60°C for 80 min.

Genotyping

The PCR products were mixed with GeneScan™ -500 LIZ ™ Size Standart (Applied Biosystems, Foster City, CA, USA) and Internal Lane Standart 600 Size Standart (Promega Corporation, Madion, WI, USA).

Capillary electrophoresis was performed on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and results were analysed using GeneMapper® ID Software Versions 3.1 by comparison with allelic ladders.

Results

Case 1

A two months old girl was kidnapped in Ulaanbaatar on 5 July 1975. In 2010, 35 years later, the criminal investigation team discovered information that the missing girl might be found.

As the father of the kidnapped girl died 5 years ago, we carried out DNA analysis by comparing the kidnapped girl's DNA to her mother's and her brothers' DNA. The result of the analysis was positive and confirmed that the 35 years old lady is the girl who was kidnapped as an infant.

DNA analysis was carried out with 16 STR loci using AmpFlSTR® Identifiler® kits and PowerPlex-16 Systems. Statistical calculation of the analysis results showed the probability of 99,99 percent accuracy.

Fifteen STR loci allele frequency distribution in the Mongolian population was used in the calculation of statistical probability [1, 2].

At the end of the DNA analysis, the missing girl's genotype was determined as shown in Table 1.

No	STR Loci	Missing Girl	Mother	Brother 1	Brother 2	Allele Frequency*
1	D8S1179	11 - 13	11 – 12	11 - 13	11 – 12	P_{11}=0.0583 P_{12}=0.1242 P_{13}=0.2792
2	D21S11	30 – 30	29 – 30	29 – 30	30 – 30	P_{29}=0.2500 P_{30}=0.3300
3	D7S820	11 - 11	10 - 11	8 - 11	11 - 11	P_{8}=0.2692 P_{10}=0.1642 P_{11}=0.2833
4	CSF1PO	10 - 11	10 - 11	9 – 10	10 - 11	P_{9}=0.0467 P_{10}=0.2600 P_{11}=0.2667
5	D3S1358	15 – 17	16 – 17	15 – 17	16 – 16	P_{15}=0.3675 P_{16}=0.3292 P_{17}=0.1867
6	THO1	9 – 9.3	6 – 9	6 – 7	7 – 9	P_{6}=0.1292 P_{7}=0.0300 P_{9}=0.3308 $P_{9.3}$=0.1200
7	D13S317	11 - 11	11 - 11	11 - 11	11 - 11	P_{11}=0.2208
8	D16S539	13 - 13	12 - 13	12 - 13	12 - 13	P_{12}=0.2283 P_{13}=0.1233
9	D2S1338	19 – 23	19 – 20	19 – 22	19 – 24	P_{19}=0.1825 P_{20}=0.1308 P_{22}=0.0600 P_{24}=0.1508
10	D19S433	14 – 14.2	12 – 14.2	14 – 14.2	14 – 14.2	P_{12}=0.0350 P_{14}=0.2717 $P_{14.2}$=0.0775
11	vWA	17 - 19	17 - 19	17 – 17	17 – 19	P_{17}=0.2683 P_{19}=0.0883
12	TPOX	11 – 12	9 – 11	9 – 12	8 – 11	P_{8}=0.5442 P_{9}=0.0983 P_{11}=0.2817 P_{12}=0.0367
13	D18S51	20 – 20	15 – 20	19 – 20	15 – 19	P_{15}=0.1408 P_{19}=0.0458 P_{20}=0.0350
14	D5S818	12 – 12	11 – 12	11 – 11	11 – 11	P_{11}=0.4058 P_{12}=0.2383
15	FGA	23 - 23	23 - 23	23 - 24	23 - 24	P_{23}=0.2208 P_{24}=0.2442
16	Amelogenin	XX	XX	XY	XY	

** Allele frequency of 15 STR loci distributed in Mongolian population*

Table 1: Results of DNA typing in Case 1.

Case 2

This case involves the cruel homicide of a 7 years old girl in the province of Mongolia in May, 2010.

Vaginal samples collected from the victim were examined microscopically and processed to extract sperm cells. Under microscopic examination of one smear sample, only one sperm cell and a large number of epithelial cells were observed (no photos available).

Sperm cells were extracted from the samples which were collected from the vagina of the victim. Male DNA was isolated from the mixed sample using the phenol chloroform method and Differex system kit. The genotype of the criminal was determined using the AmpFISTR® Identifiler®, AmpFISTR® Yfiler and PowerPlex-16 kit.

Over 25 days of investigation, we analysed approximately 100 biological specimens from all suspects. On the 25th day of the investigation, 5 more suspects' samples were delivered to the laboratory. Among these 5 samples, the genotype of one suspect matched with the genotype data from samples obtained from the victim.

Statistical analysis showed that the match probability was $5{,}17*10^{-19}$.

Upon further investigation, police found a lollipop stick under a mattress in the suspect's home. We carried out DNA analysis on the lollipop stick. The victim's DNA was detected on the evidence.

The genotype of spit found on the lollipop stick, which was retrieved from the suspect's home, matched with the victim's genotype with the probability of $7{,}31*10^{-19}$ in one person (Table 2).

No	STR Loci	Male Fraction of Swab	Female Fraction of Swab	Victim	Suspect	Lollipop Stick	Allele Frequency*
1	D8S1179	13 – 13	12 – 14	12 – 14	13 - 13	12 – 14	P_{12}=0.1242 P_{13}=0.2792 P_{14}=0.1917
2	D21S11	29 – 29	30 – 30	30 – 30	29 – 29	30 – 30	P_{29}=0.2500 P_{30}=0.3300
3	D7S820	10 – 10	11 – 12	11 – 12	10 - 10	11 – 12	P_{10}=0.1642 P_{11}=0.2833 P_{12}=0.1775
4	CSF1PO	10 – 12	10 – 11	10 – 11	10 – 12	10 – 11	P_{10}=0.2600 P_{11}=0.2667 P_{12}=0.3467
5	D3S1358	16 – 18	15 – 16	15 – 16	16 – 18	15 – 16	P_{15}=0.3675 P_{16}=0.3292 P_{18}=0.0692
6	THO1	6 – 7	7 – 9	7 – 9	6 – 7	7 – 9	P_{6}=0.1292 P_{7}=0.0300 P_{9}=0.3308
7	D13S317	8 – 8	8 - 11	8 - 11	8 – 8	8 - 11	P_{8}=0.2183 P_{11}=0.2208
8	D16S539	9 – 10	9 - 13	9 - 13	9 – 10	9 - 13	P_{9}=0.2717 P_{10}=0.1133 P_{13}=0.1233
9	D2S1338	20 – 25	18 – 23	18 – 23	20 – 25	18 – 23	P_{18}=0.0975 P_{20}=0.1308 P_{23}=0.1908 P_{25}=0.0675
10	D19S433	13 – 14	13– 14	13– 14	13 – 14	13– 14	P_{13}=0.2875 P_{14}=0.2717
11	vWA	16 – 18	14 – 17	14 – 17	16 - 18	14 – 17	P_{14}=0.1367 P_{16}=0.1983 P_{17}=0.2683 P_{18}=0.2258
12	TPOX	8 – 9	8 – 9	8 – 9	8 – 9	8 – 9	P_{8}=0.5442 P_{9}=0.0983
13	D18S51	14 – 14	13 – 17	13 – 17	14 – 14	13 – 17	P_{13}=0.1642 P_{14}=0.2758 P_{17}=0.0733
14	D5S818	10 – 11	11 – 13	11 – 13	10 – 11	11 – 13	P_{10}=0.1192 P_{11}=0.4058 P_{13}=0.1375
15	FGA	21 – 22	22 - 23	22 - 23	21 – 22	22 - 23	P_{21}=0.1083 P_{22}=0.1333 P_{23}=0.2208
16	Amelogenin	XY	XX	XX	XY	XX	

** Allele frequency of 15 STR loci distributed in Mongolian population.*

Table 2: Results of DNA typing in Case 2.

As shown in Table 2, the random match probability between the genotype of sperm cell on the victim's smear and the genotype from the sample obtained from the suspect was $5{,}17*10^{-19}$. Also, the random match probability between the sample from the lollipop stick, found in suspect's home, and the victim's genotype was $7{,}31*10^{-19}$.

No	Y-STR Loci	Evidence / Swab	Suspect
1	DYS456	15	15
2	DYS389I	12	12
3	DYS390	24	24
4	DYS389II	28	28
5	DYS458	16	16
6	DYS19	14	14
7	DYS385a	13	13
8	DYS385b	18	18
9	DYS393	12	12
10	DYS391	10	10
11	DYS439	14	14
12	DYS635	22	22
13	DYS392	14	14
14	GATA H4	12	12
15	DYS437	15	15
16	DYS438	11	11
17	DYS448	20	20

Table 3: Results of Y-STR investigation in Case 2.

DNA analysis was performed using AmpFISTR® Identifiler® and the result was controlled by PowerPlex-16, AmpFISTR® Yfiler kits.

The results of the analysis are registered and archived.

Conclusions and Discussion

1. The missing girl's DNA profile was the same as her biological mother's by Human autosomal 16 STR loci. The Probability of Paternity of the DNA evidence is at least 99.99% compared to an untested random of Mongolian population (prior probability = 0.50) (Table 1).
2. The male DNA profile from the victim's vaginal swab, determined via AmpFISTR® Identifiler®, PowerPlex-16 and Yfiler kit analysis, showed statistical probabily of $5{,}17*10^{-19}$ in a random male.

For Case 2, the main difficulty of the examination was encountered during the process of DNA purification. It was difficult to come up with the result, because the male DNA profile was masked by female DNA after PCR amplification with AmpFISTR® Identifiler® kit. Hence, we performed Y-chromosome analysis with Yfiler kit, reviewed its results with PowerPlex-16 kit and then carried out a comparison analysis.

Furthermore, there were neither any witnesses to the crime nor anyone who had noticed any suspicious activity. This had a negative impact on the investigation as there was a large number of suspects, which required a lot of sample analyses conducted by our laboratory. In other words, we had lost considerable amount of time and work effort in performing analyses on individuals who were not related to this crime.

At the moment, a genetic database and its legal conditions have not yet been established in Mongolia. Due to financial problems, we also lack software and sufficient reagents which would help in our casework. These are some challenges we face in our laboratory when handling similar cases.

References

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DNA Contamination Risks in Post-mortem Samples and Tracking the Source: A Case Report

Dr Seah Lay Hong, Ms Aedrianee Reeza Alwi, Mr Sasitaran Iyavoo, Mr Abdul Hail Bin Ahmad Tarmizzi, Ms Nur Azeelah Abdullah
Department of Chemistry, Malaysia

Abstract

DNA can be transferred at any time before, during and after a death event. The observation of mixtures in DNA profiles recovered from post-mortem samples besides providing investigative leads could also indicate post-event transfer. The potential sources of contamination must be considered before the link to a potential perpetrator. This paper discussed the risks of post-mortem contamination and illustrated a case report tracking the source of contamination to prior autopsied bodies whose DNA profiles were reconstructed from their living next-of-kin.

Assessment of Contamination Risks

Before and after a death event, there is the potential for adventitious transfer of DNA [1, 2, 3]. Contamination is transfer of DNA after the death event. Potential sources of contamination are:

a) Investigation officers and others at the crime scene;
b) Pathologists and personnel in the post-mortem room;
c) DNA Laboratory staff;
d) Cross-contamination from body fluids originating from bodies pre-autopsied on the same post-mortem table or in the same post-mortem space; and
e) Cross-contamination from samples processed in the DNA laboratory.

Whereas a), b) and c) can be eliminated by reference to staff databases and databases of investigation personnel, d) and e) are more difficult to detect but are minimised by good laboratory and sterilisation practice [4, 5].

Post-mortem samples whether in the form of tissues like hairs and nails, swabs from any body part or clothing items from the autopsied body could produce mixed DNA profiles, revealing information on the activity before death and possibly the identity of the perpetrator. However, the scientist should exercise caution in the interpretation of mixed profiles from post-mortem sources. The mixtures may show DNA profiles arising from the following possibilities:

a) The DNA of the perpetrator has been visualised.
b) Cells have been transferred by an innocent individual before the death event – 'adventitious transfer'.
c) Cells have been transferred after the death event – 'contamination'.

The circumstances of the victim leading up to the death event is unknown to the scientist, hence the possibilities of adventitious transfer cannot be directly ascertained. Once the death event has been discovered, the scene and the associated evidence enter a controlled environment, where the risks of contamination can be possibly explored.

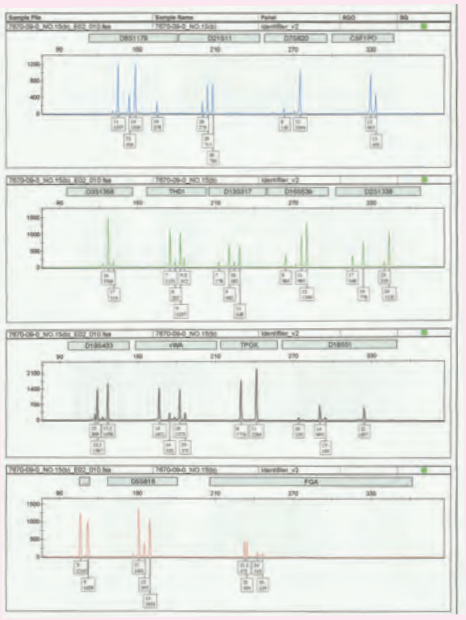

Figure 1: Mixed STR profile from swab taken at the back of the blazer. [Amplification using the AmpFISTR® Identifiler® PCR kit and analyzed by Applied Biosystems 3130xl Genetic Analyzer and GeneMapper® ID-X software].

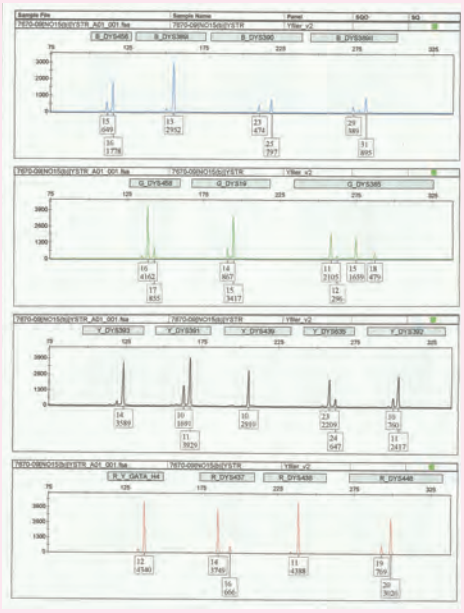

Figure 2: Mixed Y-STR profile from swab taken at the back of the blazer. [Amplification using the AmpFISTR® Yfiler® PCR kit and analyzed by Applied Biosystems 3130xl Genetic Analyzer and GeneMapper® ID-X software].

Defining Cell Origin

The association of body fluid and the DNA profile is not implicit [6]. The scientist cannot infer either the type of cell donating the DNA or the time when the cells were deposited. If a visible fresh bloodstain (giving a positive presumptive test) from an injured victim indicates a mixed DNA profile, the victim's DNA source can be reliably inferred but the cell-origin of the unknown foreign DNA is uncertain. An estimate of the quantity of DNA is useful to assist in the interpretation of the relevance of a DNA profile [7]. For example, if a sample from the victim yields a level of DNA exceeding 1 ng/µl, it is not unreasonable to associate the unknown foreign DNA with a body fluid source rather than transfer by casual contact.

A Real Case Situation

A swab taken at the back side of a blazer worn by the deceased in a death fall case produced a mixed DNA profile. The mixture was interpreted to contain the DNA of the deceased and one other unknown source (see Figures 1 and 2).

(continued on page 21)

Upcoming Events

Date	Event
20 Feb – 25 Feb 2012	AAFS 2012 Annual Scientific Meeting, Atlanta, Georgie, USA
03 Jun – 08 Jun 2012	The International Association of Forensic Toxicologists (TIAFT) 2012, Hamamatsu, Japan
01 Jul – 06 Jul 2012	The Society of Forenisc Science (SOFT) Meeting 2012, Boston, USA
18 Aug – 23 Aug 2012	The American Society of Questioned Document Examiners (ASQDE) Annual Meeting, Charleston, South Carolina, USA
20 Aug – 24 Aug 2012	6th European Academy of Forensic Science Conference (EAFS2012), The Hague, The Netherlands
23 Sep – 27 Sep 2012	21st International Symposium on the Forensic Sciences (ANZFSS), Hobart, Tasmania, Australia
26 Nov – 28 Nov 2012	AFSN 4th Annual Meeting and Symposium, Bangkok, Thailand