ForensicAsia

THE ASIAN FORENSIC SCIENCES NETWORK NEWSLETTER | ISSUE 2

AFSN President's Address

It seemed a short time ago when a few members of the Network came together and mulled over the idea of a network of Asian forensic institutes and soon we will be looking forward to the 3rd Annual Meeting in Seoul, South Korea.

Since we last met in Brunei Darussalam, we have also seen a number of transitions.

At the level of the Board, Ms Cheong Poh-Yee has retired from DSS, Brunei Darussalam, and her role on the Board is now filled by Ms Zunaidah Bte OKMB Hj Othman. Mr Primulapathi Jaya of Department of Chemistry, Malaysia has also retired and Mr Lim Kong Boon will take his place. Dr Khunying Porntip Rojanasunan represents CIFS, Thailand on the Board and Dr Chung Heesun, Director-General of the National Forensic Service (formerly known as the National Institute of Scientific Investigation), South Korea has joined the Board and is AFSN's International Liaison Officer. Dr Nguyen Van Ha from Vietnam Forensic Science Institute and Dr Romel Papa of National Bureau of Investigation, Philippines have both stepped down from the Interim Board. Ms Nellie Cheng has taken over the role of the Board Secretariat from Dr Angeline Yap. Dr Barbara Remberg has taken up her new role as Senior Technical Advisor in the Precursors Control Section of International Narcotics Control Board and our liaison with UNODC is now through Dr Justice Tettey.

We are indeed very grateful to Pathi, Poh-Yee, Angeline, Romel, Dr Nguyen and Barbara for their valuable contributions to the organisation and we wish them well in all their future endeavours.

AFSN has officially become a member of the International Forensic Strategic Alliance (IFSA) signed at Interpol in Lyon, on 5 October 2010, during the International Forensic Science Symposium. IFSA is now an alliance of five members, namely American Society of Crime Laboratory Directors (ASCLD), European Network of Forensic Science Institutes (ENFSI), Academia Iberoamericana De Criminalística Y Estudios Forenses (AICEF), Senior Managers of Australian and New Zealand Forensic Laboratories (SMANZFL) and AFSN.

The networks have committed themselves to create opportunities for strategic collaboration across the global forensic science community. The goals of IFSA are: to represent the operational forensic science community; to develop and execute a rolling agenda for strategic issues relating 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 members of the networks and other operational forensic experts. At the discussions held, it was clear that we share many challenges in common, at a time of financial stresses and increasing globalization.

Even as we are now connected into the global network, our Network will only thrive, if our members continue to enthusiastically support and contribute to the work that is being carried out in the network's workgroups and activities, in the months and years ahead. I believe that every one has a part to play, has something to share, be it experience, technical expertise or even an encounter with difficulty.

I look forward to seeing all of you in Seoul, South Korea in May 2011.

Dr Paul Chui
AFSN President

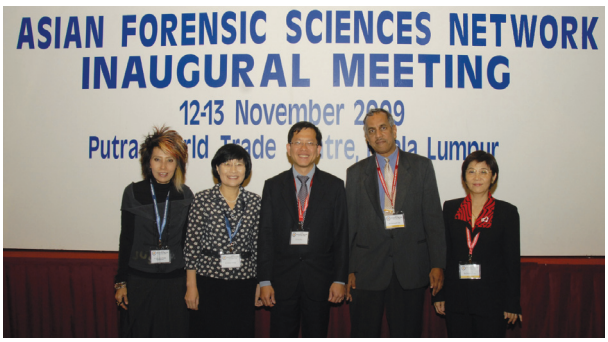
AFSN Board Members (2009-2011)

President:
Dr Paul Chui, Health Sciences Authority, Singapore

Vice-President:
Mr Lim Kong Boon, Department of Chemistry, Malaysia

International Liaison Officer:
Dr Chung Heesun, National Forensic Service, Korea

Board Members:
Ms Zunaidah Bte OKMB Hj Othman, Department of Scientific Services, Brunei Darussalam
Dr Khunying Porntip Rojanasunan, Central Institute of Forensic Science, Thailand



The elected Board Members at the 1st AFSN AGM on 13 November 2009.
From left: Dr Khunying Porntip Rojanasunan, Dr Chung Heesun, Dr Paul Chui, Mr Primulapathi Jaya (retired in November 2010), Ms Cheong Poh-Yee (retired in June 2010).

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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.

Editor's Address

Dear readers,

It has been a very exciting year since the Inaugural Issue of ForensicAsia was lauched at the AFSN Inaugural Meeting in Kuala Lumpur, Malaysia in November 2009. This second issue will bring you some snapshots of the happenings at the Inaugural Meeting in Kuala Lumpur, Malaysia in November 2009, as well as the second Annual Meeting which was held in Brunei Darussalam in June 2010.

I would like to thank all members who had enthusiastically submitted articles, as well as all Advisors and Guest Editors who tirelessly went through many rounds of reviews. It is all of you who made it possible for the publication of this newsletter!

Dr Angeline Yap
Editor

AFSN 3rd Annual Meeting and Symposium, Seoul, Korea, 25 - 27 May 2011

Registration is now open. For more details, please log on to **www.asianforensic.net/2011**

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AFSN Inaugural Meeting and Symposium in Kuala Lumpur, Malaysia, 2009

Mr Primulapathi Jaya
Department of Chemistry, Malaysia

AFSN Board meeting with UNODC and IFSA representatives.

Prof Jose Lorente was conferred as an Honorary Member of AFSN.

Dr Barbara Remberg was conferred as an Honorary Member of AFSN.

AFSN Inaugural Meeting in Kuala Lumpur, Malaysia, 2009.

After a year of preparation and hard work, the Asian Forensic Sciences Network was officially launched at the Putra World Trade Centre, Kuala Lumpur on 12 November 2009. This Inaugural Meeting of AFSN was attended by ninety-five participants from 23 organisations from 13 countries. This meeting followed the centenary celebrations of the Department of Chemistry, Malaysia.

The President of the AFSN Interim Board, Dr Paul Chui of Health Sciences Authority, Singapore, in his inaugural address, introduced the network, its aims and objectives as well as the reasons for the formation of the network. The official website of the network (www.asianforensic.net) as well as the first issue of AFSN newsletter "*ForensicAsia*" were also launched.

Two close friends who played a great role in the events leading to the formation of AFSN were honoured as honorary members – Dr Barbara Remberg of UNODC and Dr Jose Lorente of AICEF.

The Constitution and the Code of Conduct for the network was formally adopted at the First Annual General Meeting. The network also held its first election of office bearers for the Board. A total of five institutions were nominated and all were elected to serve the first term of office from 2009 to 2011. These five institutes were:

- Department of Scientific Services, Brunei Darussalam
- National Forensic Service (was then known as National Institute of Scientific Investigation), Korea
- Department of Chemistry, Malaysia
- Health Sciences Authority, Singapore
- The Central Institute of Forensic Science, Thailand

Dr Paul Chui from Health Sciences Authority, Singapore was further elected by the Members as the first President and Mr Primulapathi Jaya as the Vice President. Dr Chung Heesun of the National Forensic Service, Korea was appointed as the International Liaison Officer.

Lively discussions took place and friendships were formed between all participants.

The three workgroups (DNA, Illicit Drugs, and Trace Evidence) and the Quality Assurance & Standards Committee had held their first business meetings earlier in the day. It was an excellent opportunity for the workgroups and committee to come together to discuss common issues, draft the terms of reference for the workgroup, elect office bearers, identify immediate concerns, define workplans and plan future activities.

On the second day of the meeting, the DNA workgroup, Illicit Drugs Workgroup and Trace Evidence Workgroup all held a half-day workshop for the participants. There were many lively discussions, insightful presentations and a great deal of sharing and exchange of information.

The AFSN Board also held a meeting with Dr Barbara Remberg, representative from United Nations Office on Drugs and Crime (UNODC), and Mr Alastair Ross, representative from International Forensic Strategic Alliance (IFSA), to discuss on issues pertaining to collaborations and cooperation between AFSN, UNODC and IFSA. The idea of AFSN joining IFSA was also explored during the meeting.

The Inaugural Meeting has been a great success in bringing the regional forensic science community together for the purpose of sharing of information and strengthening the cooperation and network among these organisations. It is hoped that in the years to come more and more forensic institutions will become members of AFSN and work together in assisting the law enforcement agencies in combating crime in this region.

AFSN 2nd Annual Meeting and Symposium in Brunei Darussalam: An Unforgettable and Valuable Experience

Ms Zunaidah OKMB Haji Othman
Department of Scientific Services, Brunei Darussalam
Photos: Vianaliza Rajak (DSS)

The AFSN 2nd Annual Meeting & Symposium was held in Brunei Darussalam from 1 to 3 June 2010. Department of Scientific Services (DSS), Brunei Darussalam, one of the founding members of the network, had volunteered to host the event. The local organising committee comprised the staff of DSS, as well as staff from other departments of the same ministry: namely, the parent Department of Health Services and those outside the Ministry of Health and the committee was largely made up of non-forensic staff. The registration of participants and submission of abstracts for the scientific sessions were handled by the AFSN Secretariat at the Health Sciences Authority, Singapore. The AFSN Board and Chairpersons of the Workgroups and Committee also assisted in the organisation of the scientific sessions and planning of workshops and business meetings. The user-friendly AFSN website prepared by the AFSN Secretariat contained useful information on Brunei Darussalam, complete with information on hotels close to the venue, transport system, climate, currency, as well as meeting and symposium programmes.

The preparation was understandably hectic as the second meeting was scheduled to take place only about six months after the first Inaugural Meeting in Kuala Lumpur, Malaysia in November 2009. As AFSN is a regional network with no funding of its own, the funding for the meeting mostly originated from the kind sponsorships of local and international companies. It had been a challenge for the committee and the involved departments to observe all the rules and policies stated by the government. However the efforts and good cooperation between all parties concerned eventually paid off when the vital resource was obtained a few months before the meeting.

As this was the first international meeting organised by DSS, assistance from experienced personnel in the planning, execution and monitoring of agenda and timeline was evidently vital. A good leadership and cooperation from all staff involved to ensure efficient execution of all matters were essential.

The last few days before the event were most hectic where site visits, final discussions, logistics issues, etcetera, kept going on till the actual day.

The guest of honour for the event was the Permanent Secretary of Ministry of Health, Brunei Darussalam, Dato Paduka Haji Abdul Salam bin Abdul Momin. There was a total of one hundred and eighty-six participants from twenty countries representing fourteen local and forty-one international organisations in this meeting. Three plenary lectures were delivered by Dr Henry Lee from USA and Prof Jose Lorente of AICEF. There were in addition five parallel

Some of the Brunei AFSN committee with Dr Barbara Remberg (UNODC) and Dr Michael Tay (HSA, Singapore).

Ms Cheong Poh-Yee, Chairman of Organising Committee and AFSN Board Member, giving a welcome address.

Dr Henry Lee (left) and Prof Jose Lorente (right) captured all members' attention by their interesting and well-delivered plenary lectures.

AFSN 2nd Annual Meeting & Symposium in Brunei Darussalam, 2010.

AFSN and IFSA preliminary signing ceremony. (From left: Dr Paul Chui, Prof Jose Lorente, Dr Barbara Remberg, Mr Alastair Ross)

The cheerful AFSN President, Dr Paul Chui was having a lively discussion with Prof David Gidley (ICITAP) and Mr Saman Azhari.

workshops and scientific sessions in DNA, Illicit Drugs, Trace Evidence, Toxicology, and Quality Assurance & Standards. Dr Bruce Budowle was the invited trainer for the DNA workshop while the Trace Evidence Workshop had Dr Grzegorz Zadora. Altogether, thirty-two oral presentations and thirty-three posters were presented during the scientific sessions.

All the meetings and luncheons were conveniently held at the same venue. The local secretariat, manned by the non-forensic staff, helped to ensure that everything ran smoothly while their forensic colleagues attended the meeting and symposium.

The welcome dinner was graced by the guest of honour, Minister for Health, the honourable Pehin Orang Kaya Johan Pahlawan Dato Seri Setia Awang Haji Adanan bin Begawan Pehin Siraja Khatib Dato Seri Setia Awang Haji Mohd Yuusuf, where a most sumptuous dinner was complemented by interesting Brunei cultural performances.

On the last day, the committee stayed on at the meeting venue until all the participants had left safely with a warm memorable farewell. Tidying up began and everybody was tired but the valuable experience was well worth it.

Annual Meeting of The International Forensic Strategic Alliance and The International Forensic Science Symposium

Dr Chung Heesun
International Liaison Officer, AFSN

Annual Meeting of International Forensic Strategic Alliance (IFSA)

The 2010 annual meeting of the International Forensic Strategic Alliance (IFSA) was held at the INTERPOL building in Lyon, France on 3 October 2010. The participants were Jan DeKinder (ENFSI President), Pavel Rybicki (ENFSI Chairman Designate), Peter de Bruyn (ENFSI Secretary), Greg Matheson (ASCLD President), Susan Johns (ASCLD ILO), Jacobo Orellana Suarez (AICEF President's representative), Alastir Ross (SMANZFL Chair's representative), John Scheffer (SMANZFL Observer), Tony Raymond (SMANZFL ILO), Paul Chui (AFSN President) and Heesun Chung (AFSN ILO). Susan Hitchin from INTERPOL and Justice Tettey from UNODC also participated in the meeting as guests.

Signing of IFSA agreement by representatives from the five forensic networks.

Participants of IFSA Annual Meeting.

Jan DeKinder chaired the meeting according to the bylaws of IFSA. During the meeting, many issues were discussed, including updates on INTERPOL activities, member updates, activities of UNODC, research library, business rules/ procedures, updates on NIFS research center, position statement, twinning, gap analysis, IFSA website, NIFS standards, 2011 meeting site and future meetings.

Since there was not enough time to discuss new businesses, another meeting was organised during the lunch time on 5 October 2010. During this lunch meeting, ten new businesses were discussed and each regional organisation volunteered to take charge of a few of them.

IFSA Signing Ceremony

A significant event was the signing ceremony held at 5 pm on 5 October 2010 during the 16th International Forensic Science Symposium (IFSS) to welcome AFSN into IFSA (http://www.interpol.int/Public/ICPO/PressReleases/PR2010/PR079.asp).The expansion of IFSA (formerly comprised the European Network of Forensic Science Institutes (ENFSI), the Academia Iberoamericana De Criminalistica Y Estudios Forenses (AICEF), the American Society of Crime Laboratory Directors (ASCLD), and the Senior Managers of Australian and New Zealand Forensic Laboratories (SMANZFL) to include AFSN was greeted with much excitement and enthusiasm as this would enhance the strategic cooperation among the international forensic science and world police communities.

After the signing ceremony, there was a poster presentation session of IFSA during the delegate reception in Atrium hosted by INTERPOL. The poster presentation of AFSN was very well received by many participants, especially those from Asia, many whom expressed their desire to join AFSN. It was a very successful event. The next IFSA meeting will be held in Madeira, Portugal in September 2011.

The International Forensic Science Symposium (IFSS)

More than 170 forensic scientists, investigators and researchers from more than 50 countries participated in the 16th IFSS from 5 - 8 October 2010 at the INTERPOL premises. After the opening ceremony on 5 October 2010, many scientific sessions on criminalistics (firearms, marks, paint and glass, fibres, forensic geology), environmental crimes, biometrics (INTERPOL forensic identification), human identification, media evidences, 'Forensic Science: effectiveness and efficiency', chemical evidence, drugs and toxicology were held till the 8 October. Business meeting was also held to prepare for the next meeting in 2013.

National Forensic Service, Korea

Dr Chung Heesun
National Forensic Service, Korea

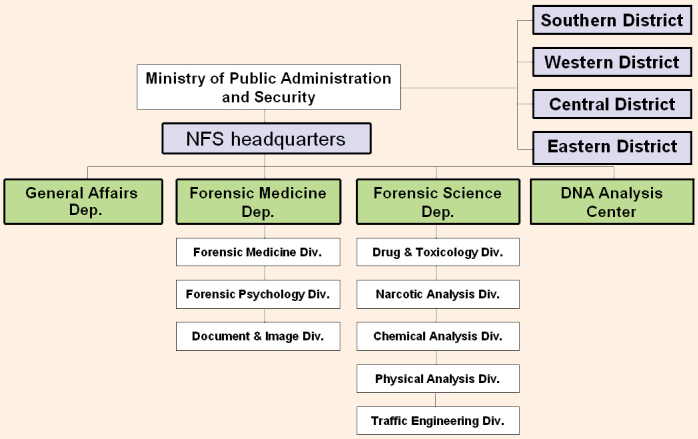
The National Forensic Service (NFS), formerly known as the National Institute of Scientific Investigation (NISI), was established in 1955. Since then, it has provided a comprehensive forensic medicine and science service to law enforcement agencies throughout the country. The NFS performs autopsies as well as scientific analysis, examination and research for forensic evidence. Its headquarters is based in Seoul and it has four branches at strategic locations in the country.

- The NFS's vision is to be a reliable forensic organisation providing the leadership in scientific investigation.
- The NFS's mission is to be a globally renowned forensic institute with cutting-edge scientific technologies and to be a forensic research centre contributing to a peaceful and just society through scientific analysis that the public can have full confidence in.
- The NFS's core values are reliability, future-oriented thinking and realisation of a just society.

History

In 1955, the NFS was established as the NISI under the Presidential Decree No. 1021 to conduct forensic examination and research. Initially, forensic service was provided mainly in the field of fingerprint identification. The service areas expanded to many disciplines including lie detection in 1980, human voice recognition in 1987 followed by DNA analysis in 1991. Four district branches, which cover the forensic service in the southern, western, eastern and central parts of the country, respectively, have been set up since 1993. In order to facilitate the forensic investigations of mass disasters, a Mass-Death Victims Service Team was established in 2002. In 2005, the 50th anniversary year of the NFS, the 43rd annual meeting of The International Association of Forensic Toxicologists (TIAFT) was organised by the NFS and held in Seoul. The NISI changed its name to the NFS in 2010 due to the high recognition given by the Korean government.

Organisation



The NFS headquarters has three departments and one center: General Affairs Department, Forensic Medicine Department, Forensic Science Department and Forensic DNA Center. Forensic Medicine Department has 3 divisions which are Forensic Medicine Division, Forensic Psychology Division and Document & Image Division. Forensic Science Department has 5 divisions which are Drug & Toxicology Division, Narcotic Analysis Division, Chemical Analysis Division, Physical Analysis Division and Traffic Engineering Division.

- The Forensic DNA Center performs personal identification by DNA analysis and establishes and maintains profiling of the DNA database as well as perform DNA analysis for missing children.
- The Forensic Medicine Division provides postmortem inspection and autopsy as well as other forensic medicine examinations including forensic pathology, anthropology and orthodontics.
- The Forensic Psychology Division conducts research on criminal psychology, crime motivation and environment and juvenile crimes as well as enhancement of victims' or witnesses' recollection using forensic hypnosis.
- The Document & Image Division examines documents and images, including handwriting, imprints, counterfeit bills, contracts, etc and also restores digital evidence.
- The Drug & Toxicology Division conducts the analysis of drugs and poisons in postmortem cases and food analysis.
- The Narcotic Analysis Division provides analysis and research on illegal drugs in biological specimens as well as seized materials and also performs drug characterisation and impurity profiling.
- The Chemical Analysis Division performs blood alcohol analysis in DUI cases, blood alcohol and carbon monoxide analysis in postmortem cases, and the analysis of trace evidences and petroleum products.
- The Physical Analysis Division conducts comparative analysis of tool marks and impressions, fire investigation and fire debris analysis, forensic phonetic and acoustic analysis, forensic firearms identification and analysis of firearm and explosive residues.
- The Traffic Engineering Division conducts vehicle safety and structural analysis, accident reconstruction and analysis of trace evidences (such as fibers and paints) in traffic accidents.

Research and Quality Assurance Activities

The current research projects conducted by the NFS include the development of new analytical and investigative methods, the construction of a video evidence analysis system, the construction of a narcotic information system and a drug characterisation / impurity profiling system, the construction of a national DNA database, the applications of stable isotope ratio mass spectrometry to trace the geographical origin of evidence, and the application of CT and MRI in autopsies.

In 2004, the NFS was accredited by KOLAS to ISO/IEC 17025 standards and in April 2009, it was further recognised and designated by KOLAS as a provider of international proficiency testing services.

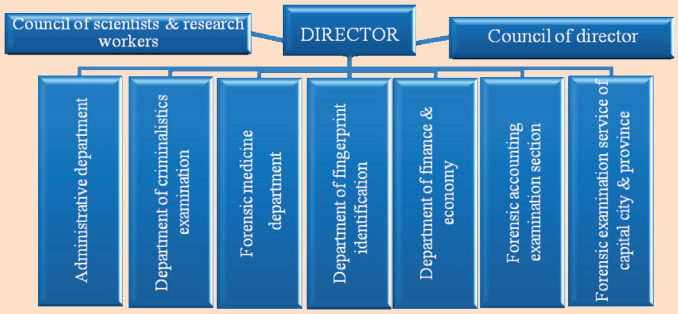
National Institute of Forensic Science, Mongolia

Mr Chimid Altankhishig
National Institute of Forensic Science, Mongolia

The National Institute of Forensic Science was established in 2005 under the authority of the Minister of Justice and Home Affairs of Mongolia. This forensic investigation organisation is a state organisation that has main direction to strengthen discovering evidence and material evidence during the control and settlement procedure of criminal, civil, administrative and other crimes, to conduct investigation of the scene of the crime and material evidence, and to make a conclusion with scientific basis.

The approval of the Law on Forensic Investigation at the autumn session of the Parliament of Mongolia in 2009 was fundamental for refining legal environment of the activity in the organisation. The Law on Forensic Investigation legalised the main principle of forensic investigation, as well as set up common rules and terms explanation for investigation, details the rights and duties of investigators, and defines the structure of the organisation and legal guarantees for staffs.

Organisation Chart



The Department of Criminalistics Examination

The Technical Section of Examination developed under the control of the State Police Department at the decision No.14 by the Minister of Domestic Affairs on 12 January 1944, and later became the official Department of Criminalistics Examination.

From its long history, the Department of Criminalistics Examination has obtained its structure today and operates with its laboratories for firearm examination, handwriting analysis and trace study.

The Department of Forensic Medicine Examination

In accordance with the resolution No.198 of Ministers' Council of the People's Republic of Mongolia on 20 April 1960, the Forensic Medicine Examination Bureau was founded under the jurisdiction of the Ministry of Health Care. Since then, it has developed into a specialised service which came closer to international standards.

The Department of Fingerprint Identification

This Department organises national fingerprint database activity, prepares experts to work in sub-stations, examines fingerprints and trace at the scene of serious crime by participating as an expert and providing professional assistance.

The "Papilon" system for automatic fingerprint comparison has been installed since 1995. In 2008, substations of the "Papilon" system were installed nationwide. The activity to develop, enhance and use of the fingerprint database was regulated by the "Rule on Fingerprint Database" which was approved by the order No.110 of Minister of Justice and Internal Affairs in 2009.

The DNA Laboratory

The DNA Laboratory uses an ABI 310 Genetic Analyser and conducts the following examinations:

- Isolation of nuclear DNA from blood, saliva, and sperm found at the scene of a crime;
- Extraction of male-specific DNA;
- Isolation of mitochondrial DNA from hair and soft tissue;
- Nuclear DNA profiling of biological samples taken from suspects, victims, and other persons concerned with a crime;
- Mitochondrial DNA analysis of ancient samples as well as hair and bone samples;
- Development of a DNA database on unidentified bodies;
- Establishment of a database of DNA profiles recovered from unsolved crimes, and also offenders and victims of major crimes;
- Determination of gender;
- Parentage analysis using 6 to 15 genetic loci.

The Physical Laboratory

In this laboratory, gunshot residue, paint from vehicles, earth, precious items such as gold, silver and gemstone, as well as other chemicals are examined. Heavy metals such as Na^{11}-U^{22} are analysed by the ELVAX roentgen- fluorescence spectrometer where more than 10 methods may be used.

The Biological Laboratory

The laboratory performs blood typing (Mn, Rh, R, and Le antigens) from liquid blood, dried blood stains, and other biological samples (e.g., hair, organs, body). The laboratory also examines for the presence of sperm on evidence.

The Chemical Laboratory

This laboratory carries out 12 types of examinations which include:

- Determination of the level of alcohol in blood and urine of suspects and persons concerned at the scene of crime, as well as determination of the level of spirits in the gastric liquid;
- Analysis of toxic substances in certain kinds of food products and unknown substances;
- Analysis of drugs and psychoactive substances in plants and synthetic products;
- Restoration of obliterated numbers on metals and alloys,
- Comparison of compositions of glue and a variety of written and painted dyes;
- Determination of alcohol levels in alcoholic beverage;
- Determination of carboxyhaemoglobin level in blood.

Since 2009, the Varian-400 CC-MC gas chromatography-mass spectrometer has been used in examinations.

The aim of the forensic science organisation is to provide individuals and legal institutions with regular and trustworthy forensic services that are of good standards quality.

Preliminary Techniques to Distinguish Methylamphetamine Synthesis via P-2-P and Ephedrine/Pseudoephedrine Routes

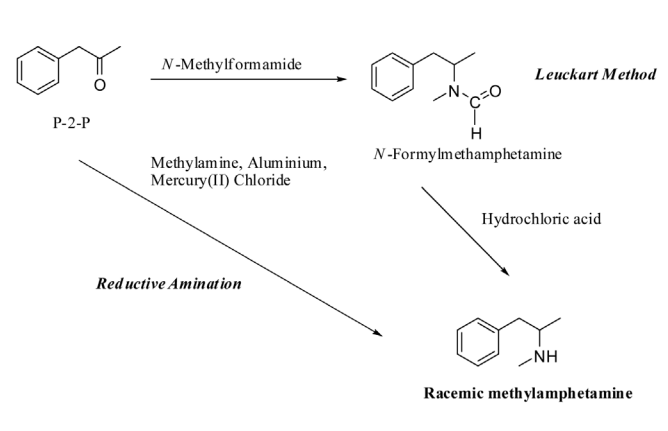
Dr Vanitha Kunalan
Department of Chemistry, Malaysia

Abstract

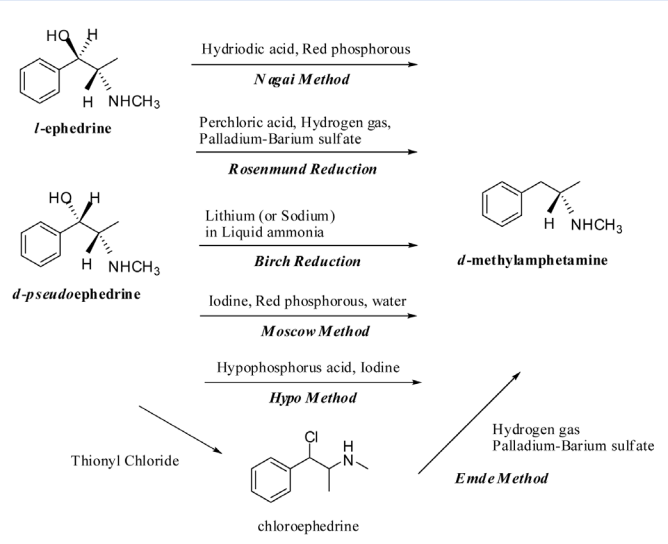
Methylamphetamine was synthesised using the eight most frequently encountered routes used in the clandestine laboratories. Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (NMR) determination were used to confirm the synthetic products. Melting point and optical rotation were used to distinguish the racemic and *d* forms of methylamphetamine produced in the synthesis.

Introduction

The illicit manufacture of methylamphetamine can be accomplished in a variety of ways, but it is produced most commonly by using either of two primary synthesis methods. The fundamental difference between the two methods is in the use of precursor chemicals. The first method requires the use of phenyl-2-propanone (P-2-P) as the precursor, while the second method uses ephedrine or *pseudo*ephedrine (known as the ephedrine/*pseudo*ephedrine reduction method). Methylamphetamine synthesis via P-2-P route yields methylamphetamine in racemic form while ephedrine/*pseudo*ephedrine route produces methylamphetamine in *d* or *l* forms.[1]



Scheme 1: Synthesis via P-2-P route.



*Scheme 2: Synthesis via ephedrine/pseudo*ephedrine *route.*

Material and Methods

Methylamphetamine was synthesised in the laboratory via each of the routes in Scheme 1 and 2. Two batches of methylamphetamine hydrochloride was synthesised by the Leuckart and Reductive Amination methods (one batch per method) using the same starting material, phenyl-2-propanone (P-2-P). Twelve batches of methylamphetamine hydrochloride were synthesised by the Nagai, Rosenmund, Birch, Emde, Moscow and Hypo methods (two batches per method) using either *l*-ephedrine or *d*-*pseudo*ephedrine as the starting material.

The exact reaction conditions of each synthesis were modified to small scale reaction from the methods presented in a commonly available book used by clandestine chemists, Uncle Fester's "Secrets of Methylamphetamine Manufacture" 5th Edition.[2] In each route, methylamphetamine was synthesised in methylamphetamine base form and converted to methylamphetamine hydrochloride. The hydrochloride salt of the methylamphetamine was obtained according to the method suggested by Uncle Fester.[2]

Infrared spectra were obtained with a Perkin Elmer Spectrum 1. The resolution was set at 4.000 cm^{-1}, with 32 scans between 450 cm^{-1} and 4000 cm^{-1}. ^{1}H and ^{13}C spectra were recorded on a Bruker DPX 400 spectrometer at 400 MHz and 100 MHz respectively. Chemical shifts are reported in parts per million (ppm). The melting point was determined using the Buchi B-545 melting point apparatus. The optical rotation was measured with Perkin Elmer 341 polarimeter using a cell with a path length of 1 dm. $[\alpha_D]^{25}$ is the observed angel of optical rotation of methylamphetamine at 25ºC.

Results and Discussion

Analysis for methylamphetamine HCl was in agreement with published data for IR,[3] ^{1}H NMR,[4] and ^{13}C NMR.[5]. IR ν_{max} (KBr)/cm^{-1}: 3419 (N-H), 2971, 2731, 2461 (C-C), 1603 (N-C) (Figure 1). ^{1}H NMR (400 MHz, D_2O): δH 1.22 (d, 3H, *J* = 8.0 Hz, CH_3), 2.64 (s, 3H, CH_3), 2.87 (dd, 1H, *J* = 24.0, 8.0 Hz, CH), 3.03 (dd, 1H, *J* = 20.0, 8.0 Hz, CH), 3.44-3.50 (m, 1H, CH), 7.25-7.38 (m, 5H, C_6H_5) (Figure 2). ^{13}C NMR (100 MHz, D_2O): δ 14.8, 29.9, 38.8, 56.4, 127.5, 129.1, 129.5, 135.8 ppm.) (Figure 3).[6]

❄ Melting Point

Table 1 shows the melting point results obtained for samples prepared in this study. Methylamphetamine synthesised from phenyl-2-propanone (P-2-P) could be differentiated from ephedrine or *pseudo*ephedrine. The tabulated result was in agreement with published data for melting point.[7]

❄ Polarimetry

Optical rotation was used to distinguish the *d* and racemic forms of methylamphetamine and, thus elucidate which

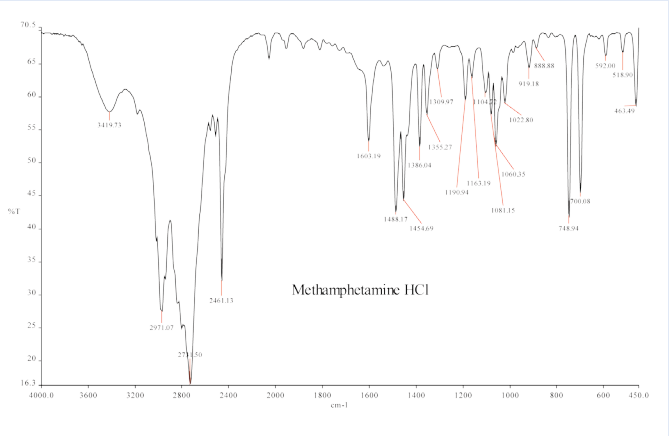


Figure 1: IR spectrum of methylamphetamine hydrochloride.

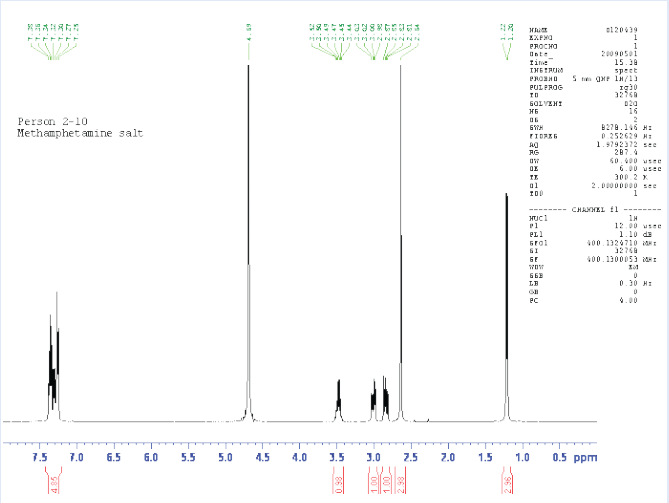


Figure 2: ^{1}H NMR spectrum of methylamphetamine salt.

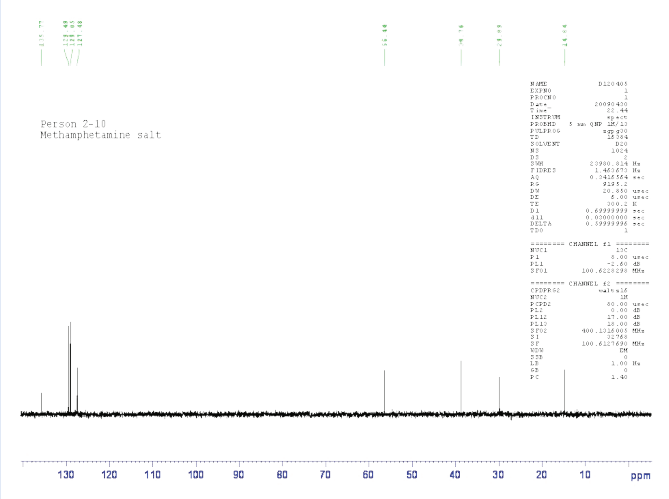


Figure 3: ^{13}C NMR spectrum of methylamphetamine salt.

methylamphetamine samples were synthesised from the P-2-P routes - Leuckart and Reductive Amination routes ($[\alpha_D]^{25}$ = 0º) and which were via the ephedrine / *pseudo*ephedrine routes - Nagai, Rosenmund, Birch Reduction, Emde, Moscow and Hypo routes ($[\alpha_D]^{25}$ = +17.7). The obtained result was in agreement with published data for optical rotation.[8]

Route	Precursors	Melting point of methylamphetamine product
Leuckart	phenyl-2-propanone	130-131ºC (*dl*-methylamphetamine)
Reductive Amination	phenyl-2-propanone	130-131ºC (*dl*-methylamphetamine)
Nagai	*l*-ephedrine HCl and *d*-*pseudo*ephedrine HCl	170-173ºC (*d*-methylamphetamine)
Rosenmund	*l*-ephedrine HCl and *d*-*pseudo*ephedrine HCl	170-173ºC (*d*-methylamphetamine)
Birch	*l*-ephedrine base	170-173ºC (*d*-methylamphetamine)
Emde	*l*-ephedrine HCl and *d*-*pseudo*ephedrine HCl	170-173ºC (*d*-methylamphetamine)
Moscow	*l*-ephedrine HCl and *d*-*pseudo*ephedrine HCl	170-173ºC (*d*-methylamphetamine)
Hypo	*l*-ephedrine HCl and *d*-*pseudo*ephedrine HCl	170-173ºC (*d*-methylamphetamine)

Table 1: Melting point results.

Conclusion

This work has reported the melting point and polarimetry of methylamphetamine hydrochloride synthesised in-house via P-2-P and ephedrine/*pseudo*ephedrine. Eight preparative methods were used during the synthesis. The melting point of a racemic mixture of *dl*-methylamphetamine is 130-131ºC while the more potent *d* isomer of methylamphetamine has a melting point of 170-173ºC. As a result, melting point and polarimetry are preliminary techniques to distinguish methylamphetamine synthesis via P-2-P and ephedrine/*pseudo*ephedrine routes. However, this method requires fairly pure samples.

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Analysis of Organic Impurities in 3,4-Methylenedioxymethamphetamine (MDMA) Tablets: A Tool to Classify and Determine Synthetic Methods and Link Samples of Ecstasy Seized in Vietnam

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Abstract

Profiling organic impurities in Ecstasy (3,4-methylenedioxymethamphetamine, MDMA) tablets by GC and GC-MS is an important tool to determine production methods and links between illegal laboratories producing Ecstasy tablets. The combination of the forensic intelligence with the investigative information shall improve the effectiveness of the battle against drugs in Vietnam.

Introduction

In the twenty-first century, Ecstasy tablets have become more popular in Vietnam, overtaking the place of "Ice" (Methamphetamine), and is about 70% of total seized. In February 2008, a big case with 57,000 Ecstasy tablets (logos: double hearts, star, music note…) together with tableting instruments were destroyed. In the black market, the price of Ecstasy tablets is now higher than that of methamphetamine. Therefore, fake Ecstasy tablets have also appeared. These contain methamphetamine and/or ketamine and caffeine instead of MDMA.

Analyses of organic impurities (adulterants, diluents and pharmaceutical substances) in Ecstasy tablets have been performed by different groups [1-5]. Other authors found different by-products in different extraction milieus [6-10]. Other published literature described different extraction conditions (neutral or different basic media). The characteristic by-products for each synthesis method are still being discussed [5], [12], [15].

The organic impurities in seized Ecstasy tablets will be analyzed. This provides vital information to support law enforcement effort in its combat against drugs in Vietnam [16].

Experimental

❄ **Reference standards**

MDMA, amphetamine, methamphetamine, dimethylamphetamine (DMA), ketamine, diazepam, nitrazepam, ephedrine, caffeine, (purity from 98.84 to 99.99 %) were obtained from Lipomed Company (Switzerland).

❄ **Samples**

400 seized Ecstasy samples from 2005 to 2009 were analyzed. The samples came from big cases of different provinces. Tablets with the same logo were randomly chosen.

❄ **Sample preparation for quantitative analysis of MDMA**

200 mg of tablet (about 20 mg of pure MDMA) were pulverized and added 3 ml buffer with pH=11.5, shaken for 10 min, extracted 2 times, each time with 3 ml diethyl ether for 5 min, separated the organic layer, filtered through a nylon membrane disc with porous size 0.20 µm diameter; dried with nitrogen, dissolved the residue in 500 µL diethyl ether containing n-dodecane (C12) as internal standard, mixed again, put into vials and injected in gas chromatograph on the same day.

❄ **Sample preparation for semi-quantitative analysis of by-products**

300 mg of pulverized Ecstasy tablets were dissolved in 2 ml buffer solution with pH=7.0; vortexed for 10 min; shaken for 20 min, added 400 µL toluene containing eicosane (C20) as internal standard, shaken again for 10 min; filtered through a nylon membrane disc with porous size 0.20 µm diameter; dried with nitrogen, dissolved the residue in 500 µL toluene, then put in micro vials for gas-chromatography mass spectrometry (GC-MS) analysis.

❄ **Instruments and operating conditions**

Agilent GC-MS 6890/ 5973 i, column DB-5 MS 30 m x 0.25 mm x 0.25 µm, temperature: column 90ºC (1 min) with 8ºC/min to 300ºC (10 min); injector: 250ºC; splitless; transfer line: 300ºC; ion source: 230ºC; injection volume: 2µL; Helium gas with flow: 0.7 ml/min (velocity 30 mL/s); TIC: 50-550 m/z; Library: NIST 02 and comparison with literatures [16].

❄ **The Limit of Detection (LOD) and the Limit of Quantification (LOQ)**

The linearity of the method was determined as follows [14]:

Y= 768.72X- 15.896; R= 0.9998

X: Concentration of MDMA; Y: Peak area.

The LOD GC/FID: 0.05 mg/ml and LOQ: 0.15 mg/ml

Reproducibility: relative average deviation of peak area is 2.7%.

Repeatability: relative average deviation of peak area is 3.3%.

Recovery: 91.7%

Results and Discussion

Based on the qualitative and quantitative analysis of MDMA, the analyzed Ecstasy tablets could be divided in 3 groups:

i) The first group contains MDMA from 28 to 70%. It represents 46% of all analyzed Ecstasy tablets. This group contains a lot of adulterants such as antihistamines: venlafaxine, diphenhydramine, chlorpromazine, clozapine, benzodiazepines (diazepam, medazolam, nitrazepam), barbiturates (phenobarbital, methylphenobarbital), anesthetics (lidocaine, procaine, ketamine), opiates (acetylcodeine, heroin) and other drugs such as etafedrine, amphetamine, methamphetamine, tenamphetamine (MDA), dimethylamphetamine (DMA) , brolamphetamine (DOB), 2,5-dimethoxy-4-bromophenethylamine (2C-B), 2,5-dimethoxy-4-ethylphenethylamine (2C-E), diethylpropione, 2,5-dimethoxy-4-ethylamphetamine

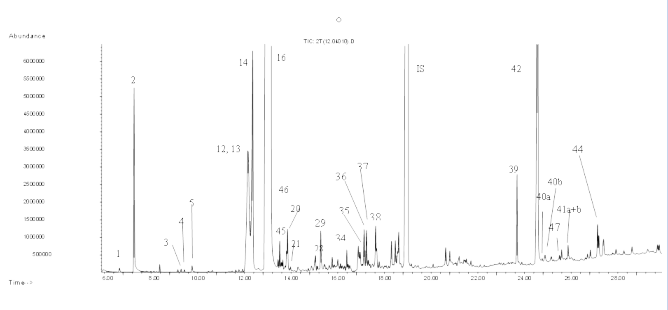

Figure 1. A GC chromatogram of an MDMA tablet.

(DOET), trifluoromethylphenylpiperazine (TFMPP), benzylpiperazine (BZP) and some pharmaceutical substances (caffeine, paracetamol, ephedrine). Some logos as well as new drugs identified in this group appeared for the first time in Vietnam. Tablets of this group are assumed to have been imported to Vietnam.

ii) The second group consists of Ecstasy tablets containing 10-25% MDMA. The group represents 37.5% of all analyzed Ecstasy tablets. Most of them were not mixed with adulterants. The kinds of logos of this group are limited to a few: two double hearts, star, music note, small arrow, Mickey mouse and petrol station. Tablets of this group were most likely pressed in the country and are more widely distributed in the north.

iii) The third group consists of fake Ecstasy tablets containing 0-5% MDMA. Most of the tablets contained large amounts of caffeine and/or ketamine. They were likely pressed in the country, and represent 16.5% of analyzed Ecstasy tablets.

After chemical analysis, the seized tablets could be classified into one of the three groups, but not based on their logos. For example, there were three kinds of tablets with double hearts logo, which contain different amounts of MDMA in the three different groups.

Based on the peaks detected in GC-MS and MS match with library, 46 by-products were identified as follows:

No.	Substance	No.	Substance
1	1,2-MD-4-methylbenzene	22	4-(3,4-MDphenyl)-5-methyl-1,3-dioxolan-2-one
2	Safrole	23	3,4-dimethoxymethamphetamine
3	1-(3,4-MDphenyl)propane	24	3,4-MDbenzylmethylketoxime
4	Piperonal	25	5-(3,4-MDphenyl)-4-methylpent-4-en-2-one
5	Piperonyl methyl ether	26	N-[(3,4-MDphenyl)-1-methylvinyl]-N,N-dimethylamine
6	Isosafrole (cis & trans)	27	N-methyl-3,4-MDphenylalanine
7	3,4-MDphenylmethanol	28	4-(3,4-MD)-but-3-en-2-one
8	N-methyl-3,4-MDbenzylamine	29	N-methyl-N-formyl-3,4-MDbenzylamine
9	3,4-MDacetophenone	30	N-[2-(5-methoxy-3,4-MDphenyl)-1-methylethyl]-N-methylamine
10	4-methoxymethamphetamine (PMMA)	31	3,4,5-trimethoxymethamphetamine
11	3,4-MDphenyl-2-propanone	32	5-(3,4-MDphenyl)-2,2,3,4-tetramethyl-1,3-oxazolidine
12	3,4-methylenedioxyamphetamine (MDA)	33	N-methyl-N-acetyl-3,4-MDbenzylamine
13	3,4-MDphenyl-2-propanol	34	N-formyl-MDA
14	3,4-MDphenyl-1-propanol	35	N-acetyl-MDA
15	MDMA	36	N-formyl-MDMA
16	1-(3,4-MDphenyl)propane-1,2-dione	37	N-acetyl-MDMA
44	Unknown 147 [16]	38	N-[β-(3,4-MDphenylisopropyl)]-3,4-MDbenzaldimine
45	3,4-methylenedioxyethylamphetamine (MDEA)	41	N-(3,4-MDphenylmethyl)-N-[2-(3,4-MDphenyl)-1-methylethyl]-N-methylamine
17	N-[2-(3,4-MDphenyl)-1-methylethylidene]-N-methylamine	39	di-[1-(3,4-MDphenyl)-2-propyl]amine (a+b) (MDA dimer)
18	Trimethyl-3,4-MDchromane	46	Unknown 192 [16]
19	3,4-MDdimethylamphetamine	40	di-[1-(3,4-MDphenyl)-2-propyl]methylamine (a+b) (MDMA dimer)
20	2-(3,4-MDphenyl)-1-methylethylacetate	42	Unknown 218 [16]
21	3-(3,4-MDphenyl)-2-oxopropanoic acid	43	Unknown 178 [16]

(Abbreviation: MD=methylenedioxy-; by-products are ordered according to increase of retention time)

Table 1: By-products in Ecstasy tablets extracted in pH=7.

The reductive amination reaction of piperonyl methyl ketone (PMK) with methylamine could happen through different reductive agents such as $NaBH_4$ or $NaCNBH_3$ or $Al/HgCl_2$. Based on compound markers (by-products) of reductive amination method [2,5] as: N-methyl-3,4-MDbenzylamine (No.8), N-[2-(3,4-MDphenyl)-1-methylethylidene]-N-methylamine (No.17), N-formyl-MDA (No.34) and N-(3,4-MDphenylmethyl)-N-[2-(3,4-MDphenyl)-1-methylethyl]-N-methylamine (No.41), the analyzed Ecstasy tablets could be classified as follows: 85% of samples were produced by the reductive amination method; 10% of samples were produced by Leuckart method with compound markers such as: N-methyl-3,4-MDbenzylamine (No.8), N-(3,4-MDphenylmethyl)-N-[2-(3,4-MDphenyl)-1-methylethyl]-N-methylamine (No.41), di-[1-(3,4-MDphenyl)-2-propyl]amine (a+b) (MDA dimer) (No.39) and di-[1-(3,4-MDphenyl)-2-propyl] methylamine (a+b) (MDMA dimer) (No.40). The rest of the analyzed Ecstasy tablets could not be classified as to their production method.

Conclusion

The qualitative and quantitative analysis of Ecstasy tablets identified three groups. One group is assumed to have been imported (high concentrations of MDMA), while the other two groups (lower MDMA concentration and fake Ecstasy tablets) were probably prepared in Vietnam.

The analysis of by-products determined that 85% of the analyzed Ecstasy tablets were synthesized by reductive amination method while 10% of them were produced by Leuckart method.

The results of the analysis of organic impurities showed the diversity of Ecstasy tablets in Vietnam and provided supporting information as to whether the tablets had likely been pressed in the country or whether they had been imported to Vietnam. This could provide law enforcement agency with important information about illicit clandestine laboratory in the country and the link between illegal gangs in the country.

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DNA Profiling: The Way Forward

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Introduction

The central goal in crime investigation is "attribution", i.e., who is the perpetrator or who is the victim of the crime. Identification of the "who" facilitates the investigation into the "why" of the crime, and forensic science through the ages has endeavoured to address this goal. An important step in "attribution" is human individualisation which can be achieved by DNA profiling. Modern DNA profiling began through the discovery of tandemly located DNA repeats in the seal myoglobin gene, and has taken huge strides forward with the discovery of the polymerase chain reaction (PCR) process. The use of PCR reduced requirements on DNA template quality and quantity, and enabled multiplexing, which increased the power of discrimination. This paper highlights the current practices of forensic biology, and the possible harnessing of rapid developments in biomedical sciences to exploitations of legal and civil nature.

Forensic Biology in Crime Laboratories Today

Forensic biological examinations begin with the exhibit submitted by the investigation officer. A typical examination starts with the screening for biological fluids, e.g., blood [7,13], seminal fluid [5,12], and/or saliva [1,2], depending on the context and nature of the case. Screening is currently performed based on enzymatic tests. Confirmatory tests would have to be conducted next to ascertain the presence of the biological fluids. The next hurdle facing the forensic biologist would be the isolation of DNA from the selected stain. Laboratories currently have various options including organic solvent-based extraction, FTA cellulosic solid phase extraction, chaotropic agent-promoted silica adsorption of nucleic acids, anion exchange chromatography, alcohol precipitation, and size exclusion filtration. The purified DNA is quantified using real-time PCR technology [10,11]. This is currently the standard technology platform adopted by the forensic DNA community. The DNA is then amplified using standard multiplex short tandem repeat (STR) kits. STR loci can be located on the autosomes, and the X- and Y-chromosomes. The amplicons are then separated and detected in a capillary electrophoretic instrument [6]. To complicate matters, forensic stains can be limiting in quantity. In such instances, the forensic biologist has to thread a fine balance between biological fluid screening which could consume the evidence material and proceeding directly to DNA extraction. Such determinations would have to be made based on the professional experience of the forensic biologist.

The next major challenge confronting the forensic biologist is the interpretation of the DNA profile. Inherent in all PCR processes are the stochasticism that occur in low DNA template conditions. These would be manifested as heterozygote imbalance, inter-loci imbalance, partial profiles, and increased stuttering. The degree of the effect is inversely proportion to the number of template molecules [8,9]. Conversely, in the presence of excessive quantities of DNA, artifacts such as spectral bleedthroughs, incomplete adenylation, and inter-loci imbalances would occur. Instrument optics, temperature and voltage fluctuations would also contribute their share of difficulties. The forensic biologist would have to navigate through the sea of artifacts and identify the correct allele. He/she must also consider the issues of chimaerism, mutations in the primer binding site, and chromosomal alterations. Other questions that must be addressed would be the number of persons contributing to the observed DNA profile and their relative levels of contribution [3,4].

Subsequent to profile interpretation, different statistical approaches must be used in the reporting of the DNA profile and the attribution of the contributor(s), e.g., random match probabilities would be appropriate in cases of one-person DNA profiles. For DNA mixtures, likelihood ratios would offer greatest information value in two- to three-person mixtures while probability of exclusion calculations would be more suited in cases of complex multi-person mixtures. In the case of Y-chromosomal STR profiles, haplotype frequencies would have to be determined along with the confidence interval. The "matching" of the observed DNA profile for attribution is predicated on the availability of a reference sample obtained from the suspect. In the no-suspect cases, attribution would depend on successful searching of a DNA database. DNA database searching is, therefore, another important dimension in forensic biology, which will not be discussed in this paper.

The Way Forward

The DNA Profiling Laboratory at Health Sciences Authority, Singapore, has seen a 3.3-fold increase in sample volume over the past three years, resulting in a growing backlog and turnaround time. Justice delayed is justice denied. Embracing automation in DNA extraction, quantitation, and amplification set-up would reduce the DNA processing bottleneck. The laboratory is also embarking on a technological horizon scan and workflow process re-engineering to operationalise future technologies that can reduce, change, or even evoke paradigm shifts in forensic DNA analytical processes.

The laboratory is also concerned about the reduction in the power of discrimination with just the 15 commonly used STR loci when dealing with relatives or when degraded samples lead to incomplete DNA profiles. In this context, the laboratory is currently validating several additional STR markers with amplicons < 200 bp. These smaller-sized PCR amplicons would also facilitate the typing of highly degraded DNA. We are also evaluating new methodologies to maximise sample recovery and DNA extraction, for reliable PCR amplification.

The DNA profiling of non-human samples such as animals, plants, microbial and food can also be of significance in crime solving. Pet hairs, plant tissue and microbial strains may form part of the physical evidence associated with crime scenes, while food authentication by DNA profiling of 'expensive' foods like sharks fin and ginseng can prevent counterfeits. There is also growing interest in the use of transcriptomic and proteomic approaches for the determination of the tissue of origin of biological fluids. Moreover, transcriptomic approaches can help investigators estimate the age of biological stains, as well as the age of the contributor of biological fluids.

All in all, the future of forensic biology looks to be an exciting and dynamic one. Numerous opportunities exist for the forensic biologist, for the exploitation of rapid biological advances coupled with technological and engineering developments to strengthen and expand the field of forensic biology.

(continued on page 2)

Tracking Standards and Trends in Trace Evidence - A Review by the AFSN Trace Evidence Workgroup (AFSN TEWG)

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The trace evidence community has been thrown into a state of flux following the National Academy of Sciences (NAS) Report: "Strengthening Forensic Science in the United States – A Path Forward" which found forensic science to be in a state of disarray. This paper aims to track the standards and trends in trace evidence in the 3 major continents: United States, Europe and Australia/New Zealand, and recommend the way forward for the trace evidence working group in Asia.

The NAS report recommended the setting up of an independent federal entity, the National Institute of Forensic Sciences, to oversee mandatory accreditation and certification in the forensic science community. It calls for standardisation in terminology used for reporting and testimony, with appropriate qualifiers as well as basic research to study the accuracy, reliability and validity of trace evidence. Other recommendations include the establishment of standard protocols for forensic examination, methods and practices; routine quality assurance and quality control measures; and an enforceable national code of ethics.

A major bulk of the papers presented in the recent Trace Evidence Symposium, held in Florida, USA, 2-7 August 2009, focused on the responses by the different agencies to the NAS Report. In response to the NAS report, the American Society of Crime Laboratory Directors (ASCLD) also made the following recommendations [1, 2]:

a) Implement mandatory accreditation of lab and certification of individuals as one of the key deliverables with the support of federal legislation and existing funding streams.
b) Prioritise funding for research efforts to crime laboratories and educational institutions to create, publish and develop the proper validation documentation to restore the trust and confidence regarding forensic science testing within the criminal justice system.
c) Implement true blind proficiency testing utilising samples previously examined by a particular examiner (where feasible) to ensure the competency of examiner(s).

Following the NAS report, the trace evidence community worldwide faces a challenging future. Fundamental changes in the way things are performed are expected to impact the trace evidence community as the working groups in the different continents carry out their assigned tasks to get the forensic science house in order. Business as usual is not a viable option if trace evidence is to have a future in forensic science.

Accreditation, certification, research and development, and the strength of the conclusions are the hot topic of discussions in the various continents. Quality assurance remains one of the most important issues to ensure that the house is in order. Accreditation and certification of individuals are the key pillars of quality assurance. Collaborative exercises between organisations and proficiency testings, especially those using blind tests are much needed.

Standardisation appears to be the key word in the forensic community. The various networks: ASCLD, ENFSI and SMANZFL are all looking into ways to harmonise their SOPs and recommend a set of standards and best practices for their community. These documents are related to recovery, examinations, instrument SOPs, report writing etc.

In USA, the current drive is towards revising the way reports are structured and written and looking into better and more consistent terminologies for reporting conclusions. The European community believes that the CAI and the Bayesian approaches are the path forward. The approaches between the two continents may be different but the focus is similar: evaluation and interpretation of evidence and setting a more unified and common scale for conclusion levels. Databases which provide invaluable information on the evaluation and interpretation of evidence will be much needed for the different types of trace evidence in the near future.

Greater emphasis has also been placed towards continuing education for the forensic scientists and the lay person, so that potential jury understands the capabilities and limitations of forensic science. There is also a move towards combined research and development and collaboration across organisations and continents. The International Forensic Strategic Alliance (IFSA), a partnership between the different networks: ENFSI, AICEF, ASCLD and SMANZFL, was set up to create opportunities for strategic collaboration across the global forensic science community. AFSN's participation in IFSA during the 2nd AFSN meeting has set the stage for representatives of the AFSN TEWG to participate in the various workgroup meetings of the other networks. This will enable the AFSN TEWG to be more attuned to the standards and trends in the trace evidence field. Partnership will also foster long term collaboration and cooperation on strategic issues and create opportunities for information sharing with the other networks.

Moving forward, the trace evidence working group in Asia should continue to keep in step with the current trends of trace evidence in United States, Europe and Australia/New Zealand by:

a) Developing best practices for the collection, sampling and analysis of trace evidence
b) Establishing guidelines for report writing in specific trace evidence disciplines
c) Establishing education and training requirements of trace evidence examiners

The AFSN TEWG does not want to re-invent the wheel to accomplish the above tasks. It hopes to review and refer to the best practices published by the other networks and adopt them with modifications, if necessary in order to suit specific needs in the Asian community. Hence, it is vital for the AFSN TEWG to have more involvement with the other international/regional networks and create opportunities for strategic collaboration across the global trace evidence community.

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Internal Validation Studies of the AmpF*l*STR® Identifiler® Direct PCR Amplification Kit: Sensitivity Study and Analysis of PCR Inhibited DNA

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Abstract

Purification of DNA is an important process for the successful direct amplification of DNA from blood or buccal sources. However, the ability to type unpurified DNA samples becomes possible with the AmpF/STR® Identifiler® Direct PCR Amplification Kit which is designed to overcome the inhibitors present when processing unpurified samples. An internal validation study was carried out on 10 fresh bloodstains and 10 aged bloodstains (bloodstains kept at ambient temperatures for more than one year) as well as on two bloodstains which encountered inhibited amplification (incomplete DNA profiles) with the AmpF/STR® Identifiler® Amplification Kit. Our results demonstrate successful complete profile amplification of unpurified 1.2-mm bloodstain discs in both models of fresh and aged bloodstains. Full STR profiles were also observed in two samples which previously exhibited partial profiles when amplified with the AmpF/STR® Identifiler® amplification kit. All samples amplified with the AmpF/STR® Identifiler® Direct PCR Amplification Kit shown complete genotype concordance with the Identifiler® results. In addition our data demonstrate that the use of a reduced 20-µL reaction volume has not compromised the quality of the profiles obtained.

Introduction

Blood or buccal cells are the most common biological samples collected for genotyping. Crude samples such as blood contain heme compounds that can inhibit the activity of the DNA polymerase [1]. It is thus necessary to remove inhibitors and purify DNA before performing PCR amplification. Blood samples archived on FTA® paper require a purification step to remove heme substances as well as chemicals in the FTA® paper before PCR. This additional step can be time-consuming and runs the risk of cross-contamination.

The AmpF/STR® Identifiler® Direct PCR Amplification Kit is a short tandem repeat (STR) multiplex assay optimized to allow direct amplification of single source blood and buccal samples on FTA® paper without the need for sample purification [2]. The AmpF/STR® Identifiler® Direct Kit amplifies 15 autosomal STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA) and the sex-determining marker, Amelogenin, in a single PCR reaction. An internal validation study was carried out to test the performance of the AmpF/STR® Identifiler® Direct PCR Amplification Kit on 1.2-mm blood FTA® discs and to optimize the conditions for use with a reduced amplification volume.

Materials and Methods

Blood FTA® cards were prepared by spotting 100 µl of human whole blood onto each sampling area of FTA® Classic cards. The blood FTA® cards consisted of 10 fresh blood specimens and 10 FTA® cards which were kept at ambient temperatures for more than 12 months. In addition, two samples which had incomplete DNA amplification with the AmpF/STR® Identifiler® were included. The 1.2-mm FTA® discs were prepared using the Harris Manual Punch. The amplification using the AmpF/STR® Identifiler® Direct PCR Kit were performed in two different reaction volumes of 20 µl and 25 µl using the following PCR conditions: 95ºC for 11 min, followed by 25 cycles (or 26 cycles) of denaturing at 94ºC for 20 s, annealing at 59ºC for 2 min, and extension at 72ºC for 1 min. A final extension was performed at 60ºC for 25 min. Applied Biosystems 3130*xl* Genetic Analyzer and GeneMapper® ID-X software were used for data collection and data analysis respectively. The FTA® discs were also amplified with the AmpF/STR® Identifiler® Amplification Kit according to manufacturer's recommendations.

Results and Discussion

❄ Concordance

There was complete genotype concordance of the DNA profiles obtained from both the AmpF/STR® Identifiler® Direct and AmpF/STR® Identifiler® systems for all samples.

❄ Reaction Volume and PCR cycles

No locus or allelic dropout was observed using a reduced reaction volume of 20 µl. A 25-cycle amplification had successfully generated full STR profiles from all the samples. The increase in PCR cycles to 26 cycles indicated no significant differences in the average peak heights (see Table 1) for most of the samples. The samples with significant differences in peak heights could be due to possible variation in the DNA amounts from different sampling positions. A representative Identifiler® Direct kit STR profile using a 20 µl reaction volume and 25 PCR cycles is shown in Figure 1.

	FAM		VIC		NED		PET	
	25 cycles	26 cycles	25 cycles	26 cycles	25 cycles	26 cycles	25 cycles	26 cycles
1	1464	1398	1995	1936	2407	2305	2065	2075
2	1032	1228	1413	1477	1758	2190	1721	1929
3	2005	2681	2452	3123	*2953	*4675	3016	3757
4	1533	1204	1763	1090	2319	1775	2350	2065
5	*876	*1111	1244	1386	1391	1735	1508	1810
6	*1341	*766	*1950	*994	2135	1623	*2081	*1170
7	*764	*1410	*993	*1459	*1181	*2270	*1123	*2030
8	*2315	*699	*3723	*1009	*3901	*1351	*3502	*1015
9	2258	2658	3219	3751	3598	4889	3498	3981
10	*366	*2684	*479	*3145	*542	*4365	*525	*3393

Average peak heights with significant differences (student's t-test, α=0.05) indicated in asterisk

Table 1 : Intracolour average peak height (rfu) with varying PCR cycles.

❄ Stutter

The stutter product formation by the Identifiler® Direct kit for all samples were less than 10 percent (data not shown).

❄ Intracolour peak balance

Intracolour peak balance was calculated by dividing the lowest peak height value by the highest peak height value

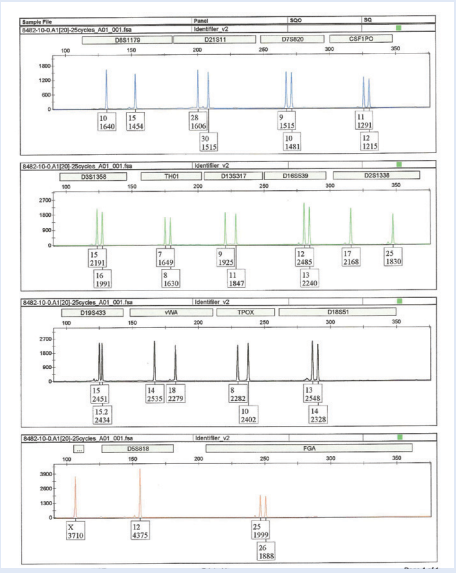

Figure 1: A representative electropherogram by the Identifiler® Direct assay of a 20 µl reaction volume and 25 PCR cycles.

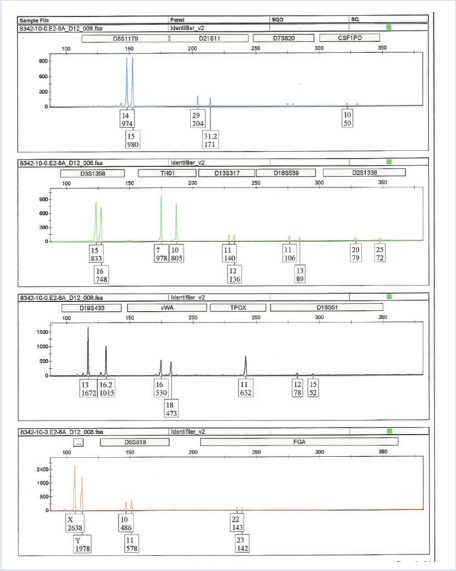

Figure 3a: Electropherogram of an inhibited amplification by Identifiler® assay.

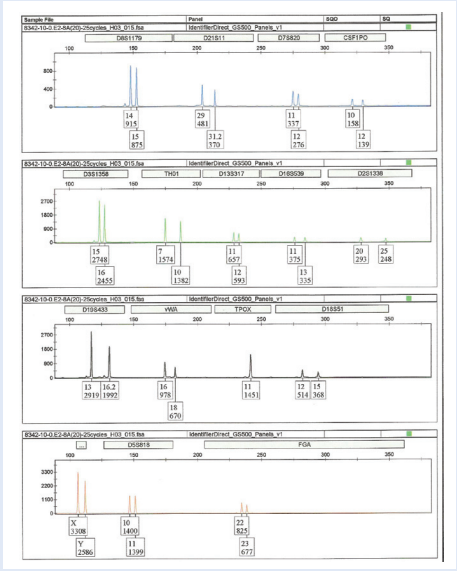

Figure 3b: Electropherogram of successful reamplification with Identifiler® Direct assay.

within a colour (homozygote peak heights are divided by two and heterozygote peak heights are averaged for each marker). Generally, the fresh bloodstains demonstrated a more balanced profile relative to the aged bloodstains. The STR profiles by the AmpF*l*STR® Identifiler® Direct PCR Kit were observed to exhibit good intracolour peak balance with peak balance exceeding 0.5 for all the bloodstains studied (see Figure 2).

Locus	Heterozygote Ratio Range (20 µl reaction volume, 25 PCR cycles)		Heterozygote Ratio Range (25 µl reaction volume, 25 PCR cycles)	
	Fresh Bloodstains Range [SD]	Aged Bloodstains Range [SD]	Fresh Bloodstains Range [SD]	Aged Bloodstains Range [SD]
D8S1179	0.886-0.979 [0.027]	0.778-0.990 [0.065]	0.845-0.996 [0.047]	0.742-0.939 [0.066]
D21S11	0.848-0.998 [0.045]	0.835-0.971 [0.043]	0.823-0.990 [0.044]	0.824-0.988 [0.050]
D7S820	0.901-0.983 [0.026]	0.807-0.991 [0.059]	0.881-0.998 [0.032]	0.848-0.987 [0.040]
CSF1PO	0.926-0.967 [0.014]	0.895-0.979 [0.032]	0.904-0.955 [0.021]	0.837-0.979 [0.057]
D3S1358	0.908-0.982 [0.020]	0.832-0.979 [0.052]	0.888-0.991 [0.033]	0.828-0.979 [0.061]
TH01	0.900-0.988 [0.026]	0.847-0.997 [0.044]	0.806-0.995 [0.063]	0.838-0.998 [0.044]
D13S317	0.911-0.970 [0.024]	0.873-0.983 [0.039]	0.811-0.995 [0.050]	0.844-0.976 [0.042]
D16S539	0.882-0.979 [0.035]	0.845-0.977 [0.039]	0.897-0.988 [0.035]	0.889-0.998 [0.037]
D2S1338	0.844-0.969 [0.037]	0.850-0.962 [0.032]	0.862-0.995 [0.040]	0.748-0.970 [0.071]
D19S433	0.888-0.993 [0.030]	0.703-0.980 [0.080]	0.858-0.991 [0.041]	0.852-0.993 [0.040]
vWA	0.899-0.955 [0.016]	0.703-0.988 [0.085]	0.907-0.981 [0.024]	0.774-0.944 [0.048]
TPOX	0.890-0.969 [0.027]	0.922-0.962 [0.014]	0.881-1.000 [0.042]	0.792-0.991 [0.068]
D18S51	0.881-1.000 [0.042]	0.663-0.963 [0.092]	0.880-0.977 [0.030]	0.882-0.986 [0.033]
D5S818	0.907-1.000 [0.029]	0.868-0.993 [0.033]	0.913-0.991 [0.026]	0.929-0.995 [0.020]
FGA	0.906-0.994 [0.028]	0.811-0.978 [0.053]	0.889-0.995 [0.030]	0.811-0.987 [0.055]

Table 2 : Heterozygote ratio range for Identifiler® Direct kit loci.

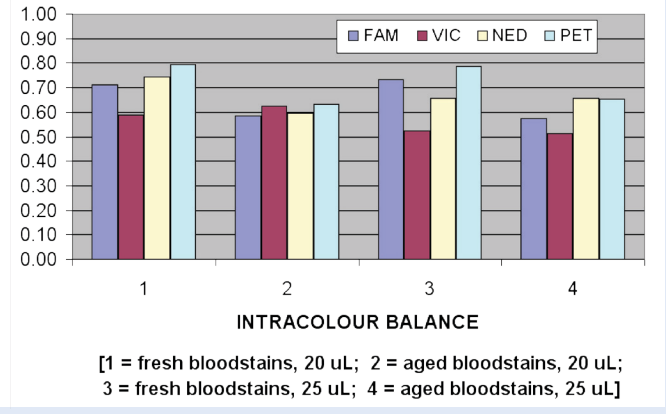

Figure 2: Intracolour balance for fresh and aged bloodstains using 20 uL and 25 uL reaction volumes.

❄ **Heterozygote ratio**

Peak height ratios were calculated by dividing the lower peak height by the higher peak height within a heterozygote at a locus. The heterozygote ratios for all the fresh bloodstains studied exceeded 0.8 and the aged bloodstains though exhibiting relatively lower heterozygote ratios generally, still demonstrated peak height ratios greater than 0.7. There is thus low stochastic variation in STR profiles amplified with the Identifiler® Direct PCR Kit. The heterozygote ratio range using 20 µl and 25 µl reaction volumes for fresh and aged bloodstains are shown in Table 2.

❄ **Inhibited amplification by Identifiler®**

Two casework samples with inhibited amplification by the Identifiler® PCR kit was reamplified successfully with the Identifiler® Direct PCR Kit as shown in Figures 3a and 3b respectively for one of the samples.

Conclusion

The results demonstrated ability of the Identifiler® Direct PCR Kit to directly amplify both fresh and aged bloodstains deposited on FTA® paper. The study demonstrated the use of a reduced 20 µl reaction volume and 25 PCR cycles had successfully generated full STR profiles of sufficiently high rfu with low stochastic variation. The ability of the Identifiler® Direct PCR Kit to overcome inhibition was only demonstrated in two samples and more samples are required to test the robustness of the Identifiler® Direct PCR Kit.

References

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Transparent Forensic Thought Processes and Crime Resolution: A Case Report of Evidence Collection from Dead Suspect's Belonging

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The goal of this short communication is to provide the thought processes in forensic science for evaluating evidences found at scenes and determining whether they are of value to investigations.

This short communication focuses on scene investigative and evidence collecting strategies for examining a case that occurred in Pattani Province, Southern Part of Thailand. A suspect was found dead from a motorcycle accident after an attempted murder on an innocent civilian.

The suspect died in a motorcycle accident while he and his pillion rider were trying to escape from the police officers, after he had shot an innocent civilian to death. His pillion rider ran away after the accident. A pistol was found on the suspect's left hip. DNA samples were collected from all the suspect's belongings with professionalism and care to preserve the integrity of the evidence. The results of the DNA analysis was surprising: a complete profile of an unidentified male who was not related to the dead suspect was recovered. A quick search on the National DNA Database matched the profile with the profile of a male who was suspected to be involved in the assassination of a military volunteer a few years ago.

Although the DNA analysis result failed to associate any individual closely related to the dead suspect to the crime, it has proven that the way to accomplish the crime resolution is to collect as much valuable evidence as possible from crime scenes and from suspects, whether they are alive or dead. The greater understanding of the transparency of the forensic thought process, the more successful one will be in resolving a crime.

The procedure below outlines the step by step forensic thought processes which should be performed by investigators at a crime scene:

1. **Information finding:** The first step in processing a crime scene is to interview the initial responding officer(s) to obtain as much information as possible to ascertain the theory of the case. This information may not be unequivocally useful but it will give us a basis to start the thinking process.

2. **Evidence consideration:** The investigator(s) in charge shall determine the value of the evidence collected. The most important consideration is the purpose of collection. The amount of items collected does not determine the success rate of resolving a crime. Finding the most crucial piece of evidence can provide strong evidence that collaborates or refutes the suspect's statements.

3. **Link up information**: Physical evidences are used to support the Criminal Justice System as the silent witnesses against suspects. Some suspects may be involved in more than one incident. The physical evidence found at the scene may have been used in past crime scenes. To increase the value of the evidence, the investigator(s) in charge of a case should input the data and information to the criminal justice data network to link, interface and exchange information with other agencies.

AFSN Members

1. Department of Scientific Services, Brunei Darussalam
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3. Eijkman Institute for Molecular Biology, Indonesia
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15. Faculty of Medicine, Chiang Mai University, Thailand
16. Central Institute of Forensic Science, Thailand
17. Ramathibodi Hospital, Thailand
18. Vietnam Forensic Science Institute, Vietnam

(as of June 2010)

Upcoming Events

Dates	Events
25 – 27 May 2011	AFSN 3rd Annual Meeting & Symposium, Seoul, Korea
08 – 11 Aug 2011	Trace Evidence Symposium 2011, Kansas City, USA
20 – 25 Aug 2011	The American Society of Questioned Document Examiners (ASQDE), Philadelphia, USA
12 – 17 Sep 2011	International Association of Forensic Science (IAFS) Triennial Meeting, Madeira, Portugal
25 – 30 Sep 2011	Joint Meeting of The Society of Forensic Toxicologists (SOFT) & The International Association of Forensic Toxicologists (TIAFT), San Francisco, USA
20 – 25 Feb 2012	American Association of Forensic Science (AAFS) Annual Scientific Meeting, Atlanta, USA