ForensicAsia

THE ASIAN FORENSIC SCIENCES NETWORK NEWSLETTER | ISSUE 7 | 2016

AFSN President's Address

Dear colleagues,

I would like to express my sincere gratitude for the support you have extended over the last year. The 7th AFSN held in Kuala Lumpur could not have been a success without your active participation and contribution.

As today's society is becoming more advanced and complex, crime is becoming more sophisticated and occurring at a larger scale. Criminal activities now are not limited to within a country's borders anymore, but are conducted on a global scale. It goes without saying that these days, the power of forensics is playing a key role in solving crimes and the importance of international cooperation and information exchange is very high. Moreover, cases where prompt correspondence on a global level are required, have to be dealt with based on mutual understanding in a unified manner and according to a clear standard operation protocol. Cooperation across borders is becoming more crucial than ever before.

As an AFSN member and one of the leading forensic scientists in Korea, I have pledged myself to uphold all of AFSN's values and standards and actively participate in AFSN's events to continually strive to enhance forensic science.

I hope the new year is as filled with progress and accomplishments as the old and this new year further strengthens the bonds of collaboration and cooperation that we have with one another.

I am looking forward to seeing you in Thailand.

Dr. Joong-Seok SEO
Director General of National Forensic Service, Korea

First of all, I am honoured to be elected as the 4th President of such a prestigious network as the AFSN. I wholeheartedly thank you all for having the confidence in me and for your strong support. Furthermore, I would like to pay tribute to my predecessors, the previous AFSN presidents and all members of the AFSN who have worked tirelessly for the establishment of AFSN and the organisation of outstanding activities and events over the past 7 years.

I would like to follow up by congratulating Dato' Dr. Yew Chong Hooi, the chairman of the AFSN 7th Annual General Meeting & Symposium organising committee, Dr. Kamarruddin Asri, Director of Forensic Division Department of Chemistry, Malaysia, and his team on successfully organising the symposium on 16th – 18th November 2015 in Kuala Lumpur, Malaysia. There were 317 participants from 25 member institutes from 12 countries. During the symposium, 59 oral presentations and 50 poster presentations from 6 workgroups/committees and several topics by distinguished speakers from around the world were presented.

An election of new board members was carried out during the Annual General Meeting. I would like to congratulate Dr. Angeline Yap Tiong Whei from the Health Sciences Authority, Singapore, who was elected Vice President. Additionally, I would like to welcome Dr. Joongseok Seo, National Forensic Services, Korea, who was the previous AFSN President, as well as the newly elected board members, Dr. Kamarruddin Asri, the Department of Chemistry, Malaysia, Mr. Liu Shuo, Institute of Forensic Science, People's Republic of China, and Ms. Zunaidah Othman, Department of Scientific Services, Brunei Darussalam. Pol.Lt.Col. Ampika Leelapojanaporn, Central Institute of Forensic Science, Thailand, will be responsible for the AFSN Secretariat.

The current board members will continue to work together and play an active role to achieve the network objectives, together with the strong support, commitment and cooperation for collaborations amongst the Asian as well as global forensic science community.

Pol. Lt. Col. Suphoj Nakngoenthong
AFSN President

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Editor's Address

Dear members and friends of AFSN,

2015 has been an exciting year, marked by the success of our first collaborative DNA testing exercise for AFSN member institutes. What better way to end the year than the 7th AFSN Annual Meeting and Symposium which was hosted by Kuala Lumpur in November 2015.

We were greatly encouraged by the response we have received for the first collaborative DNA testing exercise for AFSN member institutes and would like to take this opportunity to thank the brave souls that 'guinea-pigged' this first exercise. This exercise has provided valuable insights into the DNA extraction process by comparing the efficiencies of the different methods adopted amongst participating laboratories A second collaborative DNA testing exercise on mixture interpretation has been initiated and we look forward to more laboratories participating. We hope these collaborative exercises will enhance the quality of forensic DNA services provided, and strengthen cooperation and collaboration amongst member institutes. Perhaps it may even stimulate collaborative exercises within the other workgroups.

This current issue of the AFSN newsletter features a compilation of 16 articles, including an interesting article on the analysis of blood stains on clothing. In the laboratory, we look at the clothing in a 2-dimensional static manner on the bench top. In reality, the blood stains are deposited onto 3-dimensional humans in a dynamic manner. We also have a contribution from INTERPOL regarding the training of officers from Malaysian Maritime Enforcement Agency and the Singapore Police Coast Guard to provide them with the necessary skills to face the challenges of processing evidence at a maritime crime scene.

Lastly, I would like to extend my appreciation to members of the editorial committee for their support and assistance in reviewing the articles that we have received. We hope that you will enjoy reading the interesting articles in this issue and we look forward to receiving your submissions for our next issue. We welcome your feedback and comments at hsa_asg@hsa.gov.sg.

Assoc Prof Christopher Syn
Editor

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.

Testing Exercise for AFSN

The DNA Profiling Laboratory at Health Sciences Authority, Singapore will be organising the third AFSN Inter-Laboratory Collaborative DNA Exercise for the member institutes of the AFSN. The objectives in conducting the exercise are:

- to collect and exchange information among the member institutes,
- to provide insight to the competence within the region, and
- to provide assistance and enhance cooperation among member institutes to meet quality standards.

The third exercise is expected to commence in June 2016. Laboratories that would like to participate may contact the organiser at **tan_wei_jie@hsa.gov.sg**. Only one representative from each member institute is required to register. Please do not hesitate to contact the organiser if you have any queries.

7th Asian Forensic Sciences Network: Annual Meeting & Symposium

KUALA LUMPUR – 7th Annual Meeting and Symposium of Asian Forensic Sciences Network (AFSN) was held at Pullman Hotel Kuala Lumpur from 16th till 18th November 2015 with the theme of "Necessity vs Luxury; Where do we draw the line". The objectives of the event were to gather forensic scientists, academicians and researchers especially from the Asian region to communicate and share knowledge, as well as to learn about state-of-the-art forensic analytical methods. The meeting also served as a good platform for young forensic scientists to gain knowledge and exposure at the international level.

The annual meeting and symposium was officiated by Dato' Dr Zulkifli Mohamed Hashim, representative from the Ministry of Science, Technology and Innovation (MOSTI), Malaysia. This event gathered around 300 participants from 17 countries. The plenary speakers were Tan Sri Dr Muhammad Shafee Abdullah, Mr. Tim Schellberg and Mr. Scott R. Oulton who shared their invaluable knowledge and vast experience. There were also six parallel Workgroups sessions for DNA, Illicit Drugs, Trace Evidence, Toxicology, Crime Scene Investigation and Quality Assurance and Standards Committee.

The key points that emerged from the meeting were: to invite more institutes to join and actively participate in AFSN activities, encourage more research in forensic analyses methods, and explore the creation of a new Workgroup for Digital Forensics and Document Examination. The AFSN website will also be updated at least twice a year with the latest information.

This three-day symposium comprised of a pre-symposium workshop, 59 oral presentations, 50 poster presentations and 15 booths. Although the preparation for the event was made at short notice, the response from speakers and audience was encouraging. The unwavering support of the main organising committee; the Forensic Science Society Malaysia (FSSM) in collaboration with Department of Chemistry Malaysia (KIMIA Malaysia), Royal Malaysian Police, MOSTI and local universities USM, UTM and UKM, is also deeply appreciated. This event was also made possible with the support and assistance given by the various Workgroup Chairs and Vice Chairs from various countries, and by the presence of the many participants.

Cannabis Workshop 2015, Health Sciences Authority, Singapore

Ms Merula Mangudi, Ms Wendy Lim Jong Lee
Health Sciences Authority, Singapore

In collaboration with AFSN IDWG, a Cannabis workshop was held from 10th to 13th March 2015 at the Health Sciences Authority (HSA), Singapore. The aim of this workshop was to build up the competency and knowledge of forensic scientists in performing chemical and botanical identification of cannabis. In addition to the thirty seven participants from HSA, five participants from AFSN member institutes (Department of Scientific Services, Brunei Darussalam; Department of Chemistry, Malaysia; Bureau of Drug and Narcotic, Department of Medical Sciences, Thailand) also joined the workshop.

The 4-day workshop began with a day of training on chemical analysis conducted by Mr Chan Kee Bian, Consultant Scientist, HSA, Singapore. This was followed by three days of training on botanical identification of cannabis conducted by Ms Sue Fiddian, Manager, Botany Branch, Victoria Police Forensic Services Department, Australia.

The chemical analysis workshop consisted of a lecture on the chemistry of cannabinoids, abuse of cannabis and the legislation of cannabis in Singapore. This was followed by hands-on practical sessions on the various analytical techniques. These included identification techniques such as Duquenois-Levine colour test, Thin-Layer Chromatography and Gas Chromatography/Mass Spectrometry, as well as a quantitative technique using Gas Chromatography.

The botanical identification workshop had various lectures covering the basic knowledge of botanical concepts, botany of cannabis, microscopic identification of cannabis and differentiation of cannabis from its relatives and cannabis look-alikes. Information on cultivation of cannabis, occupational health and safety issues associated with examination, handling and storage of cannabis and other plant-based hallucinogens were also shared. There were also practical sessions where participants conducted microscopic examination of cannabis and non-cannabis vegetable matters. It was indeed a great learning experience for the participants to examine the microscopic features of cannabis and to compare these features with non-cannabis plant material under the guidance of Ms Sue Fiddian.

This workshop provided a rare opportunity for AFSN IDWG members to share, learn and discuss the analytical techniques used for the identification of cannabis. This workshop would not have been successful without our dedicated trainers Mr Chan Kee Bian and Ms Sue Fiddian, and support from HSA and AFSN IDWG member institutes.

The 27th ISFG World Congress in Seoul, 2017

Dr Lee Seung Hwan, Forensic Research Institute,
NDFC of Supreme Prosecutors' Office, Korea

Dear AFSN colleagues,
We are pleased to inform you that the 27th International ISFG Congress 2017 will be held in Seoul, Korea, organised by the Supreme Prosecutors' Office. This article aims to raise members' awareness of the ISFG congress; and to seek your interest and support for this event.

What is ISFG?

Founded in 1968, the International Society for Forensic Genetics (ISFG) is an international association dedicated to the research of forensic genetic technologies. The society now comprises of more than 1,100 members from over 60 countries. The ISFG is the leading society in forensic genetics, which provides scientific recommendations on new technologies. In addition, the ISFG was also pivotal in the launch of a highly regarded journal, FSI Genetics. FSI Genetics attained an impact factor of 4.604 in 2014, the highest value known to be obtained by a forensic journal in this field.

The International congress of ISFG

ISFG congresses are held every two years at various host cities around the world. Since the first congress in Germany in 1969, the congress has been ongoing, with the recent 26th congress held in Krakow, Poland in September 2015. The congress usually attracts more than 500 international forensic geneticists, who present their research findings as well as engage in discussions on novel ideas in the field of forensic genetics.

So far, the 26 congresses have been hosted by cities in 15 different countries (22 times in 12 European countries, twice in USA, once in Argentina and once in Australia). It is disheartening that the Congress has yet to be held in a city in Asia, as this would definitely help in fostering working relationships between forensic laboratories in Asia and other continents.

The 2017 ISFG Congress - a milestone in 'Bridging East & West'

The host city of ISFG 2017 was decided at the 2013 congress held in Melbourne, Australia. According to ISFG rules, the host country is selected four years in advance by the General Assembly of ISFG.

We presented a passionate proposal suggesting 'Bridging East & West' as our vision, emphasising the need for enhancing cooperation and building networks between Asia and other continents. With a supporting testimonial from the then AFSN President Lim Kong Boon, we successfully won the bid against Montreal, Canada to host the 27th ISFG Congress in our country.

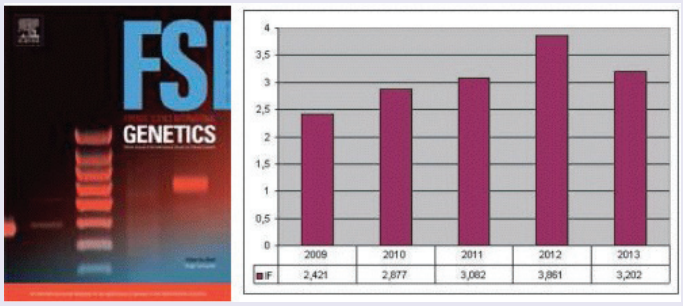
We strongly believe that this global event will be a milestone in which forensic genetics in Asia can benefit from because this field needs diverse collaborative research among nations with diverse ethnic origins.

Planning and preparing for the 27th ISFG Congress

As the main organiser, the Supreme Prosecutors' Office will do its best to make the 2017 ISFG congress an unforgettable experience from both a scientific and cultural point of view as it is the very first time that an ISFG Congress will be held in an Asian country.

We attended the 2015 ISFG congress to promote the next meeting by having a series of activities. Now, we are gearing up the preparations by forming an organising committee that is made up of other relevant national agencies and academia.

We would like to invite all AFSN members to the 2017 congress. Your keen interest and participation is crucial towards the success of the event. We will keep AFSN colleagues updated with the latest information.



The impact factor of FSI genetics from the year 2009 to 2013


Korean speaking workgroup & the ISFG board (ISFG, Vienna, 2011)

International Symposium on Forensic DNA in Law (Supreme Prosecutors' Office, Seoul, 2012)

Proposal for ISFG 2017 at Melbourne (ISFG, Australia, 2013)

Asian Forensic Sciences Network First Inter-Laboratory DNA Collaborative Exercise: Efficiency of DNA Extraction

Mr Tan Wei Jie, Ms Lim Xin Li, Mr Goh Sze Kae
Health Sciences Authority, Singapore

Introduction

Isolation of DNA from evidence is the first step of forensic DNA analysis. The efficiency of the extraction method is crucial as recovery of good yields of high molecular weight DNA would determine the quality and, consequently, evidential value of the DNA profile. In recent years, the forensic community has been switching their primary extraction protocols from laborious conventional methods, e.g. phenol-chloroform extraction, to more efficient spin column-based or paramagnetic particle-based commercial kits. An increasing number of automated platforms for DNA extraction also allow the laboratories to shorten the turn-around time of cases and reduce backlogs. With numerous extraction kits and platforms available in the market, laboratories often opt for the systems that best suit their process and their choices vary. As a result of the different methods employed by the laboratories, variations in the efficiency of the extraction processes are observed.

The purpose of the first Asian Forensic Sciences Network Inter-Laboratory Collaborative Exercise, organised by the DNA Profiling Laboratory (DNAPL), Health Sciences Authority, Singapore, was to determine and compare the efficiencies of the DNA extraction methods used by the participating laboratories.

A total of 10 laboratories of the AFSN member institutes participated in the exercise. They are (in alphabetical order of name):

- Central Institute of Forensic Science, Thailand
- Centre of Medicine and Health Services, Indonesia
- Department of Chemistry, Malaysia
- Eijkman Institute for Molecular Biology, Indonesia
- Faculty of Medicine, Mahidol University, Thailand
- Forensic Laboratory Centre of Indonesia National Police, Indonesia
- Institute of Forensic Medicine, Police General Hospital, Royal Thai Police, Thailand
- National Institute of Forensic Science, Mongolia
- Natural Sciences Research Institute, University of the Philippines, Philippines
- Supreme Prosecutor's Office, South Korea

Study Design

Equal volumes of diluted blood from female person X were stained onto two cotton swabs marked A1 and A2, i.e. A1 and A2 are replicate samples. Equal volumes of diluted blood from male person Y were stained onto two cotton swabs marked A3 and A4, i.e. A3 and A4 are replicate samples. Samples A1 to A4 were sent to the laboratories and participants were instructed to perform DNA extraction on the entire swab head as per their laboratory protocol for blood swabs and collect the extracted DNA in 50 µL of Low TE buffer (10 mM Tris, 0.1 mM EDTA). The laboratories were not informed that A1 and A2 or A3 and A4 were replicate pairs. The liquid DNA samples returned to the DNAPL were weighed to determine the volume. This was done to account for possible sample loss due to evaporation when the final volume received was less than 50 µL. The samples were quantitated in duplicates with Applied Biosystems Quantifiler Duo kit to obtain the concentration. The DNA yield for each sample was determined by multiplying the volume and concentration.

For background DNA quantitation, DNAPL performed DNA extraction with 5 replicates of blood swabs from person X and 5 replicates of blood swabs from person Y. Three different methods of DNA extraction were used:

1. Promega Casework Extraction kit and Casework Pro kit with Maxwell 16 instrument
2. Organic Extraction (Proteinase K-SDS, phenol-chloroform, ethanol precipitation, further clean up with illustra MicroSpin G-50 gel-filtration columns)
3. Qiagen QIAamp DNA Investigator kit with QIAcube instrument

The liquid DNA was eluted in a final volume of 50 µL of Low TE buffer and quantitated twice with Applied Biosystems Quantifiler Duo kit. After quantitation, the liquid DNA samples were amplified with Applied Biosystems Identifiler Plus STR-PCR amplification kit with an input DNA of 1 ng. Separation and detection were performed on the Applied Biosystems Genetic Analyser 3500*xL* with an injection parameter of 1.2 kV 24s and an injection volume of 3 µL.

Results and Discussion

The details on DNA yields were listed in the summary report that was made available to the participating laboratories. Each laboratory was assigned a unique code (LAB01 to LAB10) and the results were reported based on that code for the purpose of anonymity.

In the comparison of DNA yields amongst the participating laboratories (Figure 1), it should be noted that degradation of blood sample and/or DNA due to non-ideal environmental conditions could have occurred to varying extents during the shipment between the DNAPL and the participating laboratories. Sample loss was also observed across all laboratories, possibly due to evaporation.

As A1 and A2 were replicates and A3 and A4 were replicates, similar DNA recoveries would have been expected for the sample pairs. That was, however, not observed. Most laboratories appeared to have DNA yields within a two-fold range and the background quantitation data suggested that this level of variation could be expected. DNA recoveries from laboratories using paramagnetic particles and spin columns were compared, and no significant difference was observed. Similar results were also obtained in the DNAPL.

The liquid DNA samples from the 2 participating laboratories with the lowest yields (LAB03 and LAB07) produced partial or no profiles (Figures 2 and 3). DNA mixtures were obtained from all samples from LAB09 (Figures 4 and 5). The alleles from the major contributor corresponded with the expected profile but the minor alleles could not be accounted for. Full concordant profiles were obtained from the other laboratories.

The participating laboratories provided information on the other extraction kits and platforms that are available in their laboratories and their responses were consolidated in the summary report (Tables 1 and 2).

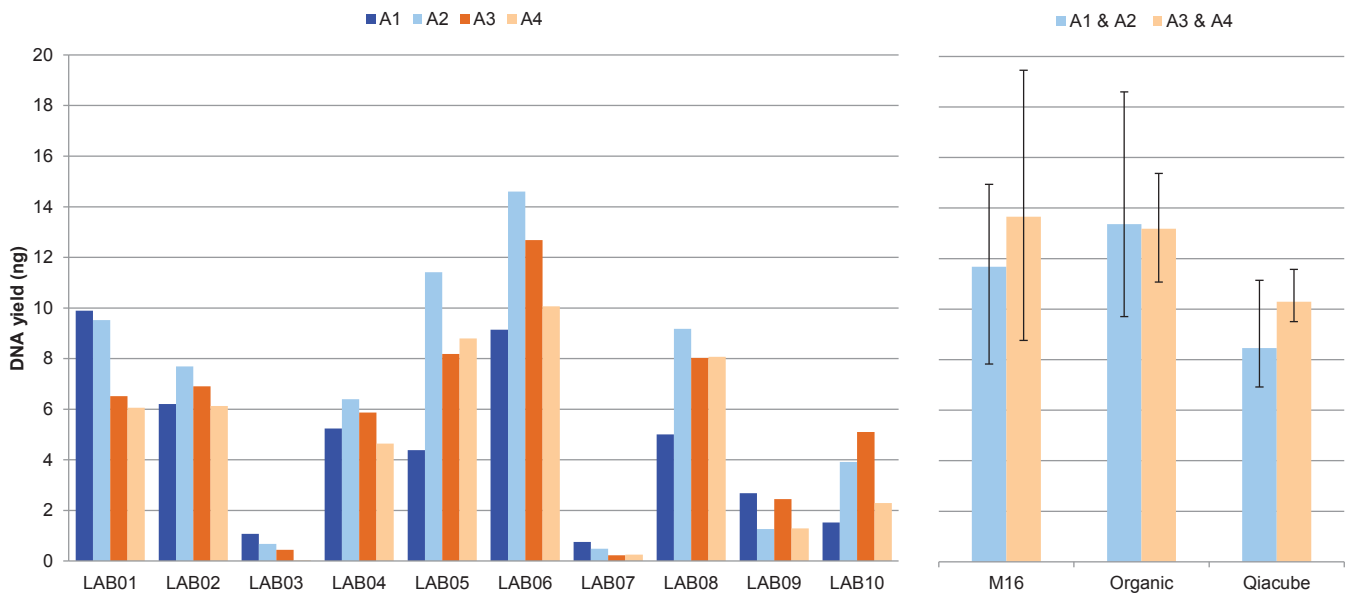
The exercise provided valuable insights into several aspects of the DNA extraction process amongst the participating laboratories. The exercise achieved its benchmarking purpose by comparing the efficiencies of the extraction systems used. Although it was explained that the differences in DNA yields were not entirely attributed to the extraction methods, the results of this exercise could provide an indication of the performance of the methods used.

Variations between reactions can always be expected from any kind of DNA extraction methods and platforms. There was, however, little information about the typical range of the variation. The quantitation results of this exercise suggested that a two-fold difference in DNA yields could be expected if extractions were to be performed on samples that contained the same amount of DNA with the above-mentioned kits and platforms.

Furthermore, the profiles obtained from the DNA extract can highlight areas for improvement. For example, the observation of DNA mixtures from single source blood samples could suggest possible contamination which would warrant further investigation.

All in all, the first inter-laboratory collaborative exercise was a good learning experience for both the participating and organising laboratories. DNAPL would like to thank the participating laboratories for their time and effort in making the exercise a success. Going forward, DNAPL seeks the support of more DNA laboratories in the AFSN family to participate in future exercises. We believe that these exercises help the network achieve its objective to enhance the quality of forensic services by allowing laboratories to benchmark their processes, develop protocols, and to strengthen cooperation in the region.

Laboratories that would like to take part in future exercises may contact the DNAPL at Tan_Wei_Jie@hsa.gov.sg.



Method	Manual	Automated	Manual	Manual	Manual	Automated	Automated	Automated	Manual	Manual
Platform	-	Automate Express	-	-	-	Maxwell 16	Tecan 150 Evo Freedom	EZ1 Advanced XL	-	-
Technology	Spin Columns	Para magnetic Particles	Para magnetic Particles	Spin Columns	Spin Columns	Para magnetic Particles	Para magnetic Particles	Para magnetic Particles	Spin Columns	-
Kit	QIAamp DNA Micro	Prepfiler Express Forensic DNA Extraction	DNA IQ System	QIAamp DNA Micro	QIAamp DNA Investigator	Casework Pro	DNA IQ System	EZ1 DNA Investigator	QIAamp DNA Micro	Chelex

Automated	Manual	Automated
Maxwell 16	-	QIAcube
Paramagnetic Particles	-	Spin Columns
Casework Pro	Organic	QIAamp DNA Investigator

Figure 1: DNA yields obtained from participating Laboratories and DNAPL (with vertical bars showing the minimum and maximum yields).

Figure 2: Partial profile from LAB03 (Sample A3).

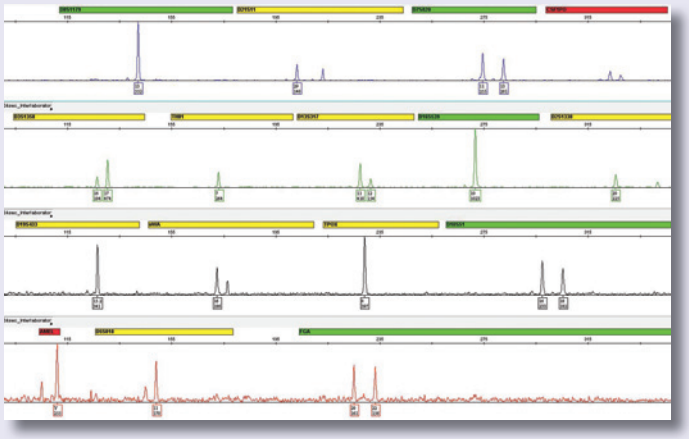


Figure 3: Partial profile from LAB07 (Sample A1).

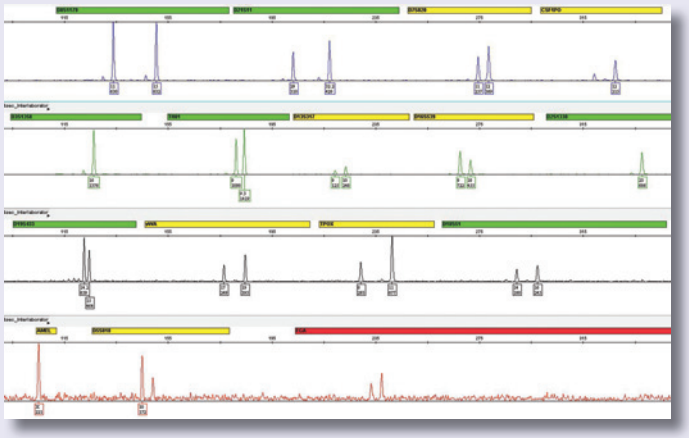


Figure 4: Mixture from LAB09 (Sample A1).

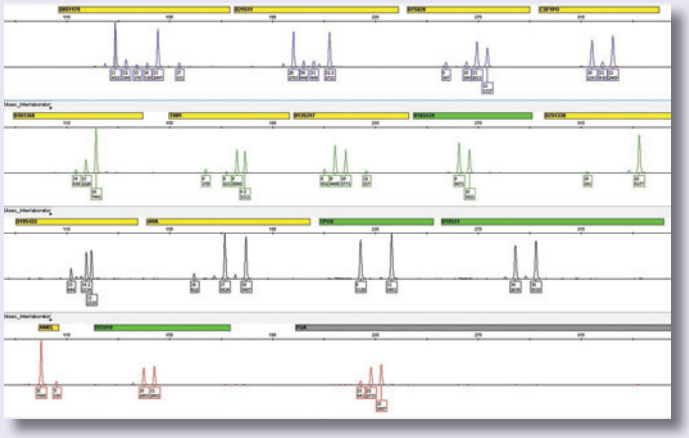


Table 1: Extraction kits available in participating Laboratories and DNAPL.

Kit	No of Lab	Kit	No of Lab
Chelex	5	ABI Prepfiler Express Forensic DNA Extraction	2
Organic	5	ABI Prepfiler Forensic DNA Extraction	2
Qiagen QIAamp DNA Micro	5	Qiagen QIAamp DNA Blood Mini	2
Promega DNA IQ System	4	Promega Differex System	1
Qiagen QIAamp DNA Investigator	4	E-Prep	1
Qiagen QIAamp DNA Mini	3	GeneAll DNA	1
Promega Casework Pro	2	Qiagen QIAamp DNA FFPE Tissue	1
Qiagen EZ1 DNA Investigator	2	Salting Out	1
ABI Prepfiler BTA Forensic DNA Extraction	2		

Table 2: Extraction platforms available in participating Laboratories and DNAPL.

Platform	No of Lab
Promega Maxwell 16	3
ABI Automate Express	2
Qiagen EZ1 Advanced XL	2
Qiagen QIAcube	2
Tecan 150 Evo Freedom	1
Tecan HID EVOlution	1

A Bloody Good Time… Clothing Bloodstain Pattern Analysis

Mr Lionel Lawrence Nonis, Mr Heng Baoqiang, Mr Holden Lim, Ms Nicole Tang
Biology Division, Health Sciences Authority, Singapore

A bloodied body was found in the bedroom of an apartment. The deceased was observed to have multiple incision wounds and several lacerations. Bloodstains were observed on the walls and on the floor. Such is a common scene presented on American crime thrillers. Based on the blood spatter patterns, is it possible to infer what happened? Did the slashing happen before or after the stabbing?

Over a period of two weeks (26-30 January 2015 and 22-26 June 2015), two Fabric Blood Spatter Pattern workshops were conducted for 48 staff of the DNA Profiling Laboratory of the Health Sciences Authority, Singapore by Dr. Mark Reynolds and Mr. Ted Silenieks. Dr. Reynolds is a Forensic Science Consultant with the Western Australia Police and Subcommittee Chair of SWGSTAIN. Mr. Silenieks is the Coordinator of Evidence Recovery from the Forensic Science South Australia and Vice-President of the International Association of Bloodstain Pattern Analysts.

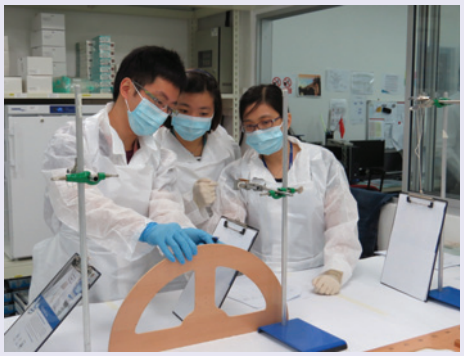
There was much excitement and buzz in the air as preparations were coordinated and essential items obtained. Each workshop incorporated a series of lectures covering topics on the formation of stains, fabric influence, blood dynamics, region of origin determination, and types of injury.

The practical component saw the laboratory being transformed into a gruesome murder scene. Staff were able to experience first-hand, how various spatter stains were formed with the use of different implements such as a golf club, metal pipe and hammer.

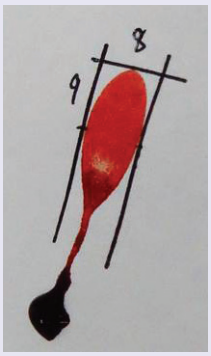
Physical activities to generate various blood spatter patterns.

The practical was not all gore and glory. There were also ‘cleaner’ activities designed to facilitate the understanding of the physics of blood and fluid dynamics that affect the resultant stain shape. Generally, by measuring the width and length of the stain, the angle of impact can be determined. Smaller angles of impact usually result in more elliptical stains while greater angles give a rounder shape. The region of origin can then be established with the aid of lasers, strings or computer software. A microscopy exercise further enhanced the understanding of the influence different textiles and fabrics have on bloodstain formation and eventual appearance. These sessions enabled the better appreciation of how each stain was formed with the many intricate forces such as air resistance and gravity at work.

The workshop also emphasised the need to visualise stains from a 3D perspective – what is seemingly illogical when observed in 2D, i.e. flat on a bench top, can make perfect sense when considered in 3D. To illustrate the value of the 3D perspective, participants examined pre-stained clothing with passive drips, cast-offs, transfers, impact spatters and saturation stains, and attempted to infer the activities/events that led to the formation of those stains. This is of particular importance as it is impractical for all stains to be sampled for DNA profiling; hence, the need to select the appropriate stains that would be of evidential value.

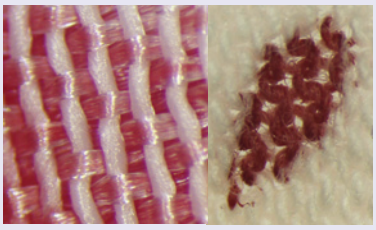


Understanding the physics and fluid dynamics.



The elliptical shape of a blood stain and determining the angle of impact.

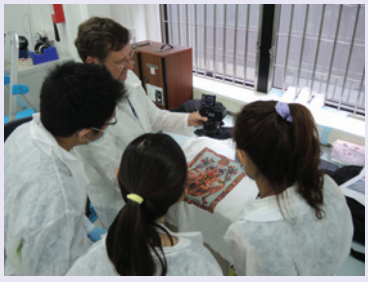
Microscopy visualisation of bloodstains on different fabrics and textiles.



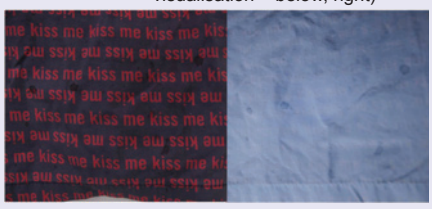
Observing the difference in the spreading of a bloodstain due to a fabric weave (above, left) versus a knit.

The trainers also shared valuable and timely advice on the use of infrared (IR) photography. A camera, with its IR blocker removed, is able to facilitate the visualisation of bloodstains on dark fabrics. Bloodstains absorb IR and would appear as dark patches, while the fabrics reflect IR and appear lighter. This could reduce the time needed to examine dark clothing for the presence of blood. There are, however, some fabric dyes that also absorb IR and would render this approach ineffectual. Additionally, this technique is less sensitive when compared to the classical blood detection method of using peroxidase activity.

This workshop was a thoroughly enjoyable and enriching one, enabling the staff of the DNA Profiling Laboratory to be more knowledgeable and better equipped to identify and select the stains of maximal evidential value.



Subjecting a blood stained clothing to IR inspection. (above)



IR inspection of a blood-stained clothing.
(Original clothing – below, left; IR visualisation – below, right)

On the Standardisation of Criminal Science and Technology in China

Dr Jiao He-juan
Institute of Forensic Science, Ministry of Public Security, China

With widespread economic globalisation, international competition is more intense, standardisation has become one of the basic elements of the national core competitiveness, and it is the technical regulations that economic activities and social life follow. With technological advancements, standardisation is the technical support of national economy and for social development. As an important component of the socialist modernisation, it plays a more and more important role in the process.

The standardisation law of PRC was issued and implemented, making standardisation part of the legal system. In China's accession to the WTO in 2001, the government conducted institutional reform and set up Standardisation Administration of China (SAC), strengthening standardisation with organisation guarantee. As a result, standardisation and normalisation of criminal science technology also had an unprecedented development, and it was a golden opportunity. The establishment of SAC/TC179 in 1992 then filled the gap between criminal science and technology standardisation in China.

Standardisation Technical Committee is a technological organisation, engaged in drafting and reviewing technical standards in professional and technical areas. Entrusted to the State Bureau of Technical Supervision (CSBTS) and SAC, led and managed by the Ministry of Public Security PRC, SAC/TC179 is the one and only standardisation organisation of criminal science and technology in China. The Secretariat of SAC/TC 179 is located in the Institute of Forensic Science, Ministry of Public Security (IFSC). With its development, SAC/TC179 had established a total of 10 sub-Technical Committees, namely Poison Analysis (drug test), Criminal Information, Physical And Chemical (trace evidence) Examination, Evidence Photography and Video Investigation, Forensic Medicine Examination, Fingerprinting, Marks, Document Examination, Electronic Evidence, Criminal Technology Products, and 3 Working Groups, namely DNA, Crime Scene Investigation and Intelligent Speech Technology. Currently, in the 10 sub-Technical Committees and 3 Working Groups, SAC has a total of 473 members and experts, distributed in all fields of forensic science, public security, procuratorial, judiciary, legal, medical and health fields, etc., who are responsible for the analysis of standardised requirements, studies and proposals, standards system, technical standard projects, and offering suggestions of technical committees for its running, management and reformation.

Members and experts of SAC/TC179

With the development of the SAC/TC179, a total of 743 criminal technical standards have been approved by Standardisation Administration of PRC (国家标准委) and Ministry of Public Security PRC (公安部), including 59 national standards and 684 industry standards. In addition, a total of 295 projects of criminal technical standards were released and published, including 26 national standards and 269 industry standards.

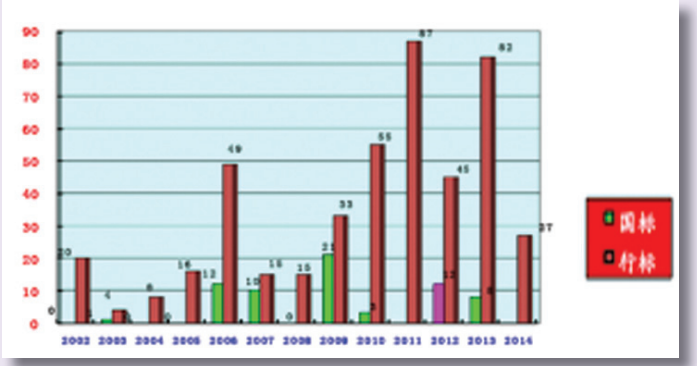


Figure 1: Approved projects annual statistics of criminal technical standards

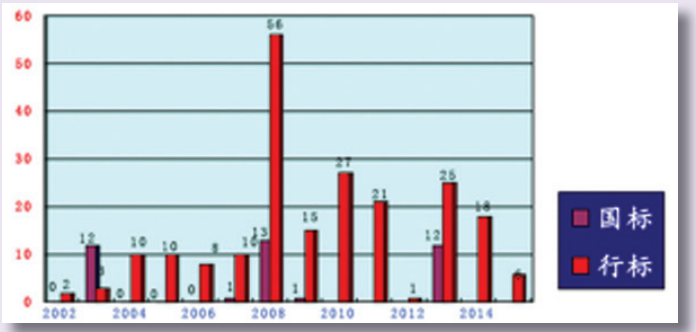


Figure 2: Released and published projects of criminal technical standards

In October 2006, management by SAC/TC179, series standards Specifications for examination of forensic DNA laboratory (GA/T383-2002), Specifications for construction of forensic DNA laboratory (GA/T382-2002), Criterion for forensic DNA database (GA/T418-2003) won the third prize of "China Standard Innovation and Contribution Award".

SAC/TC179 has been in effect for more than 20 years. With the standardisation, SAC has formed an organised, scaled and guaranteed professional association. The following are several prominent features.

China Standard Innovation and Contribution Award

First, organisational guarantee is powerful. In 2012, SAC/TC179 (IFSC) strengthened and managed standardisation work through "cleaning" and "construction", which obtained unprecedented progress. 2013 witnessed the start of re-establishment for SAC/TC179, SAC/TC179SC1-10 and SAC/TC179/WG1-3. SAC employed 24 more people, and sub-committees and work-groups added about 380 people, thus strengthening the power of secretariat. IFSC set up the Department of Standards and Specifications in 2013, constantly increasing the force. By now, more than 30 people are working at the secretariat of SAC/TC179.

Personnel of SAC/TC179 secretariat

Secondly, there has been an increase in project grant funding. From 2012 to 2014, each standard has the average funds of 7200 Yuan. On the other hand, IFSC set aside funds of 2 million Yuan, as a special fund for criminal science and technology standardisation.

The third is the establishment of specification files of standardisation system. In recent years, SAC has approved the specifications of forensic science standards name writing, the method for grant funds management, the requirements of the national standards and industry standards approval of material, the assignment book of standard projects, the method for conference management of standard projects, the program on awards of standard projects, the announcement rules for standard projects, the quality management method for standard documents, etc.

Next, training for all members and experts were completed. From October 2013 to September 2014, SAC/TC179 and IFSC had successfully held two courses on criminal technical standardisation of trainings. More than 400 members and experts, the secretariat staff of more than 20 people of SAC/TC179 participated in these trainings, organized by SAC and the Ministry of Public Security in 2014.

Training for all members and experts of SAC/TC179

Fifth, the application system for standardisation was developed. It is a network information system of forensic science and criminal science and technology, serving SAC/TC179, SC1-10 and WG1-3, in order to construct efficient management approach of standardisation.

Next, establishing and improving of the technical standard system was done. Following public security requirements, to establish standard system is essential, forensic science standard system had covered all areas of professional development, suffice for basic demand.

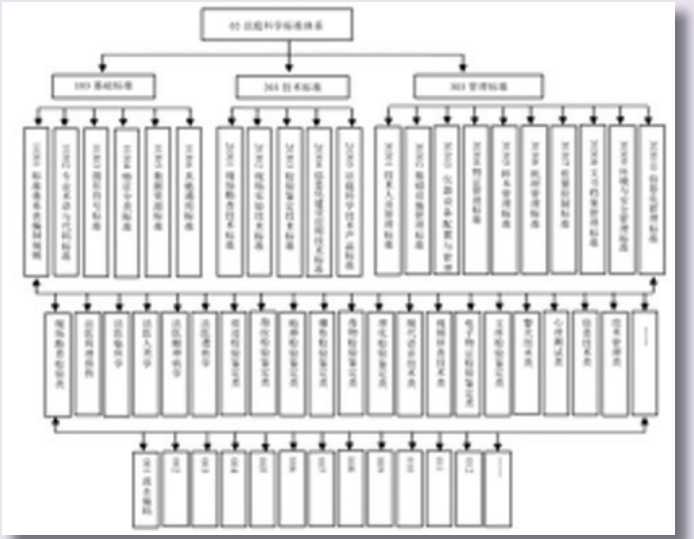


Figure 3: Structure chart for standard system of forensic science

Finally, the formulation and revising of forensic science technical standards was sped up. On one hand, outdated and delayed projects were removed, resulting in 89 projects being cancelled. On the other, by ensuring progress on scheduled projects, 119 pending projects were completed. Completion rate increased from less than 30% in 2012 to 65% in 2014. Among them, 24 have been approved and released.

All in all, although standardisation has obtained unprecedented development and remarkable achievements, in comparison, criminal science and technology is still relatively slow. The status is not clear, the dominant position of the national standard is not prominent, formulation and revision on industry standard program is not flexible, open, and transparent. Enterprise technical methods is unable to be on track with national and industry standards, as a result, being uncoordinated with technical standards is inevitable. Standardisation is not universal and omnipotent. Due to its hysteresis, standards are not able to satisfy the subsistent demands. Innovation and improvements are constantly needed.

Theoretical analysis and data provided here are from the Secretariat of SAC/TC179 and Department of Standards and Specifications of IFSC.

Proficiency Testing Program for Paint Analysis in Collaborative Testing Services

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Abstract:

Collaborative Testing Services (CTS) is a company that offers proficiency testing programs for forensic laboratories. Paint analysis is one of the testing programs in trace evidence analysis. This article provides an overview of proficiency testing programs for paint analysis from 2009 to 2014. The performance of the participants, analytical methods used and results are summarised in this paper. Based on the statistical results, a sequence for paint analysis is proposed.

Introduction

Collaborative Testing Services (CTS) was created in 1971 to meet the growing commercial demands for inter-laboratory testing. Testing programs in the areas of agriculture, colour, containerboard, fasteners and metals, forensics, paper, plastics and wine are offered to meet the specialised needs of different organisations, including proficiency tests for laboratory accreditation. This enables organisations to achieve excellence in measurement quality through inter-laboratory testing. Forensic testing programs are also provided by CTS, including forensic biology/DNA, drug analysis, latent prints and impressions, firearms and toolmarks, trace evidence, questioned documents, toxicology, crime scene and research tests. Paint analysis is one of the testing items offered in the trace evidence category. Additionally, glasses, fibers and flammables are also included.

This article provides an overview of proficiency testing programs for paint analysis performed by the laboratory from 2009 to 2014. By analysing the results and the analytical methods, a sequence for paint analysis is proposed.

Sample composition

Samples used in the CTS proficiency tests for paint analysis comprise of 2-3 “questioned” paint chips and 1-2 “known” paint chips. Participants are required to determine whether the questioned paints originate from the known paints.

Participants

The paint analysis testing program is held twice a year. Samples are sent to participants (not limited to USA laboratories) from countries all around the world.

Number of participants

More than 200 laboratories participate in the paint analysis program every year. This is shown in Figure 1.

Performance of participants

An overview of participants’ performance in paint tests is presented in Table 1. The results showed that more than 90% of participants could maintain proficiency except in 2012 for reasons unknown.

Table 1: Participants’ performance in proficiency testing programs for paint analysis from 2009 to 2014

Year	Number of participants	Number and proportion of participants with correct identification			
		Item 1	Item 2	Item 3	Item 4
2014	86	Known sample	—	83（94.2%）	—
		—	Known sample	84（97.7%）	—
	119	Known sample	119（100%）	119（100%）	—
2013	85	Known sample	81（95.3%）	82（96.5%）	
	119	Known sample	115（96.6%）	114（95.8%）	
2012	85	Known sample	83（98%）	81（95%）	—
	115	Known sample	—	100（87%）	112（97%）
		—	Known sample	95（83%）	89（77%）
2011	90	89（99%）	89（99%）	Known sample	—
	143	Known sample	139（97%）	139（97%）	—
2010	84	Known sample	79（94%）	83（99%）	79（94%）
	147	Known sample	143（97%）	143（97%）	—
2009	85	Known sample	82（96%）	81（95%）	—
	140	Known sample	135（96%）	140（100%）	—

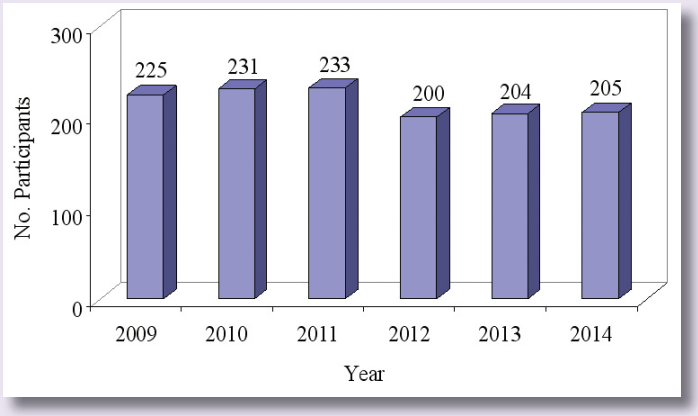

Figure 1: Number of participants in the paint analysis program offered by CTS

Analytical methods overview

The analytical methods used in paint analysis are summarised in Figure 2. Pre-testing by stereomicroscope is essential in paint analysis, and 98% of participating laboratories employed this method. By stereomicroscope analysis, the following types of paint can be distinguished: pure-coloured, pearl effect and metallic. In addition, the colour and number of paint layers can also be compared. The results of the analysis were not further examined except when obvious differences were observed.

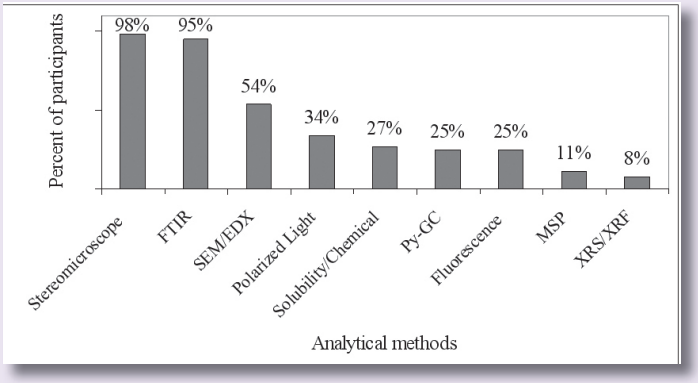

Figure 2: Percentage of participants using different analytical methods

In forensic laboratories, Fourier Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) are the most frequently used techniques to distinguish paints [1-3]. FT-IR can provide a vast amount of information on the chemical composition of paint, including the component of resins, fillers and main inorganic pigments [4]. Approximately 95% of participants employed this method. On the other hand, SEM/EDS can provide useful information about the elemental composition, size and distribution of fillers [2, 5].

Pyrolysis-gas chromatography (Py-GC) or pyrolysis-gas chromatography/mass spectroscopy (Py-GC/MS) are also applied in paint analysis [2]. However, these methods are destructive and consume a significant amount of samples during the analytical process.

Polarised light, fluorescence and microspectrophotometry (MSP) are also used to detect the physical characteristics of paints. However, only a small portion of participants used these methods. Lastly, X-Ray fluorescence (XRF) is another useful technique which is similar to EDS that is used for characterising the elemental composition of paints. Although the detection limit of XRF is higher than EDS, the percentage of participants using this method was low, because the sample consumption amount of XRF is higher than EDS.

Suggested sequence of analysis

Based on the statistical results and the advantages and disadvantages of the different methods discussed above, a sequence for paint analysis is proposed. Figure 3 shows the flow chart for paint analysis.

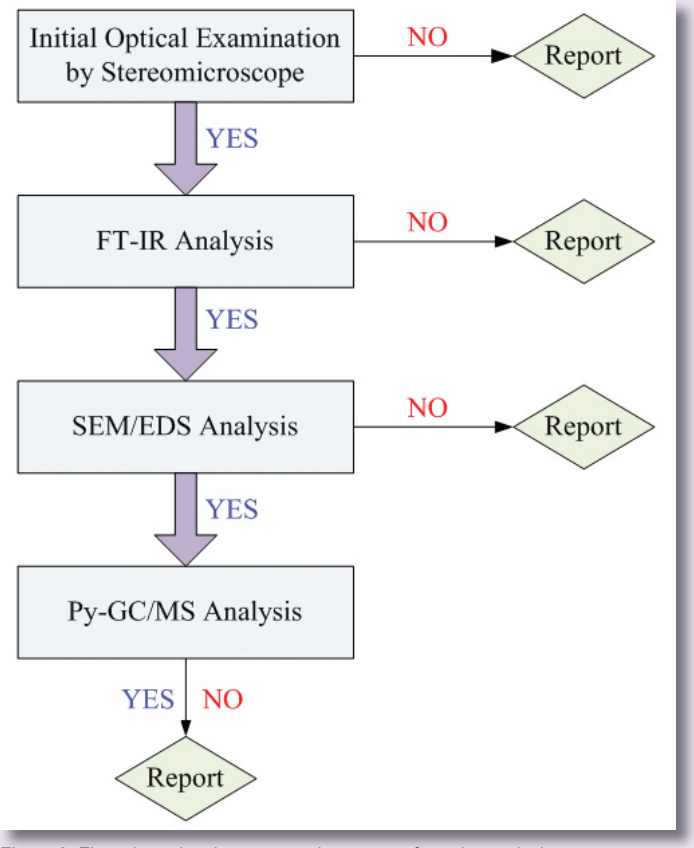

Figure 3: Flow chart showing proposed sequence for paint analysis

In forensic science, as more analytical methods are used, the higher the reliability of the conclusion. As such, different analytical methods should be applied together to improve the discriminating power of paint analysis. Results of the different examination methods can then be corroborated, which increases the reliability of expert opinions in court.

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The Assessment of Bloodstains on Fabrics

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When blood is shed during a violent crime against a person, the clothing of the victim(s), the perpetrator(s) and any nearby witness(es) may become bloodstained. Whilst DNA profiling of any bloodstains located on the clothing (source level evidence) is now a routine and scientifically reliable procedure, the determination of the behaviour of those involved in the bloodshed event (activity level evidence) often relies, wholly or in part, on the correct evaluation and interpretation of the bloodstains and bloodstain patterns on the clothing item(s) examined. Often those bloodstains likely to give good source level evidence are not the same as those capable of providing more probative activity level evidence. Without an understanding of the mechanism(s) responsible for the deposition, thus an ability to correctly classify the bloodstains observed on the clothing, the activity evidence bloodstains can easily be overlooked during the sample selection process.

Whilst bloodstains and bloodstain patterns can potentially provide a retrospective window to the physical events that have occurred during a bloodshed event, the theoretical concepts that govern the evaluation and interpretation of bloodstains and bloodstain patterns on smooth, static non-porous surfaces found at crime scenes often cannot be used to underpin the assessment and interpretation of bloodstains deposited on fabrics. Currently little scientific research exists regarding some of the challenges encountered in this area of Bloodstain Pattern Analysis. Variables such as fabric construction, composition, history, treatment(s), moisture content, resultant vector influences and fabric surface curvature or folding can add additional levels of complexity to the reliable determination of the mechanism(s) responsible for the deposition of the bloodstains and bloodstain patterns observed. Obviously these variables and their effect permutations are many and cannot all be described here.

In some circumstances, the influence of these variables can be dramatic (see Figures 1 and 2). Images show drip stains on 100% polyester (Figure 1) and 100% (treated) cotton (Figure 2).

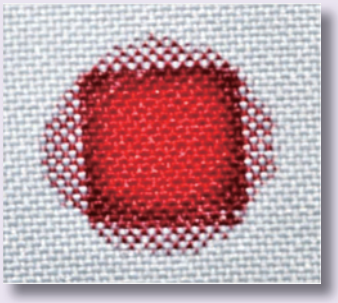

Figure 1

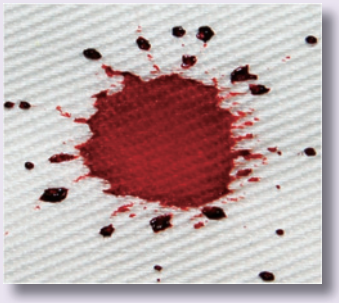

Figure 2

In Figure 1 the blood has preferentially diffused along the non-absorbent synthetic filament fibres, giving a stain appearance more akin to that of a transfer. Figure 2 however shows how the blood has been absorbed by the natural staple cotton fibres and as such the bloodstain exhibits an improved shape correlation to what might be expected given the deposition mechanism. In a general sense, research and training observations demonstrate that fabrics composed of natural fibres will more closely reflect the original size and shape of the bloodstains than fabrics composed of synthetic fibres.

In simplistic terms it can be said that all fabrics will absorb blood, however in a more technical sense the same cannot be said about all fibre types. Whilst natural fibres generally absorb blood, with a cross sectional view of the fibre showing blood drawn in towards the fibre core, synthetic fibres do not. The interaction of blood with synthetic fibres involves a phenomenon known as adsorption. In a fibre context, adsorption is surface coating by a fluid, whereby the non-permeating nature of the synthetic fibre means that the blood will only coat the surface of the fibre. A cross sectional view of the fibre would show the unstained non-penetrated core surrounded by a ring-like coating of blood. Subsequently the wicking rate and direction of blood flow through fabrics composed of synthetic fibres can be vastly different to that of fabrics composed of natural fibres. The wicking rate and direction of blood flow for fabrics composed of fibre blends (that is varying percentages of natural and synthetic fibres) may differ again.

These changes to the geometric display of the bloodstains and bloodstain patterns due to the aforementioned variables can have significant practical consequences. Of note, these bloodstain shape changes often begin immediately after blood deposition with major changes to stain shape occurring within minutes of blood deposition. Obviously the assessment of bloodstains and bloodstain patterns rarely, if ever, occurs within the timeframes just mentioned, thus the clothing examiner or bloodstain analyst is presented with a historic view of the bloodstain which can often bare little shape resemblance to that of the bloodstain immediately following deposition. Some examples of these changes include, but are not limited to, multiple pattern transfers blending into one unremarkable bloodstain thus masking the pattern transfer source (see Figures 3 and 4) or pattern evidence changing shape and configuration to such an extent as to possibly render an exclusion of the contacting surface (see Figures 5 and 6).

Figure 3

Figure 4

Figure 5

Figure 6

The basal unit for any fabric is the fibre which composes the yarn. Yarns are comprised of single or multiple fibre types and most commonly are either natural or synthetic in composition. To form the broad layer of a fabric the yarns are then either woven, a minimum of at least two sets of yarns interlaced perpendicular relative to each other, or knitted, a minimum of at least a single yarn forming interlocking loops (see Figure 7). In a general sense any garment that can stretch is a knit. Weaves are typically more robust than knits and exhibit greater mechanical strength, thus less stretch.

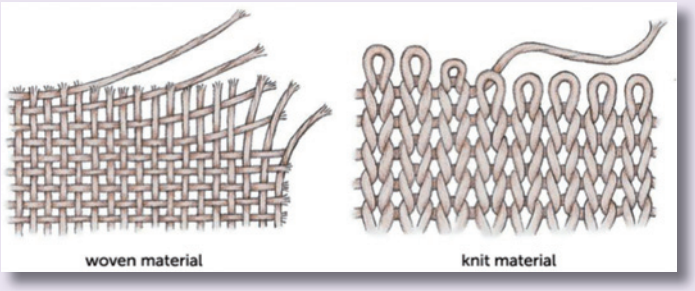

Figure 7: (Available at: http//:www.one_little_minute_blog.com/knits.vrs.wovens. Accessed 25th June 2015)

The footprint of synthetic fibre use in the clothing industry is increasing dramatically and, unfortunately for the clothing examiner or bloodstain analyst, clothing manufactured from synthetic fibres, or high synthetic fibre blends, represent the most challenging clothing category with regards to bloodstain evaluation and interpretation. What is also becoming clearer is that whether knitted or woven, bloodstains deposited on clothing items manufactured from synthetic fibres, or high synthetic fibre blends, will almost certainly undergo some form of the aforementioned bloodstain shape change(s) following deposition.

Of additional concern regarding the assessment of bloodstains and their deposition mechanism(s) is the fact that under certain circumstances transferred bloodstains on fabrics can mimic impact spatter especially when the bloodstains are only viewed at the macroscopic level (see Figures 8 and 9). Figure 8 shows an impact spattered bloodstain (approximately 3.0mm in diameter) deposited on a 50% cotton / 50% polyester blend fabric. Figure 9 shows a transferred bloodstain (approximately 2.0mm in diameter) deposited on 100% polyester fabric. Note in Figure 8 that apparent air bubbles are present in the bloodstain. The presence of air bubbles within bloodstains is often used as a key to diagnose an expirated deposition mechanism. In this instance however the likely cause was air entrapment at the time of bloodstain formation due to the irregular receiving surface.

Whilst recent research and training observations indicate that apparent discernible differences (such as surface coating features and micro-spatter) which may lead to separation criteria between spatter and transferred bloodstains seem to exist at the microscopic level, further dedicated research is required to confirm that these differences exist in all instances. The experience of the authors is however that in some instances these potential separation criteria are not always seen.

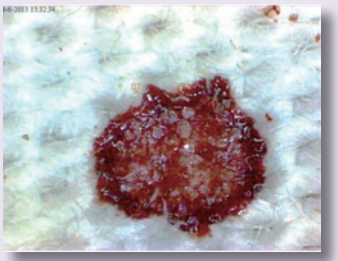

Figure 8

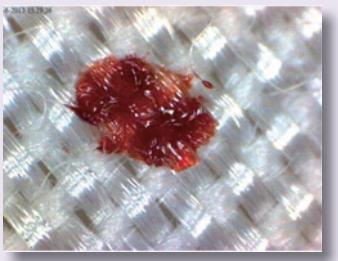

Figure 9

When large numbers of spattered bloodstains are present on clothing the correct categorisation of deposition mechanism (spatter versus transfer) can often be made. However when only small numbers of bloodstains are present the appropriate classification of deposition mechanism becomes more problematic with a misidentification likely to have considerable implications within the judicial environment.

Subsequently, when assessing bloodstains on fabric, caution must be exercised in the use of shape as a predictor of deposition mechanism. It appears that in many laboratories little bloodstain pattern analysis training is provided to clothing examiners or bloodstain analysts, especially those responsible for activity level evidence sampling, with regards to the complexities and challenges involved in assessing bloodstains and bloodstain patterns on fabrics. In instances where no training is provided or the training has a concentration on smooth, static non-porous surfaces there is a very real potential for inappropriate sampling and / or analysis to occur. In instances of violence that result in multiple items of bloodstained clothing or that involve more than one bloodstain contributor the results of inappropriate sampling or analysis are likely compounded.

As research and training in the area of assessing bloodstains and bloodstain patterns on fabrics progresses, it is becoming evident that increased recognition and consideration of the potential influence of the variables mentioned earlier on the size, shape and distribution of the bloodstains and bloodstain patterns observed must occur. Failure to understand and recognise the potential influence of these factors may lead to erroneous stain sampling and worse, scientifically unsupportable interpretative conclusions.

INTERPOL workshop in Singapore aims to strengthen regional forensic capabilities

Dr Susan Hitchin, INTERPOL

Strengthening the forensic capabilities of counter-terrorism investigations was the aim of a three-day INTERPOL Forensics Workshop for Southeast Asia and the Pacific.

Attended by some 100 delegates from 18 countries, the workshop allowed delegates from the INTERPOL National Central Bureaus, forensic experts and forensic managers of participating countries to familiarise themselves with the different INTERPOL forensic tools and services that can enhance their national forensic capabilities and counter-terrorism initiatives.

These include INTERPOL's AFIS and DNA databases and Gateways on a secure network for sharing data, INTERPOL's DNA matching software that can be used in various countries for their national use, the new facial recognition database and missing persons DNA database, and assistance to countries' capacity building activities for improving disaster victim identification (DVI) responses.

Held at the INTERPOL Global Complex for Innovation (IGCI) in Singapore in partnership with ASEANAPOL, the workshop was jointly funded by INTERPOL and the Government of Canada as part of the INTERPOL Capacity Building Programme on Improving Counter-Terrorism and International Collaboration in ASEAN member states.

Representing Cambodia's Ministry of Interior, and ASEAN's Senior Officials Meeting on Transnational Crime (SOMTC), Sieng Lapresse opened the workshop which also sought to encourage the continued international exchange of information and biometric data for counter-terrorism efforts.

"The sharing of knowledge on enhancing forensic capabilities with a focus on counter-terrorism is very timely given the backdrop of recent terrorist-related crimes in the region and beyond. I congratulate all concerned in achieving the objectives of this workshop and sincerely look forward to more training activities in tandem with the excellent facilities available at the IGCI in Singapore," said Pengiran Dato Paduka Haji Abdul Wahab bin Pengiran Haji Omar, Executive Director, ASEANAPOL Secretariat.

With terrorism investigations often requiring extensive forensic capabilities and depending on access to tools which enable the gathering, recovering and preserving of evidence, Dale Sheehan, INTERPOL's Director of Capacity Building and Training, said: "INTERPOL's capacity building activities draw from trainings and workshops to strengthen the sustainability and operational capacity of member countries.

"We will strive to further develop the forensics component in the framework of our counter-terrorism programmes and we have called upon the countries from the Southeast Asian and Pacific regions to include a stronger forensics element as part of their training curricula," added Mr Sheehan.

In this respect, the forensics workshop focused on five key areas: fingerprint analysis, DNA, facial recognition, DVI and digital forensics. Breakout sessions were held for each discipline for participants to share national practices and discuss how to more effectively share data in the region.

"The Southeast Asian and Pacific countries have shown a strong desire to improve international police cooperation, in particular in relation to the wider use of biometrics and forensic capabilities to tackle the evolving terrorist threat," said INTERPOL's Director for Operational Support, Michael O'Connell.

"INTERPOL has launched its regional forensic strategy through this workshop at the IGCI, demonstrating that its modern biometric capabilities can deliver these improvements and enhance our response in partnership with regional actors such as ASEANAPOL and ASEAN," added Mr O'Connell.

The following countries attended the workshop: Australia, Brunei, Cambodia, Fiji, Indonesia, Korea, Laos, Malaysia, Myanmar, New Zealand, Papua New Guinea, Philippines, Vietnam, Singapore and Thailand.

The workshop was run in parallel with the INTERPOL Advanced Course on Forensic Examination, providing forensic officers from the Malaysian Maritime Enforcement Agency and the Singapore Police Coast Guard with the necessary skills to face the challenges of processing evidence at a maritime crime scene.

Enhancing forensic skills for maritime security focus of INTERPOL training

Dr Susan Hitchin, INTERPOL

Providing forensic officers with the necessary skills to face the challenges of processing evidence at a maritime crime scene was the focus of an INTERPOL training course in Singapore.

Maintaining the chain of custody required for successful prosecutions was also a key element during the INTERPOL Advanced Course on Forensic Examination held at the INTERPOL Global Complex for Innovation (IGCI) in Singapore, which brought together officers from the Malaysian Maritime Enforcement Agency (MMEA) and the Singapore Police Coast Guard.

Practical crime scene investigation training was provided by INTERPOL and the National University of Malaysia using the mock ship facility of the Singapore Police Coast Guard, in addition to a simulated trial hosted by the National University of Singapore.

First Admiral Maritime Dato Zulkifli bin Abu Bakar, Director of the Maritime Criminal Investigation Department of the MMEA said: "The MMEA is in the process of developing a standard operating procedure in forensics which will enable swift decision-making processes and pre-planned responses for any eventualities.

The INTERPOL Advanced Course on Forensic Examination was held at the INTERPOL Global Complex for Innovation (IGCI) in Singapore.

"By attending the Advanced Course on Forensic Examination, coordinated by a leading law enforcement organization such as INTERPOL, the MMEA's forensic officers will be able to increase their proficiency in law enforcement, while at the same time validating the standard operating procedures that have been developed," concluded the First Admiral.

INTERPOL's Director of Capacity Building and Training, Dale Sheehan said: "With the globalisation of criminal syndicates, strong infrastructures with sustainable capacities are key to successful law enforcement.

"Pirates will always be looking for easy targets and easy money. To strike at the heart of maritime piracy, we must ensure that all actors involved in maritime law enforcement are able to deal with the increasingly sophisticated piracy network," added Mr Sheehan.

Practical crime scene investigation training was provided to the Malaysian Maritime Enforcement Agency by INTERPOL and the National University of Malaysia (UKM) using the mock ship facility of the Singapore Police Coast Guard.

Building on a basic training programme held in Malaysia earlier this year, the advanced five-day (7-11 September 2015) course was run in parallel with the INTERPOL Forensics Workshop for Southeast Asia and the Pacific, which enabled participants to liaise with INTERPOL officials and experts from across the region.

Forensic equipment and materials have also been donated to the Malaysian Maritime Enforcement Agency in order to support the agency's enhanced specialised forensic capabilities. Specialised equipment such as portable CNA fuming cabinets, Ninhydrin spray, Amido Black spray and SPR spray for developing fingerprints in difficult scenarios were donated and training was given on their use. These items will greatly enhance the possibility of finding potentially vital evidence in the difficult humid atmosphere of the agency's operations.

The training is part of the INTERPOL Capacity Building Programme to Foster Maritime Security in Malaysia funded by the Department of Foreign Affairs, Trade and Development, Canada.

The programme aims to improve maritime piracy and armed robbery related investigation capacity in Malaysia, as well as to support increased investigative resources, specialised forensic capabilities and improve information-sharing amongst national law enforcement agencies.

Course participants took part in practical exercises in crime scene investigation, including the retrieval of fingerprints and other evidence.

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Prof Hong Sungwook, Ph.D

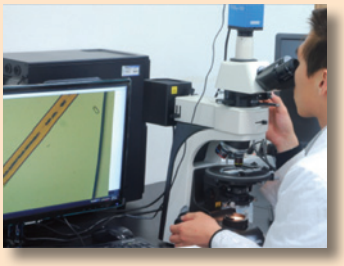
Background

In order to discover 'real' truth and to uphold justice in criminal investigation, scientific approaches in collecting, analysing and systematic management of evidence are the key elements of success. The Graduate School of Forensic Science was established in 2011 to educate students in the theoretical and application methods of scientific crime investigation. The education program focuses on the application of the physical, biomedical, and social sciences to the analysis and evaluation of physical evidence, human testimony and criminal suspects. This field-oriented program gets huge benefits from collaborations with the three major police training institutes, namely the Korea National Police University, Police Training Institute, and Korean Police Investigation Academy, which are within 1 mile from Soon Chun Hyang University. With excellent curriculum and facilities, the graduate school proudly offers a Master's degree for individuals seeking advanced knowledge in scientific crime investigation. Students with a Bachelor's degree or similar qualifications can apply for a Master's degree program and students with a Master's degree or similar qualifications can apply for a Doctorate degree program.

Curriculum

The course begins on 1 March 2016. It is a 2-year course (4 semesters) comprising of:

- Forensic professional modules: evidence law, court testimony
- Forensic science research modules: research methods, statistics, reasoning, thesis
- Forensic evidence modules: photography, fingerprint, impressions, DNA, blood pattern, trace evidence, crime scene reconstruction, etc.
- Crime and criminal justice modules: criminology, decision-making, justice



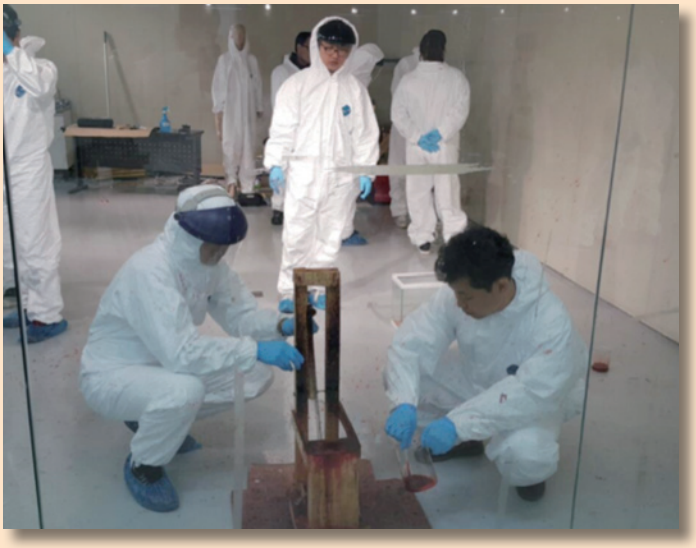
Facilities

The Graduate School of Forensic Science has three specialised laboratories for forensic training:

- **Evidence Processing Laboratory**
  The latest equipment and facilities like microscopes (stereo and polarising), forensic light sourcing and fuming chambers are ready to use for observing and experimenting on latent fingerprints and footprints, trace evidence, etc.

- **Moot Courtroom**
  The lecture room is designed like a real courtroom to give the trainees an opportunity to present their findings like a real specialist giving a testimony in a real court hearing. These facilities are set up to train skilled specialists into becoming skilled witnesses.

- **Blood Pattern Analysis Laboratory**
  All the walls of the laboratory are made of tempered glass which will make reenacting of bloodstain patterns easier. Wireless communication is also integrated in all laboratory and classroom interiors. This is where bloodstain pattern analyses required by the courtroom are reenacted and tested. In addition, students will be trained in site reconstruction in pre-designed mock sites.



Research

Interdisciplinary research is active and funded by national and local government agencies. About 10 articles are published every year in peer-reviewed journals of Korea and other countries. Recent projects have included “Korean Characteristics of Ridge Density”, “Development of Artificial Fingerprint”, “Transfer and Persistence of Trace Evidence”, “Enhancement of Fingermarks in Blood Deposited on the Thermal Paper”, and “Child Pornography Investigation”.

Open Training Programs

The Graduate School of Forensic Science provides diverse short-term training programs to law enforcement officers in the following areas:

- Forensic photography
- Forensic light source and evidence collection
- Fingerprint development and enhancement
- Footprint development and enhancement
- Trace evidence for criminal investigation

It also provides diverse Korea International Cooperation Agency (KOICA) programs to law enforcement officers of many countries. The Crime Prevention and Forensic Investigation Program for the Indonesian police force is currently in progress (2014-2016). The Cybercrime Investigation Capacity Improvement Program for Bangladesh police was completed in 2014.

KOICA implements grant aid and technical cooperation programs for the Korean Government, thereby contributing to strengthening the cooperative relationship between Korea and developing countries, and promoting socio-economic advancement of the recipient countries (For more information, visit http://www.koica.go.kr/)

Admission Requirements

- Major: BS in physical or natural sciences (e.g., biology, chemistry, physics, forensic science)
- Language: TOPIK (Test of Proficiency in Korean) level 4+ or English speaker

Contact: forensics@sch.ac.kr

Sensitivity Study of 30 Indels in the Investigator® DIPplex Kit For Forensic Casework Applications

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Abstract

A marker system based on the presence or absence of small insertion or deletion alleles (INDEL) provides a useful alternative to the STR-based typing platform, particularly for biological samples containing relatively small amounts (< 250 pg) of compromised DNA. A sensitivity study was performed on a panel of 30 INDELs (or DIPs) and Amelogenin using the Investigator® DIPplex Kit. The assay was able to type DNA and obtain full profiles from 60 pg of template DNA and partial profiles with as little as 10 pg of DNA. An assessment was made on the quality of the profiles and the stochastic variations for DNA template range of 10 pg to 1 ng. The high sensitivity, lack of stutter peak formation and simple analysis protocol allowed the Investigator® DIPplex Kit to be easily implemented for forensic casework.

Introduction

The STR typing systems work well with relatively small quantities (250 pg to 1 ng) of low quality DNA [1-3]. While STR-based typing remains the standard DNA typing system utilised by forensic laboratories, it has some limitations [4,5]. Target amplicons in highly degraded DNA are usually less than 200 bp in length [6,7], thus they will not likely be typeable by STR-based markers which require amplicons of 100-400 bp size. In addition, STR profiles produce stutter peaks which can add ambiguity to mixture analysis [4,5] and STRs have relatively high mutation rates (approximately 10-3), which can reduce the power of kinship analysis [8].

The Investigator® DIPplex Kit consists of 30 biallelic deletion/insertion polymorphism (DIP or INDEL) loci (distributed over 19 autosomes) plus Amelogenin. INDELS can be captured in smaller amplicons than STRs; they do not produce stutter peaks during PCR; and their mutation rates are relatively lower than that of STRs [9]. The presence or absence of a small insertion or deletion in INDEL alleles enables an analytical process based on size which is readily resolvable using capillary electrophoresis. Analytically, INDELs perform similarly to that of STRs and instruments for INDEL analyses is commonly found in forensic laboratories. It is expected that the smaller amplicons of INDELs would allow successful typing of smaller quantities of challenged DNA.

Methods and Materials

PCR Amplification

Template DNA (Control DNA 9947A) at two template ranges: 0.1 to 1.0 ng, and 10 to 100 pg were used for amplification. The PCR mix in the Investigator® DIPplex Kit contained 5.0 µL Reaction Mix A with dNTP mix, MgCl2 and bovine serum albumin, 5.0 µL primer mix and 0.6 µL 2.5 U/µL Taq DNA polymerase. 10 µL of DNA template was added to the PCR mix to a final volume of 20 µL. The PCR was performed in a GeneAmp® PCR System 9700 thermal cycler (ABI) with the following conditions: denaturation at 94ºC for 4 min followed by 30 cycles of 94ºC for 30 sec, 61ºC for 120 sec and 72ºC for 75 sec, followed by 60 min at 68ºC.

Electrophoresis using the ABI 3130xl Genetic Analyzer

The PCR products were prepared for capillary electrophoresis by adding 1 µL of amplified product to 12 µL of a mixture of 24:1 Hi-Di formamide (ABI) and DNA size standard 550. Separation and detection were performed with an ABI 3130xl Genetic Analyzer with 36 cm capillary arrays and POP-4 polymer (ABI). Electrophoretic data were analysed by GeneMapper ID v3.2.1 software using 50 RFU as the minimum detection threshold.

Results and Discussion

Reproducibility

The assay using the Investigator® DIPplex Kit showed reproducible genotypes. The allele calls for Control DNA 9947A are shown in Table 1. Figure 1 illustrates a DIPplex profile from 100 pg DNA.

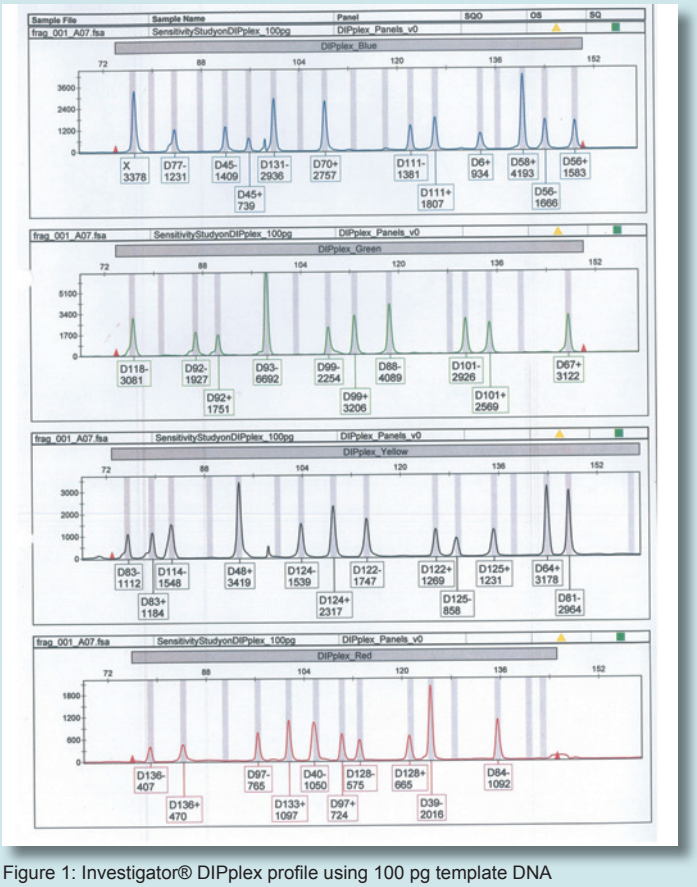

Figure 1: Investigator® DIPplex profile using 100 pg template DNA

Reaction Volume and PCR Cycles

The Investigator® DIPplex assay performed well with a reduced reaction volume of 20 µL. The assay was able to type DNA and obtain full genotypes with 60 pg of template DNA. The standard cycling protocol of 30 cycles recommended by the manufacturer worked well with the low template range of 10 to 100 pg DNA. Attempts with a 28-cycle protocol caused allele drop-outs with less then 100 pg DNA (data not shown).

Table 1 Investigator® DIPplex assay allele calls for 9947A DNA for the two template range: 0.1 ng to 1.0 ng and 10 pg to 100 pg.

Locus	1.0 ng	0.8 ng	0.6 ng	0.5 ng	0.4 ng	0.3 ng	0.2 ng	0.1 ng	100 pg	80 pg	60 pg	40 pg	20 pg	10 pg
Amelogenin	X/X	X/X	X/X	X/X	X/X	X/X	X/X	X/X	X/X	X/X	X/X	X/X	X/X	X/X
HLD77	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	*/*
HLD45	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	*/+
HLD131	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
HLD70	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
HLD6	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
HLD111	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+
HLD58	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
HLD56	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	*/+	-/*
HLD118	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
HLD92	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/*
HLD93	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
HLD99	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+
HLD88	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
HLD101	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+
HLD67	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
HLD83	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/*	-/*
HLD114	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
HLD48	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
HLD124	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+
HLD122	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/*
HLD125	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/*	-/+
HLD64	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
HLD81	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
HLD136	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	*/*	*/+
HLD133	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
HLD97	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/*	-/+
HLD40	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
HLD128	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	*/+	-/+	-/+
HLD39	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
HLD84	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-

Indel allele with deletion denoted by " - " and insertion by " + ". Allele drop out denoted by " * ".

Intra-colour peak balance

Intra-colour peak balance was calculated by dividing the lowest peak height value by the highest peak height value within a colour (homozygote peak heights are divided by two and heterozygote peak heights are averaged for each marker). The DIPplex profiles were observed to exhibit fair intra-colour peak balance with peak balance exceeding 0.4 for most profiles (see Table 2).

Table 2: Intracolour Balance of Investigator® DIPplex Assay

DNA Template	Intracolour Balance: DIPplex Blue (6-FAM™)	Intracolour Balance: DIPplex Green (BTG)	Intracolour Balance: DIPplex Yellow (BTY)	Intracolour Balance: DIPplex Red (BTR)
1 ng	0.530187	0.326639	0.405217	0.549770
0.8 ng	0.585128	0.395100	0.353681	0.525282
0.6 ng	0.532082	0.385825	0.334439	0.563908
0.5 ng	0.537357	0.368474	0.377407	0.433449
0.4 ng	0.465466	0.384134	0.485211	0.425107
0.3 ng	0.367096	0.502367	0.430694	0.484577
0.2 ng	0.380460	0.608700	0.439220	0.429379
0.1 ng	0.262382	0.485637	0.373109	0.438252
100 pg	0.222752	0.460400	0.401452	0.435020
80 pg	0.382827	0.679667	0.508016	0.440467
60 pg	0.269540	0.575336	0.496665	0.566467
40 pg	0.240453	0.504249	0.412412	0.259766
20 pg	0.305903	0.458671	0.239936	0.389286
10 pg	0.277647	0.091232	0.218997	0.140212

Heterozygote ratio

The heterozygote ratio for the heterozygous loci in 9947A Control DNA is illustrated in Table 3. The mean heterozygote ratio for DNA template range of 0.1 ng to 1.0 ng exceeded 0.6 for all loci. For the low template range of 60 pg to 100 pg, except for locus HLD136 with a mean heterozygote ratio of 0.5885, all other loci exhibited ratios exceeding 0.6.

Table 3: Heterozygote ratio of Investigator® DIPplex Assay for 9947 A Control DNA

Locus	1.0 ng	0.8 ng	0.6 ng	0.5 ng	0.4 ng	0.3 ng	0.2 ng	0.1 ng	100 pg	80 pg	60 pg	40 pg	20 pg	10 pg
HLD45	0.9322	0.8811	0.8458	0.7144	0.7868	0.7038	0.9969	0.7080	0.5245	0.7606	0.6240	0.5883	0.4951	AD
HLD111	0.9532	0.8009	0.8188	0.8197	0.8390	0.8933	0.8554	0.6174	0.7642	0.6078	0.9164	0.6873	0.6228	0.1756
HLD56	0.7671	0.5652	0.6134	0.8727	0.8940	0.9371	0.6808	0.9259	0.9502	0.7215	0.7601	0.8191	AD	AD
HLD92	0.9704	0.9749	0.9528	0.9439	0.8495	0.9911	0.8426	0.8875	0.9087	0.7896	0.3501	0.8103	0.7542	AD
HLD99	0.9156	0.8621	0.6920	0.5427	0.5942	0.5446	0.6997	0.9056	0.7030	0.8967	0.8788	0.4330	0.7704	0.8190
HLD101	0.9450	0.8344	0.7938	0.8090	0.8018	0.8449	0.6268	0.3411	0.8780	0.7622	0.9101	0.8477	0.4739	0.2715
HLD83	0.6619	0.9494	0.8611	0.6752	0.7657	0.8687	0.9050	0.9318	0.9392	0.8414	0.8322	0.8143	AD	AD
HLD124	0.7750	0.7177	0.8383	0.8778	0.8566	0.9183	0.9135	0.9336	0.6642	0.8108	0.9586	0.9240	0.9697	0.5628
HLD122	0.8632	0.8455	0.9321	0.9406	0.9042	0.9121	0.9183	0.8937	0.7264	0.5892	0.7213	0.4717	0.8426	AD
HLD125	0.4196	0.3693	0.8077	0.7594	0.9110	0.6536	0.7044	0.7367	0.6970	0.4889	0.6933	0.9430	AD	0.6419
HLD136	0.9407	0.7837	0.7635	0.7257	0.8045	0.8599	0.7846	0.7055	0.8659	0.6311	0.2684	0.5027	LD	AD
HLD97	0.9206	0.9742	0.9886	0.9924	0.9372	0.9857	0.9170	0.5790	0.9464	0.4072	0.7713	0.6462	AD	0.8151
HLD128	0.4762	0.5972	0.5284	0.8346	0.7413	0.8200	0.8392	0.8602	0.8647	0.8872	0.4036	AD	0.6829	0.6395

AD = Allele dropout; LD = Locus dropout

Artefacts and Stochastic Variations

The DIPplex profiles for DNA above 100 pg were characterised by pull-ups and split-peaks, particularly for DNA template exceeding 500 pg where enhanced pull-ups and split-peaks rendered some difficulty in the reading of the alleles. The artefacts were significantly absent in profiles from DNA template of less than 100 pg. However, allele and locus drop-outs were observed for DNA template of less than 60 pg.

Conclusion

The Investigator® DIPplex Kit has the advantage of having a similar analytical protocol to that of STR analyses. The small amplicon size of INDELS make them highly desirable for low template DNA analysis. This study has demonstrated that the Investigator® DIPplex assay performs well for low-template DNA. Work is ongoing for testing low template DNA samples with the Investigator® DIPplex Kit where standard STR analyses have failed. It is expected that the Investigator® DIPplex Kit will provide a useful alternative to the STR-based typing platforms. Population studies are also being carried out using the Investigator® DIPplex Kit.

Acknowledgements

Our sincere thanks goes to QIAGEN Biotechnology Malaysia for the start-up kit and technical assistance. Many thanks to the staff of the Kuching DNA Laboratory for their support and assistance.

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Three cold cases solved by touch DNA STR analysis using the combined DNA database in China

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[#] Contributed equally

Abstract

Touch DNA is thought to be difficult to test, especially for old biological evidence from a decade ago. In this paper, the Chinese police used DNA technology to yield full profiles of touch DNA in three cold cases which had occurred 10 years ago and matches were made between the case's DNA profiles and the convicted offender DNA database. Following more investigations, it was demonstrated that the persons matched were the real murderers.

Keywords: Forensic science; Touch DNA analysis; STR; Cold Case

A "cold case" is defined as a case where the police investigation has been scaled down and the case is effectively no longer active and still sub-judiciary [1]. DNA has proved to be a powerful tool in the fight against crime, as DNA evidence can identify suspects, convict the guilty, and exonerate the innocent. DNA technology development has shed light on the examination of cold cases. Biological evidence that was previously deemed unsuitable for DNA testing as the sample was too little or degraded may now yield a DNA profile. USA and UK have initiated the plan of using DNA to solve cold cases and have made much progress. In China, after years of development, the National DNA Database of China is the biggest database in the world, and now contains the DNA profiles of more than 30 million individuals, of which convicted offenders account for a significant proportion. DNA technology and the success of DNA database systems have inspired the reevaluation of DNA evidence for cold cases throughout China. Touch DNA is the transfer of trace quantities of DNA through skin contact. It is cellular material transferred from the skin of an individual who has simply touched or handled objects, such as weapons and other items associated with a crime, touching surfaces, or wearing clothing [2]. As we know, there are only several cold cases solved by touch DNA testing, such as the murder of Krystal Beslanowitch in 1994 in America. In that case, the stone collected at the crime scene was supposedly held by the suspect. A single male DNA profile was obtained in 2013 when the evidence was retested. In China, a cold case project was initiated in 2013 and here we report the three cold cases that occurred 10 years ago which have been successfully solved by touch DNA analysis.

Case 1

This case happened in 1999, and a female was killed in her home. The key evidence item was a male black suit jacket found at the crime scene (Figure 1A), on which there should have been more blood. However, it had been washed when local police found it. Up to thirty different areas of the jacket were cut into the 1.5 mL tubes for DNA testing.

Case 2

The murder case happened in 2002, and a woman was killed on the streets. The key evidence was a felt hat that was found at the crime scene (Figure 2A), which most likely belonged to the suspect. There was a short cotton thread on the hat eye side. The short cotton thread and other parts of the hat were cut into the 1.5 mL tubes for DNA testing.

Case 3

The murder case happened in 2003, and a man was killed by two other men who were wearing headgears. The two headgears were found at the crime scene (Figure 3A). Parts of the headgears close to the mouth were cut into the 1.5 mL tubes for DNA testing.

Materials and Methods

DNA extraction

The DNA extraction was performed manually using MagAttract® DNA Mini M48 kit (Qiagen), not using the workstation. The modified manual method follows this procedure: 300 µL diluted Buffer G2 and 30 µL proteinase K (10 mg/ml) was added to the 1.5 mL tube containing cut items, and mixed thoroughly. The tube was incubated at 56°C for 1 h, then centrifuged at 12000 RPM for 10 mins. The supernatant was transferred to another 1.5 mL sample tube. 900 µL buffer MTL and 30 µL MagAttract Suspension B was added and mixed thoroughly. After 10 mins of binding, the sample was washed 3 times with 80% ethanol solution using Magnetic shelf (Qiagen). The genome DNA was eluted in 30 µL ddH2O.

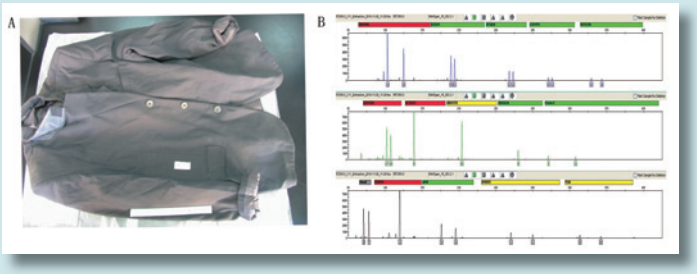

Figure 1: A the male black suit jacket found in the crime scene; B the single male STR profile

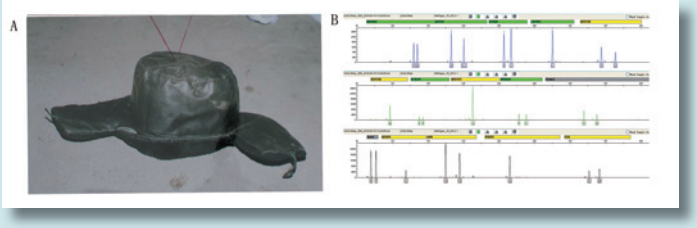

Figure 2: A the felt hat found in the crime scene; B the single male STR profile

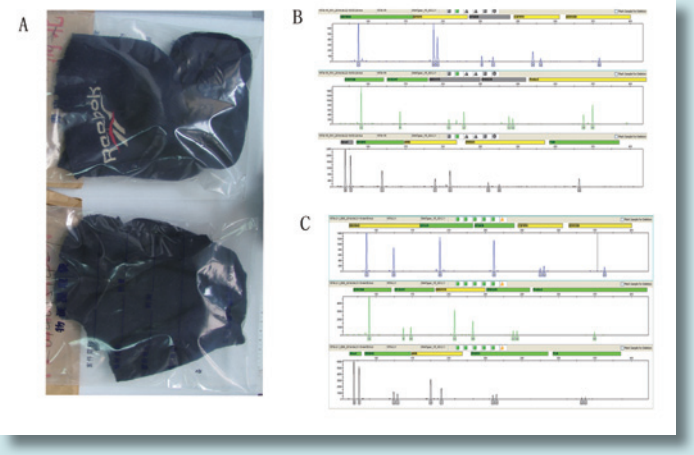

Figure 3: A two headgears found in the crime scene; B, C two male STR profiles

STR typing

DNATyper™ 15 STR typing was carried out following manufacturer's guidelines except for the use of 10 reaction volumes. The DNA templates were 1 μL and 2μL respectively, and amplified 30 cycles. 1 μL PCR products were denatured in 10 μL of loading buffer composed of 9.8 μL HI-DI™ formamide (Warrington, UK) and 0.2 μL LIZ™-500 size standard mixture (Warrington, UK). DNA was separated by capillary electrophoresis using an ABI 3500xl Genetic Analyzer (Applied Biosystems). The peak detection threshold was set at 50 relative fluorescence units (RFU), and data analysis was carried out using GeneMapper™ ID-X (Applied Biosystems) software.

Results

In the first case, as the jacket was washed and it was not easy to distinguish the bloodied parts. According to our experience, we cut thirty areas of the jacket, including sleeves, cuff, pocket, collar and so on, which were most likely to be in contact with skin. Either no DNA was detected or only the victim's DNA STR were obtained from most tests. A single male STR profile was detected only at the front of the garment (Figure 1B). In the second case, the short cotton thread and other areas of the hat were tested and a single male STR profile was detected (Figure 2B). In the third case, the two headgears were tested and two single male STR profiles were detected (Figures 3B and 3C). All the STR profiles obtained were searched in the National DNA Database of China, and matched three persons in the convicted offender DNA database. Following more investigations, it was demonstrated that the matched persons were the real murderers.

Discussion

Compared to blood, semen, sweat and saliva, trace DNA is invisible and more difficult for DNA analysis, especially for trace DNA from ten years ago. After immediate contact, the likelihood of success of obtaining a profile indicative of the holder was approximately 9% for glass samples, 23% for fabric and 36% for wood [3]. Old trace DNA faces a higher chance of degradation. In our lab, we retested the touch DNA obtained from 19 cold cases that happened at least 10 years ago. The ratio of achieved STR profiles with more than nine genotyped loci was 29.4% (9 of 33 evidence items). The other evidences included cigarette butts extracted from crime scene, victim blood and blood extracted from crime scene. For cigarette butts, the ratio of achieved STR profiles with more than nine genotyped loci was 80% (12 of 15 cigarettes); for victim blood, it was 66.7% (2 of 3 items); and for blood extracted from crime scene, it was 77.8% (7 of 9 samples).

In these three cases, it is important that the evidence items were properly stored and evaluated for probative DNA evidence. As a result of development of low template (LT) DNA testing technique, as low as 100 pg DNA could be successfully profiled, which means only few cells could possibly yield DNA profiles [4-6]. The evidence items were properly cut, instead of swabbing the surface, which ensured we got the single DNA profile that was viable for search in the database. Like all cold case testing, the possibility of contamination must be considered carefully, as evidence in older cases may have been collected in ways that lacked the appropriate contamination or cross-contamination safeguards.

In these three cases, there were many other evidence items which may have DNA. An expert working group was set up, and the members included DNA expert and a criminal investigator expert. They reanalysed the cases carefully to determine the key evidence items and to make the DNA testing plan.

Acknowledgements:

We would like to thank Prof. Guiqiang Wang for helpful discussions.

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The Identification of Cannabinoids in Chocolate Chip Cookies

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Abstract

Cannabinoids were recently detected in cookies by the Narcotics Section, Forensic Division (Department of Chemistry Malaysia). The dark brown coloured cookies (shown in Figure 1) were sent to the laboratory by a government hospital doctor in Malaysia for analysis. The patient was found to be in a delirious state and a preliminary urine test tested positive for cannabinoids. The presence of cannabinoids in the cookies was confirmed using GC/MS and TLC analysis. This is the first report of the substance in a food product submitted to the laboratory for analysis through a government hospital.

Introduction

Cannabinoids are categorised as terpenophenolic compounds found in hemp plants Cannabis sativa L.(cannabaceae) [1,2]. The three major psychoactive components present are, Δ9 –tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN). It has been reported since the mid-1990s that hemp products have gained popularity and have made its way into natural produce stores and food stores specialising in nutrition and health benefits. Some of the popular hemp based products sold are hemp leaves (tea), hemp seeds and derivatives such as oil, flour, beverages (beer, lemonade) and cosmetic products [3,4].

Δ9 –tetrahydrocannabinol (THC) is present in both the drug and fibre type hemp. Studies found that the analysis of hemp seed oil has THC concentrations between 11.5-117.5 mg kg^{-1} and 7- 150 mg kg^{-1}. It was also reported by Swiss working groups that the seeds from drug-type plants was found to contain 4.1-880 mg kg^{-1}, 3-1500 mg kg^{-1} and 2-3568 mg kg^{-1} of THC [5,6].

The method of choice in the analysis of cannabinoids present in hemp food products is gas chromatography coupled with mass spectrometry (GC/MS). Other methods such as thin-layer chromatography (TLC), liquid chromatography coupled with ultraviolet and fluorescence has been reported in hemp food product analysis. The European community, for instance, uses gas chromatography with flame ionization detector as the official method for the quantification of THC in hemp varieties [7,8].

Sample preparation for the above mentioned instrumental analysis is carried out using traditional liquid-liquid extraction (LLE). The sample preparation varies depending on the type of product obtained for analysis. For example, for the baked cookies found to contain cannabinoids, the hexane-methanol and hexane acetonitrile extraction was found to be effective in extracting out the cannabinoids [9].

Materials and Methods

Sample Preparation (Liquid-Liquid Extraction)

The chocolate chip cookies were homogenised using a mortar and pestle. Two representative samples between 1.5 and 2 gr were taken for the liquid-liquid extraction process. For both representative samples, 6 mL of hexane and 3mL of acetonitrile was added. The samples were vortexed and centrifuged. The hexane phase was then removed and another 3 mL of hexane was added to the acetonitrile phase. The samples were again vortexed and centrifuged. The hexane phase was removed and the acetonitrile layer was evaporated to dryness. For both of the samples, (a) one of the samples was reconstituted in 0.5 mL hexane and run on a Thin Layer Chromatography plate (sample A) and (b) the other sample was reconstituted in 0.25 mL hexane and run on gas chromatography mass spectrometry (GC/MS) (sample B).

GC/MS Instrumentation

Agilent 6890N GC coupled with 5975B mass–selective detector with HP-5MS column (30 m × 0.25 mm × 0.25 μm); GC condition:

- Injection mode = split mode
- Injection volume = 1 μL
- Column flow rate = 1.0 mL/min, Carrier gas = helium
- Injector temperature = 270°C
- Oven temperature = 80°C (5 min)
- Temperature programme; initial temperature = 20°C/min to 150°C for 0 min; ramp to 280°C (10°C/min) for 5 minutes and ramp to 300°C (20°C/min) for 16 minutes

MSD condition:

- Electron energy = 1.2 kV
- Scan mode = 50-500 amu
- Solvent delay = 3 min

Thin Layer Chromatography

Thin layer chromatography (TLC) plates should be silica gel or equivalent and sufficient to resolve the three major cannabinoids. Sample A was spotted on the TLC plate with the cannabinoid standards. Mobile phase was petroleum ether:ether (80:20). The visualisation spray used in this method was Fast Blue BB salt. Approximately 50 mg of fast blue BB was dissolved in 20 mL of 0.1 N sodium hydroxide. This solution was freshly prepared. Results obtained from this analysis showed that the three cannabinoids migrated and developed in the following order;

- Top spot – Cannadiol (CBD)-orange
- Middle spot – Tetrahydrocannabinol (THC)-red
- Lower spot – Cannabinol (CBN)-purple

Results and Discussion

The three major cannabinoids were found to be present in the chocolate chip cookies which were sent for analysis. The TLC analysis clearly showed the presence of the three cannabinoids, THC, CBD and CBN, as shown in Figure 2. Liquid-liquid extraction using hexane-acetonitrile was successful in extracting the cannabinoids present in the cookies.

From the GC/MS analysis the three cannabinoids were also detected using the same liquid-liquid extraction method. The spectra of sample B and ion spectra for the cannabinoids are shown in Figure 3 and Figure 4, respectively.

Comparing the results of the two different analytical techniques, it was found that the THC peak in the GC/MS and the THC spot in TLC plate were strong compared to the other two cannabinoids, CBD and CBN. However, no quantitative comparison was done.

The cannabinoids are defined as “cannabis resin” in Section 2 of the Malaysian Dangerous Drugs Act (DDA) 1952.

Conclusion

This study demonstrated the usefulness of a simple liquid-liquid extraction method used in extracting out cannabinoids present in the cookies which were then identified using straightforward analytical techniques such as GC/MS and TLC analysis. In this case the methods were applied to a real case sample and proved to be accurate and sensitive.

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Figure 1: Close up of the cookies

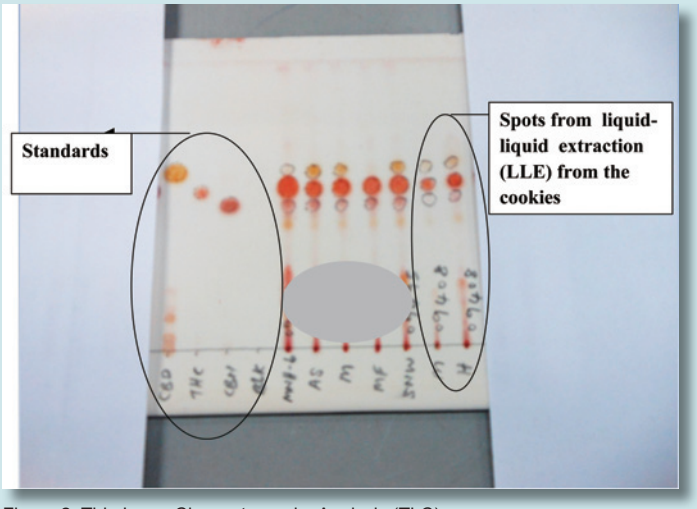

Figure 2: Thin Layer Chromatography Analysis (TLC)

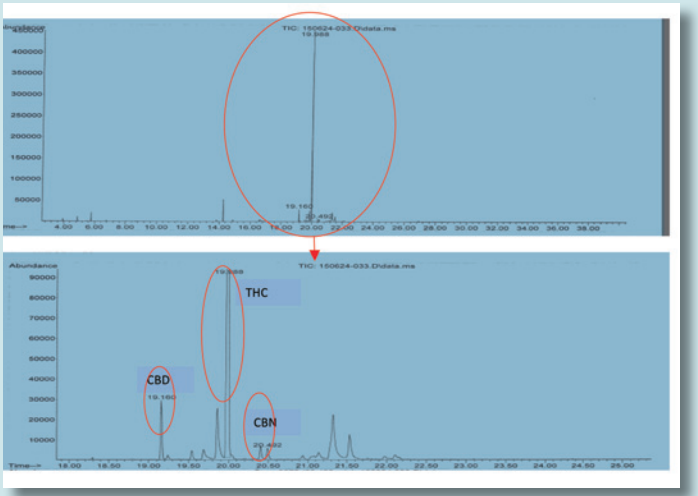

Figure 3: GC/MS spectra of Sample B

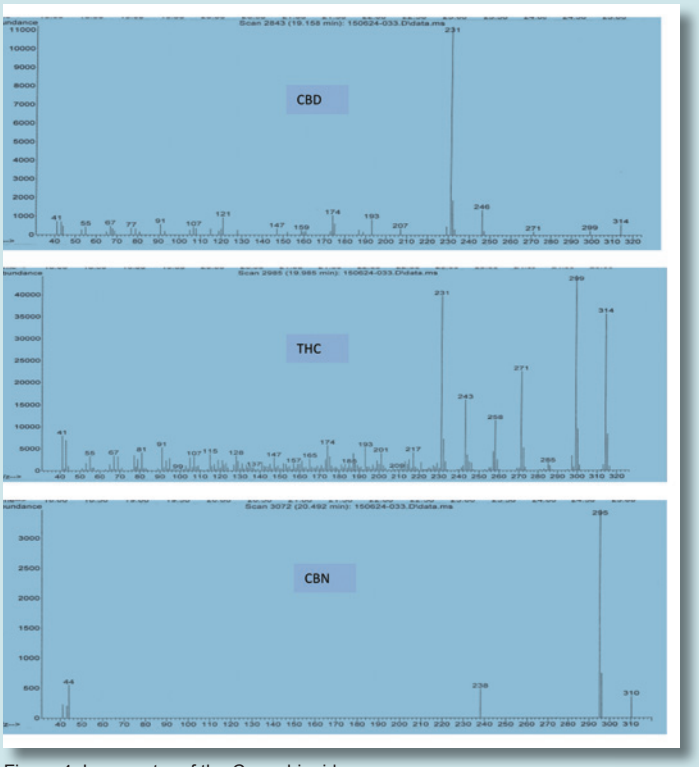

Figure 4: Ion spectra of the Cannabinoids

Identification of α-pyrrolidinovalerophenone in unknown jelly sample

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Abstract

α-pyrrolidinovalerophenone (PVP) was detected in a sample recently by the drug laboratory of the Institute of Forensic Science (IFS), Ministry of Public Security, P. R. China. The sample was in the form of a jelly, and it was delivered by the police to our laboratory. Using basic and acid extraction, the presence of α-pyrrolidinovalerophenone was identified and confirmed by GC-MS and NMR. This is the first report of the detection of this substance in Mainland China. More attention should be paid on pyrrolidinophenone derivatives, a new group of synthetic cathinones for new psychoactive substances in Asia.

Introduction

α-pyrrolidinovalerophenone (PVP) is a new designer drug and is a derivative of the synthetic cathinones. It is assumed to be taken orally, similar to other pyrrolidinophenone derivatives, which have been distributed among drug abusers in the form of tablets, capsules or powders. So far, little information is available about the dosage and the pharmacological and toxicological effects of these derivatives. However, they may be expected to be very similar to those of pyrovalerone (4-methyl-α-pyrrolidinovalerophenone) because of their close structural relationship to this drug. According to reports, pyrovalerone and several structural analogs, among them PVP, are inhibitors of dopamine, serotonin, and norepinephrine transporters[1,2].

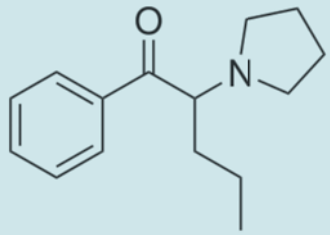


Figure 1. Structure of α-pyrrolidinovalerophenone

Definitive Identification by GC-MS and NMR

GC-MS

NaOH solution was added to approximately 1 g of the sample to obtain a pH value of greater than 9. The sample was extracted by liquid – liquid extraction with hexane. Next, hydrochloric acid was added to the aqueous solution to obtain a pH value of less than 4. NaOH solution was then added to obtain a pH value of greater than 9 and was extracted with hexane again. Two drops of hydrochloric acid was added into methanol to extract the desired product from the hexane layer. The residue was left to dry. Finally, the sample was dissolved in 1 mL of methanol and then subjected to GC-MS analysis. Figure 2 shows the appearance of the sample, which is a brown jelly-like substance.

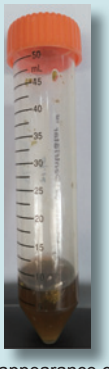


Figure 2. The appearance of the sample

Table 1. GC-MS (EI) analysis conditions

Instrument	Agilent GC-MS 7890/ 5975C
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
Ionization energy	70 eV
Injection volume	1 μL
Helium gas with flow	1.0 mL/min
Scan parameter	40-500 m/z

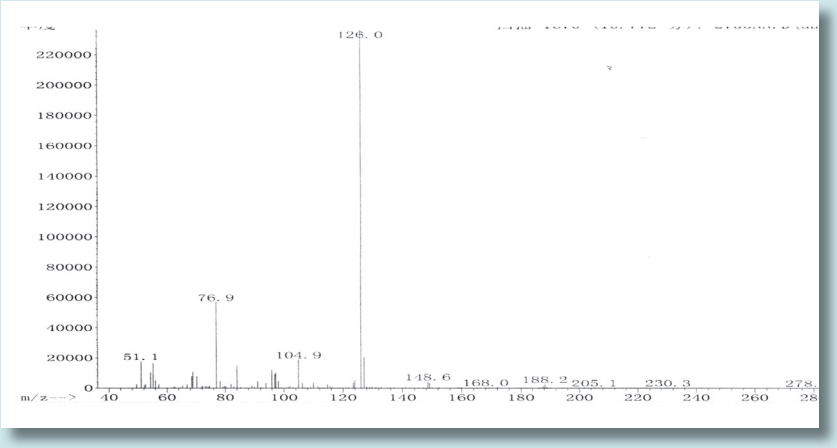


Figure 3. GC-MS spectrum of the substance.

α-pyrrolidinovalerophenone in the sample was identified by GC-MS (Figure 3) with fragmentation ions of 126, 77, 84, 105 and 188. Figure 4 shows proposed fragment ions which characterise PVP.

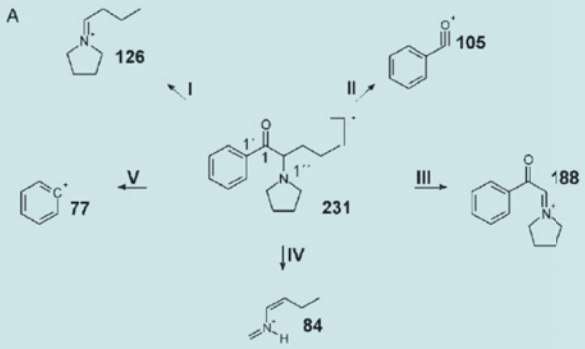
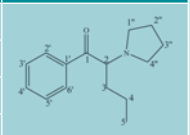


Figure 4. Proposed fragment ions characterising PVP

^{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 Methanol-d4. 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 2.

Table 2. Data of ^{1}H and ^{13}C NMR.

Position	^{13}C(ppm)	^{1}H(ppm)	Multiplicity	Structure Assignment
4’	136.3149	7.758	m	
3’,5’	130.4962	7.618	m	
2’,6’	130.0961	8.119	m	
1’	135.6336			
1	197.4122			
2	70.3536	5.473	dd	
1’’	56.2713	3.676;3.104	m;m	
2’’	24.2106	2.075~2.245	m	
3’’	24.1106	2.075~2.245	m	
4’’	52.2497	3.732;3.422	m;m	
3	33.5372	2.055	m	
4	18.4854	1.248	m	
5	14.1753	0.869	t	

Conclusion

This is the first time our laboratory has detected α-pyrrolidinovalerophenone (PVP) in a case sample. GCMS and NMR were used for identification of α-pyrrolidinovalerophenone hydrochloride salt. More attention should be paid to pyrrolidinophenone derivatives, a new group of synthetic cathinones for new psychoactive substance in Asia.

References:

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2 A. Servin, J. P. Fauquet, C. Jacquot, J. R. Rapin. Effects of pyrovalerone on peripheral noradrenergic mechanisms. Biochemical Pharmacology [J]. 1978 27:1693.

The Evidential Value of Coloured Staples in Forensic Examination

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Dr Alaric Koh Chin Wai, Ms Li Yihua, Mr Lim Thiam Bon
Health Sciences Authority, Singapore

Background

FCPL rarely receives submissions from law enforcement agencies requesting for the examination of staples. In the past, the examination of staples relied on the chemical analyses of the coating and the metal substrate of the staples. Recently, a case involving illicit drug packages sealed with blue coloured staples was seized by the Central Narcotics Bureau (CNB), Singapore, and submitted to the laboratory for examination to associate the coloured staples found on the drug packages to the unused staples found at the suspect's house. The use of tool marks on the surface of the staples to associate to the stapler had previously been reported [1, 2]. However, in this particular case, the surfaces of the staples were examined for tool marks that might have originated from a stapler but none was found.

In order to increase the conclusion levels achieved for the associations, there was a need for the laboratory to perform an in-depth study of the variations of the chemical composition of the staples (coatings and metal substrate), and the presence of manufacturing marks found on the staples.

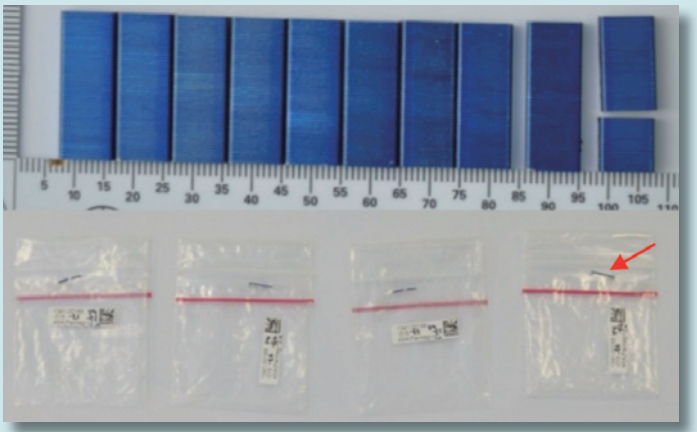

Figure 1: (Top) Unused blue coloured staples seized from suspect's house. (Bottom) Questioned blue coloured staples stapled on zip-lock bags.

Objective

The objective of the study was to examine the variations between different brands of blue-coating staples and to determine the discriminating powers of several characteristics of staples that might be of forensic value. The examined characteristics were:

(i) physical characteristics (width, weight and thickness)
(ii) chemical compositions of the coloured coating
(iii) elemental compositions of the metal substrate
(iv) manufacturing marks on staples.

Methodology

A total of 42 boxes of blue coloured staples (consisting of 14 brands) were purchased from shops in Singapore and Malaysia. Brief details of the 42 boxes of staples are listed in Table 1.

Table 1: Details of 42 boxes of blue staples

Table 1: Details of 42 boxes of blue staples

Brand	Identifier	No. of boxes	Country	Brand	Identifier	No. of boxes	Country
AOS Grapas	AOS	1	Singapore	Pop Bazic	PPB	2	Malaysia
DAISO	DSO	9	Singapore, Malaysia	Pop Zen	ZEN	2	Singapore
DAISO Japan	DSJ	2	Singapore	Popular	POP	4	Singapore
Decamax	DMX	1	Malaysia	Sanko Star	SKS	2	Singapore
Deli	DEL	4	Singapore	SureMark	SMK	4	Singapore
Etona	ETN	1	Singapore	UMOE	UMO	2	Malaysia
kangaro®	KGR	4	Singapore	Unicorn	UNC	4	Malaysia

The average width, weight and thickness of each staple were measured using a Mitutoyo 342-361 Digimatic Point Micrometer, a Sartorius MSA3245-100-DA Analytical Balance and a VWR 12777-830 Digital Caliper 8", respectively. Fourier transform infrared spectroscopy (FT-IR) and Raman spectroscopy of the blue coatings on the staples were performed using a Thermo Nicolet 6700 and Continuum FT-IR microscope and a Thermo DXR Raman microscope (780 nm laser), respectively. The elemental compositions of the blue coatings and the metal substrate of the staples were obtained using a JEOL JSM-7610F field emission scanning electron microscope equipped with an Oxford Instruments X-MaxN silicon drift detector (SEM/EDX). The manufacturing marks of the staples were examined using Leica FSC comparison microscopes.

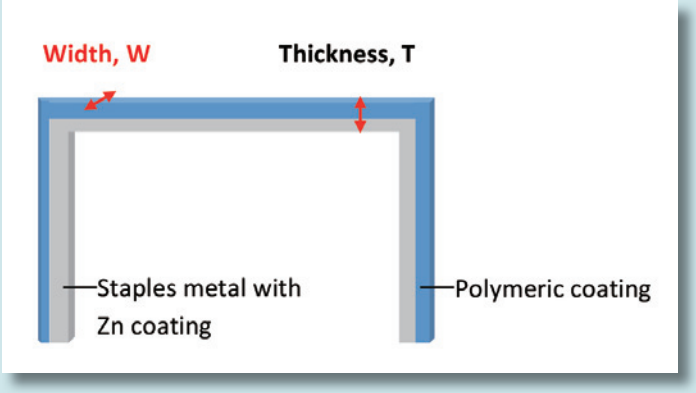

Figure 2: Width and thickness measurement

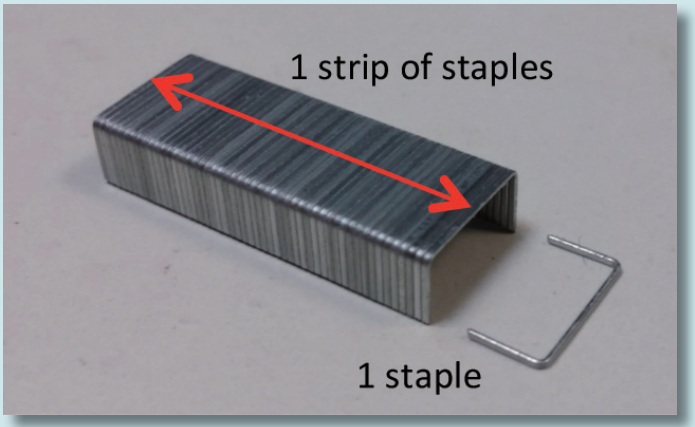

Figure 3: A strip of staples

In order to objectively assess the potential of the different characteristics at maximising long-term average discrimination, their "discriminating powers" [3] (DPs) could be compared:

$$Discriminating\ Power = 1 - \frac{2M}{N(N-1)}$$

where M denotes the number of non-discriminated pairs in the assessed characteristic and N denotes the total number of samples.

For physical characteristics, the mean ± 3SD was calculated for each box of staples. For the purpose of this study, the staple strips from two boxes were considered to be non-discriminated when their mean ± 3SDs overlap. The DPs for FT-IR, SEM/EDX and Raman analyses were calculated based on the results obtained independently from each brand of staples.

Results and Discussion

Manufacturing marks

One box from each of the 14 brands was selected for manufacturing marks examination. The manufacturing marks were generally found on the underside of the metal strips. These marks could possibly be imparted onto the staple during the bending of wires to form the characteristic U-shape of staple strips. In this study, all 14 brands of staples examined could be differentiated based on manufacturing marks. Within each strip, the marks on the individual staple were found at the same relative location and bear similar marks. Twelve boxes of staples were found to have similar marks among the staples strips in the same box. The staple strips within some of these boxes could be further divided into sub-groups based on the variations in sub-class manufacturing marks. The remaining two boxes of staples were each found to have two groups of manufacturing marks. Our findings, in terms of the location of manufacturing marks observed and the usefulness of these marks in forming associations, are consistent to those reported by Majamaa for a case in Finland [4].

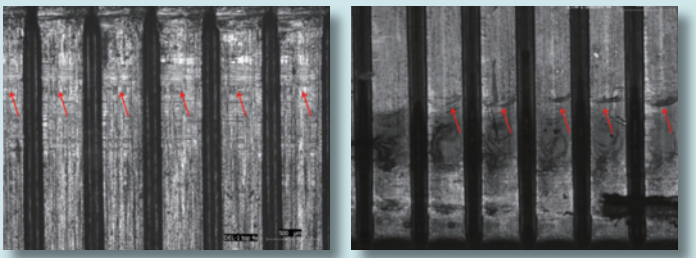

Figure 4: (Left) Manufacturing marks on the underside of coloured staples strips of DEL brand. (Right) Manufacturing marks on the underside of coloured staples strips of ETN brand.

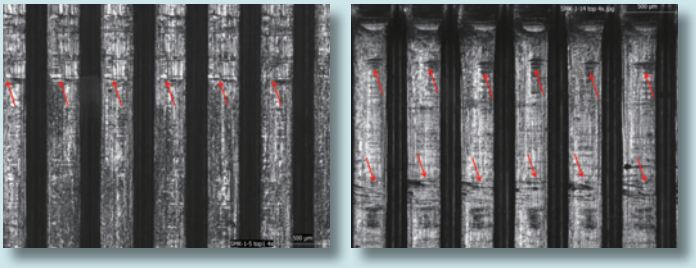

Figure 5: Manufacturing marks on the underside of different coloured staples strips in a box of SMK brand.

Physical characteristics

Among the three physical characteristics measured (width, weight, and thickness), weight measurement had the highest discriminating power. Five of the 14 brands of coloured staples could be differentiated based on Principle Component Analysis (PCA) using the Unscrambler X multivariate analysis software version 10.1 [Figure 6].

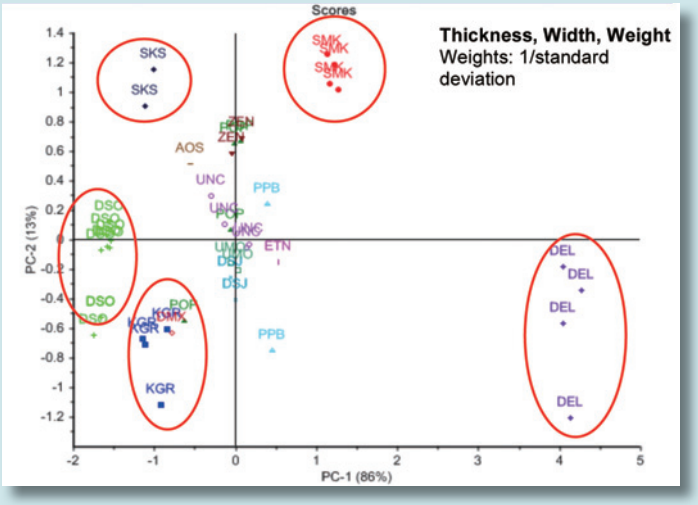

Figure 6: DEL, DSO, SKS, SMK and KGR brand could be differentiated based on a combination of width, weight and thickness characteristics

Chemical and elemental compositions of the staple blue coating

Eleven types of profiles of the blue coatings were observed following FT-IR analysis [Figure 7]. Although the spectra of Type 1 to Type 4 and Type 11 had very similar general profiles, they could be differentiated based on small variations at the 1600-1500cm-1 and 700-650cm-1 regions, as shown in Figure 8.

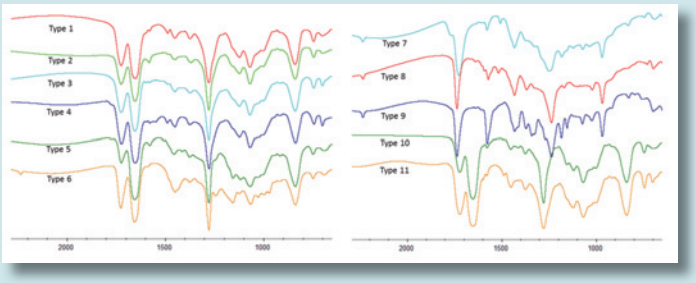

Figure 7: FT-IR fingerprint region of the blue coating spectra. 11 types of profiles were obtained.

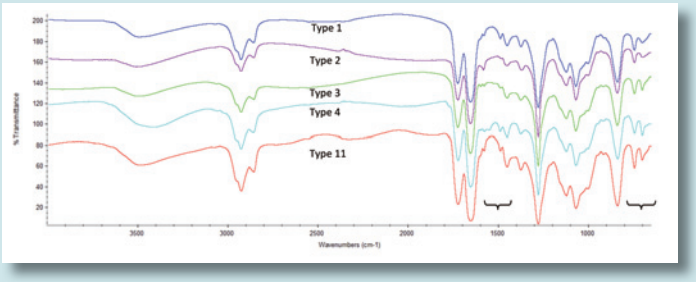

Figure 8: Small variations at 1600-1500cm^{-1} and 700-650cm^{-1} regions of Type 1, 2, 3, 4 and 11 FT-IR profiles.

The classification of the 42 boxes of staples based on FT-IR analysis of the blue coatings is shown in Table 2. It was observed that some staples (AOS, KGR, ETN and DSO brands) could be differentiated from those of the other brands based solely on this technique. Unsurprisingly, there were also different brands sharing the same profile. For example, eight of the 14 brands were found to have at least one box of staples with "Type 1" blue coatings. Within the same brand, more than one type of profile might also be observed. For example, three boxes of POP brand were of "Type 1" profile and one box was of "Type 10" profile, while two boxes of KGR brand were of "Type 5" profile and another 2 boxes were of "Type 6" profile.

Table 2: FT-IR profile types of 42 boxes of blue staples

FT-IR	Brands		FT-IR	Brands
Type 1	POP (3), ZEN, DEL, DSJ, UNC (2), UMO, PPB, DMX		Type 7	ETN
Type 2	AOS		Type 8	DSO (3)
Type 3	SMK (1), SKS		Type 9	DSO (6)
Type 4	SMK (3)		Type 10	POP (1)
Type 5	KGR (2)		Type 11	UNC (2)
Type 6	KGR (2)			

For Raman analysis, only staples in some boxes of KGR brand gave Raman signals. No profile was observed for other brands. The Raman spectroscopic technique was not found to be useful in this study.

SEM/EDX analysis was found to be more promising than Raman spectroscopy, with six types of elemental composition profiles found [Table 3 and Figure 9]. SEM/EDX could not differentiate between the brands with "Type 1" to "Type 3" and "Type 11" FT-IR profiles, and brands with "Type 7" to "Type 9" FT-IR profiles. The other brands of "Type 5", "Type 6" and "Type 10" FT-IR profiles could be differentiated by SEM/EDX.

Table 3: SEM/EDX profile types of 42 boxes of blue staples. The underlined are the brands that could not be differentiated by SEM/EDX analysis.

SEM	Brands
Type 1	SMK (1), POP (3), SKS, ZEN, DEL, AOS, DSJ, UNC, UMO, PPB, DMX
Type 2	SMK (3)
Type 3	KGR (2)
Type 4	KGR (2)
Type 5	ETN, DSO (9)
Type 6	POP (1)

In summary, the blue coatings of the staple strips in the

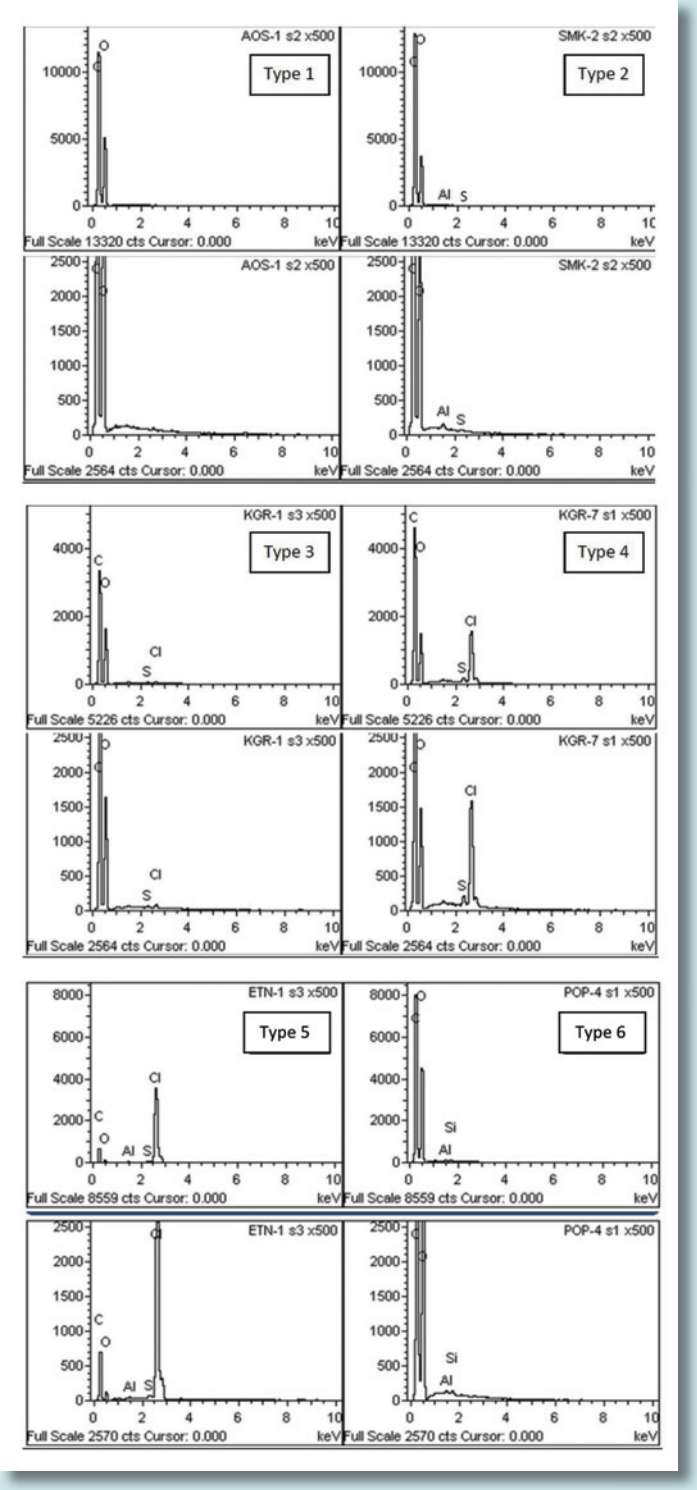

Figure 9: 6 types of SEM/EDX profiles. (Top) Full scale spectrum (Bottom) Zoom-in scale spectrum

42 boxes could be categorised into eleven types of profiles based on FT-IR spectroscopy, six types of profiles based on their elemental compositions, while for Raman spectroscopy, only staples of the "Type 6" FT-IR had a Raman profile. Hence, among these three techniques used for examination of blue coating on the staples, FT-IR spectroscopy was found to the most discriminating in our study.

Determination of discriminating power

Table 4: Discriminating power of each physical and chemical characteristic of the coloured staples and the overall discriminating power of combined physical characteristics and chemical compositions.

Physical characteristics	DP		Chemical compositions	DP
Width	52.6%		FT-IR	78.4%
Weight	84.4%		SEM/EDX	62.1%
Thickness	59.0%		Raman	9.3%
Overall	89.3%		Overall	78.5%
Overall DP physical characteristics and FT-IR only: 94.0%				
Overall DP physical characteristics and FT-IR, SEM/EDX and Raman: 94.0%				

An overall discriminating power of 94% was obtained when all three physical characteristics of the coloured staples and FT-IR analysis of the staples coatings were considered. No difference was observed in the overall discriminating power when the physical characteristics of the coloured staples and all chemical compositions (FT-IR, SEM/EDX and Raman) of the staple coatings were considered together. SEM/EDX and Raman spectroscopy were not useful in contributing to the overall discriminating power in this study.

Elemental composition of the staples

The elemental compositions of the metal strips of all 42 boxes of staples were found to contain major elements such as zinc and iron. This was consistent with them being made from galvanised steel [5]. It was found that the amount of zinc and iron detected was inconsistent between the staple strips within a box. This could possibly be due to uneven application of the zinc coating during galvanisation, damage to the zinc coating during the manufacturing process and exposure to environmental effects such as corrosion during use. Hence, the relative amount of major elements such as zinc and iron could not be used to differentiate staples in this study. A more sensitive instrument (such as inductively coupled plasma mass spectrometry, ICP-MS) is probably required to detect the trace elements in the metal strips in order to be able differentiate the staples.

Conclusion

Based on the examination of manufacturing marks on the underside of the staples, all 14 brands of coloured staples could be differentiated. Hence, microscopic examination of manufacturing marks was an excellent technique to associate questioned staples (used, single staple) to known (unused strips of staples) staple samples (both with and without coloured coatings). Based on physical characteristics (width, weight, thickness) and chemical compositions (mainly FT-IR spectroscopy) of the coloured staples, an overall discriminating power of 94% was obtained. A combination of these techniques was therefore of good evidential value in associating questioned and known source of staples, especially when marks on staples are insufficient or lacking.

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A survey of plant pollens in mountain soils

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Introduction

The pollen complexes contained in soils show the characteristics of the ecological habitat. Thus, the soils can be distinguished according to the specific distribution of pollen complexes in them.[1,2] However, till date there is no thorough and detailed research about the distribution of plant pollen complexes in soils. The pollen complexes in mountain soils were studied in this paper.

Materials and Methods

Surface soil samples were extracted from Nanshan Park in Changle, Mount Long in Fuqing and Forest Park in Fuzhou respectively. Five grams of each soil sample was collected and extracted five times. Soil samples were soaked in ethyl acetate and 30% hydrochloric acid solution for 10 minutes respectively, and the plant pollens in the soils were collected after filtering out the mixed supernatants. Morphological characteristics of pollens were analysed by SEM.

Results and discussion

1. Pollen complexes in soils from different mountains

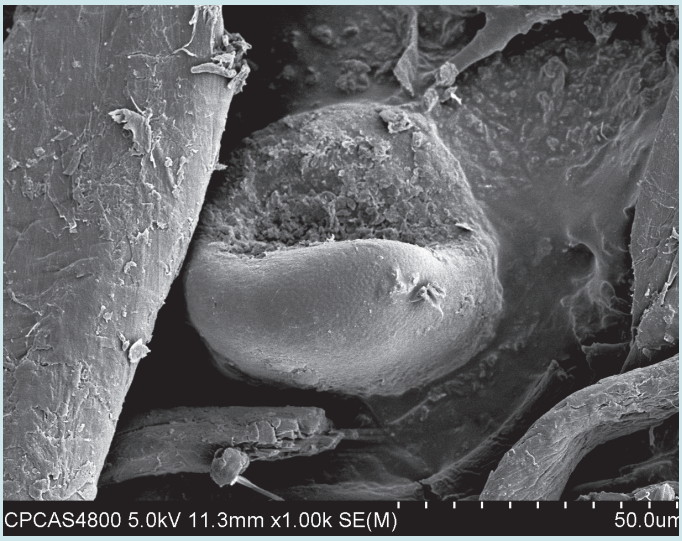


Figure 1: SEM photographs of pollens in soil from Nanshan Park (Larch).

The morphological characteristics of the plant pollen in the surface soil from the mountain foothills of Nanshan Park in Changle are shown in Figure 1. The plant pollen exhibits a spherical shape (the diameter is 53.4 μm) with horseshoe hollowness and verrucate sculpture on the exine, and it is confirmed to be the Larch pollen after comparison with the standard pollen flora[3].

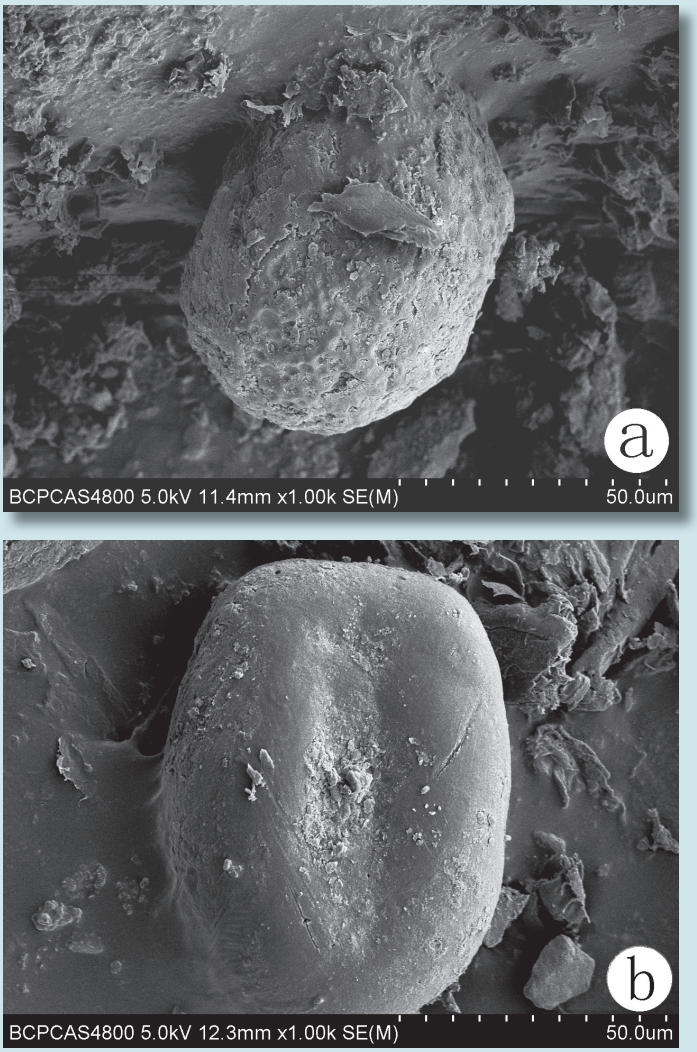


Figure 2: SEM photographs of pollens in soil from Mount Long Park (a. David Elm; b. David Peach).

The morphological characteristics of plant pollen in the surface soil from the mountain foothills of Mount Long in Fuqing are shown in Figure 2. The plant pollen shown in Figure 2(a) is almost spherical (the diameter is 62.5 μm), and the extine is dotted with verrucate and has a granulated sculpture. It is confirmed to be the David Elm pollen after comparison. The plant pollen shown in Figure 2(b) exhibits a spheroid shape (the size is 64.7 μm × 84.4 μm) with a striate sculpture on the extine, and it is confirmed to be the David Peach pollen.

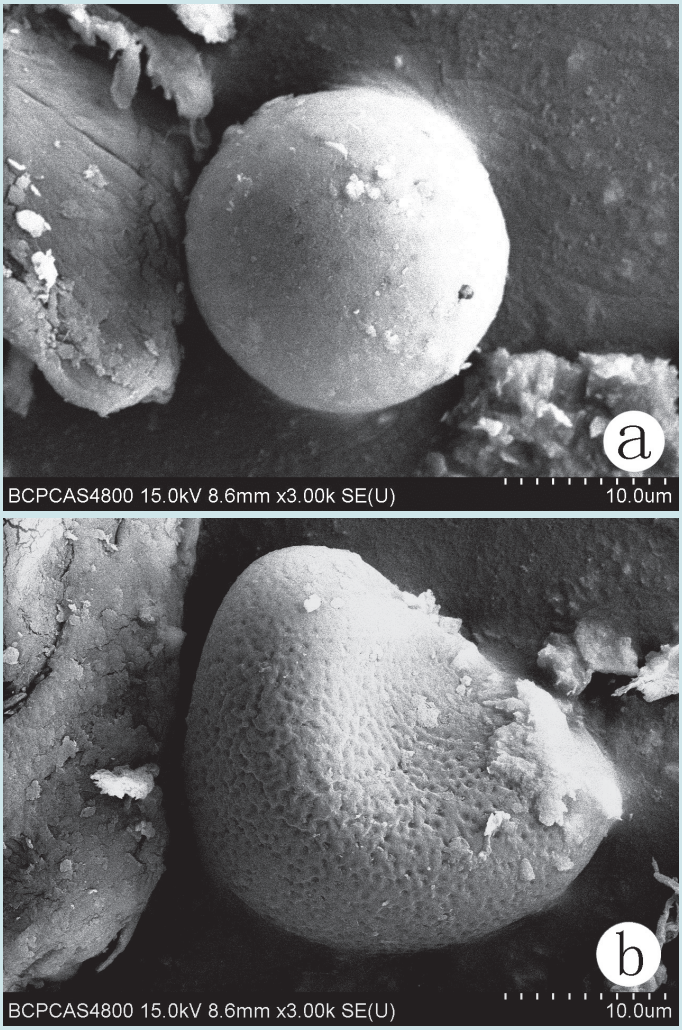


Figure 3: SEM photographs of pollens in soil from Forest Park (a. Cypress; b. Pitard Camellia).

The morphological characteristics of plant pollen in the surface soil from the mountain foothills of Forest Park in Fuzhou are shown in Figure 3. The plant pollen shown in Figure 3(a) exhibits a spherical shape (the diameter is 20.2 μm) with granulated sculpture on the extine, and it is confirmed to be the Cypress pollen. The plant pollen shown in Figure 3(b) exhibits an oblate spheroid shape (the size is 28.1 μm × 21.2 μm) with a rugulate sculpture on the extine, and it is confirmed to be the Pitard Camellia pollen.

2. Pollen complexes in soils from different locations of the Forest park mountain

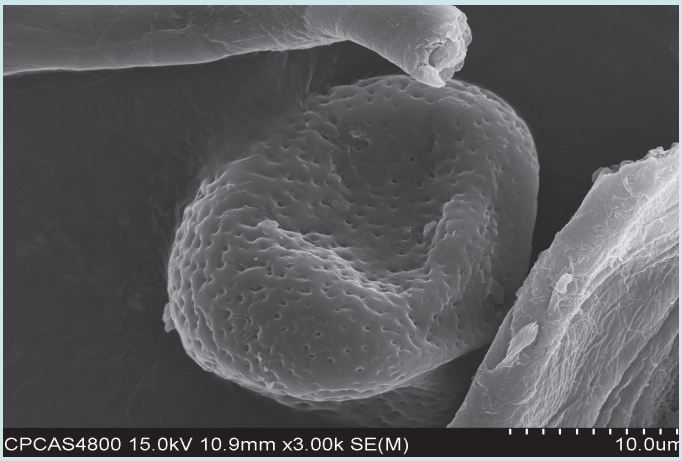


Figure 4: SEM photographs of pollens in soil from the mountainside of Forest Park (Beautyberry).

The morphological characteristics of plant pollen in the surface soil from the mountainside of Forest park are shown in Figure 4. The plant pollen exhibits an oblate spheroid shape (the size is 20.0 μm × 24.7 μm) with a reticulate sculpture on the extine, and the lumina exhibits a foveolate shape. It is confirmed to be the Beautyberry pollen.

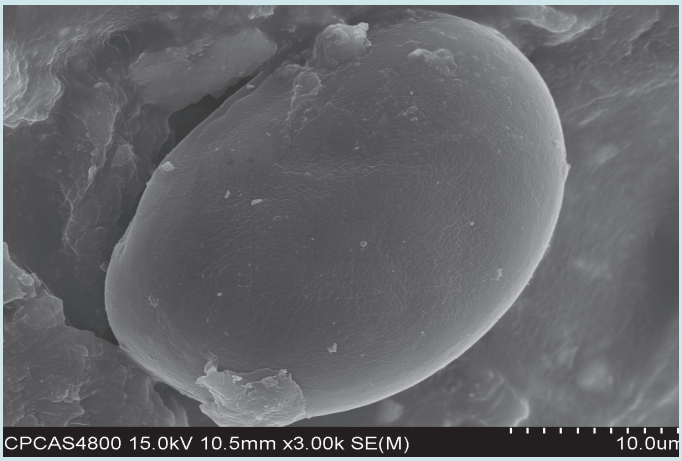
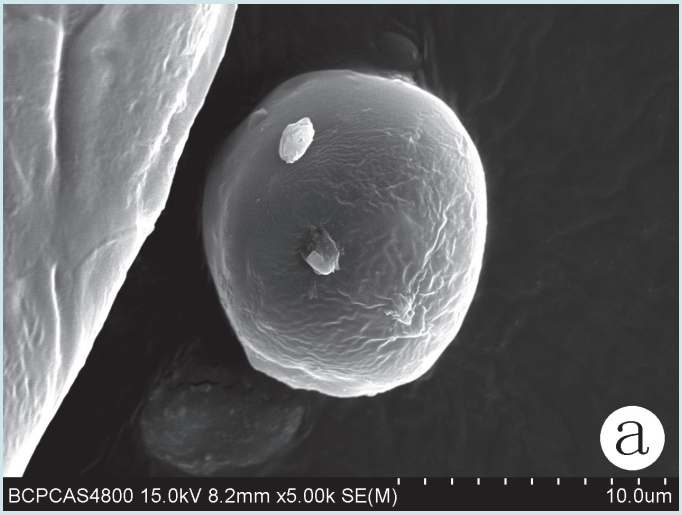


Figure 5: SEM photographs of pollen in soil from the mountaintop of Forest Park (Chinese Arborvitae).

The morphological characteristics of plant pollen in the surface soil from the mountaintop of Forest Park are shown in Figure 5. The plant pollen exhibits an oblate spheroid shape (the size is 22.2 μm × 30.9 μm) with a verrucate and granulated sculpture on the extine, and no aperture exists. It is confirmed to be the Chinese Arborvitae pollen.

As shown in Figure 3, the pollen complex in the surface soil from the mountain foothills of Forest Park comprises the Cypress pollen and the Pitard Camellia pollen.

3. Pollen complexes in soils from different seasons of the Forest park mountain



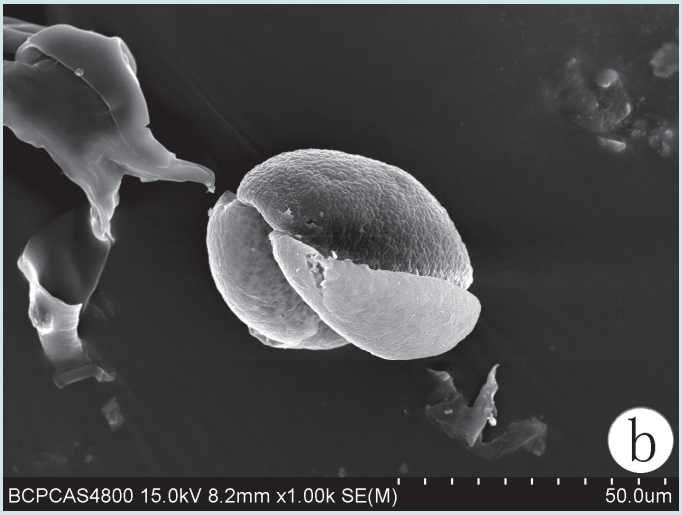
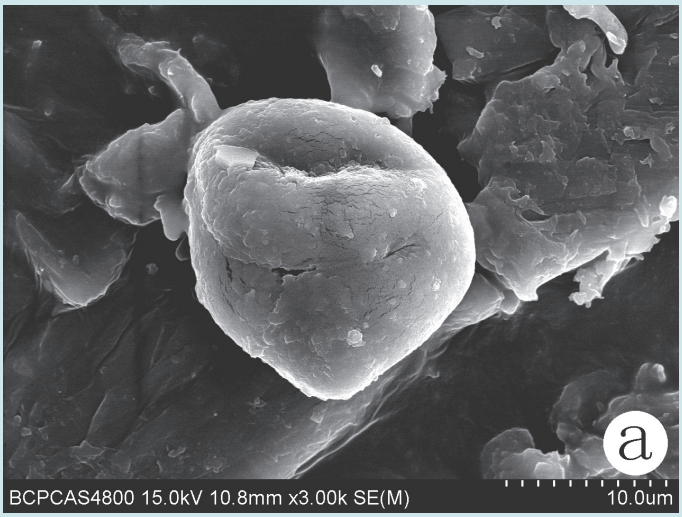


Figure 6: SEM photographs of pollens in soil from Forest Park in April (a. Chinese Banyan; b. Pine).

The morphological characteristics of plant pollen in the surface soil from the mountain foothills of Forest Park in April (spring) are shown in Figure 6. The plant pollen shown in Figure 6(a) exhibits a spheroid shape (the size is 10.6 μm × 12.1 μm) with a rugulate sculpture on the extine, and it is confirmed to be the Chinese Banyan pollen. The plant pollen shown in Figure 6(b) is formed by a body (the size is 19.8 μm × 47.3 μm) and two spheroid airsacs, and there is a verrucate sculpture on the extine. It is confirmed to be the Pine pollen.



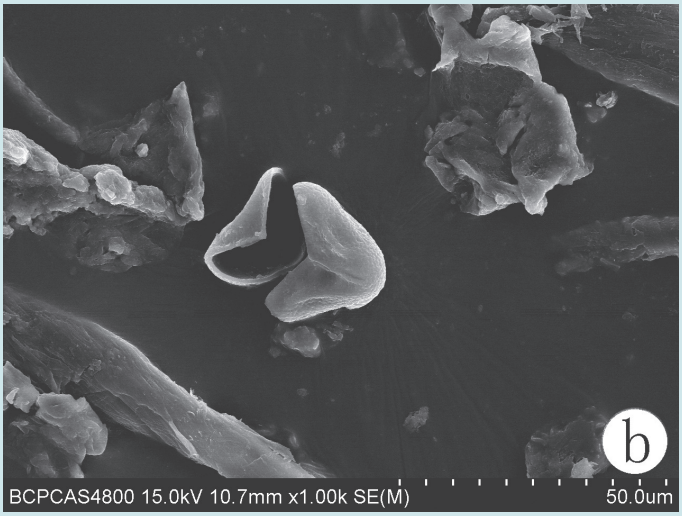


Figure 7: SEM photographs of pollens in soil from Forest Park in August (a. Chinese Weeping Cypress; b. Chu-lan).

The morphological characteristics of plant pollen in the surface soil from the mountain foothills of Forest park in August (summer) are shown in Figure 7. The plant pollen shown in Figure 7(a) exhibits a spheroid shape (the size is 18.2 μm × 18.7 μm) with hollowness and granulated sculpture on the extine, and it is confirmed to be the Chinese Weeping Cypress pollen. The plant pollen shown in Figure 7(b) exhibits an oblate spheroid shape (the size is 26.3 μm × 29.9 μm) with foveolate sculpture on the extine, and it is confirmed to be the Chu-lan Tree pollen.

The morphological characteristics of plant pollens in the surface soil from the mountain foothills of Forest Park in December (winter) are shown in Figure 3, and the pollen complex comprises the Cypress pollen and the Pitard Camellia pollen.

Conclusion

Plant growth is regional, and plant pollen can be dispersed by wind over different distances. Thus, different mountain soils can be distinguished according to pollen complexes found in them. The mountain soils from Forest Park in Fuzhou, Nanshan park in Changle, and Mount Long mountain in Fuqing can be distinguished according to the specific distribution of pollen complexes found in them, as listed in Table 1. Also, because of the vertical distribution of mountain plants and their seasonal characteristics of blossoming and reproduction, pollen complexes found in Forest Park soils may vary according to different mountain locations and seasons of the year. Soils from different mountain locations and seasons can be distinguished according to the specific distribution of pollen complexes found in them, as listed in Table 2.

Table 1: Pollen complexes found in different mountain soils.

Mountains	Sources of pollen complexes
Forest Park	Cypress, Pitard Camellia
Nanshan park	Larch
Mount Long mountain	David Elm, David Peach

Table 2: Pollen complexes found in Forest Park soils from different mountain locations and different seasons.

Locations and seasons	Sources of pollen complexes
Mountaintop	Chinese Arborvitae
Mountainside	Beautyberry
Mountain foothills	Cypress, Pitard Camellia
April (spring)	Chinese Banyan, Pine
August (summer)	Chinese Weeping Cypress, Chu-lan
December (winter)	Cypress, Pitard Camellia

Acknowledgements

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Upcoming Events

Date	Venue
7 Aug – 13 Aug 2016	International Association for Identification (IAI) 101st International Educational Conference. Cincinnati, Ohio, USA.
16 Aug – 19 Aug 2016	Asian Forensic Sciences Network (AFSN) 8th Annual Meeting and Symposium. Bangkok, Thailand
20 Aug – 25 Aug 2016	American Society of Questioned Document Examiners (ASQDE) 74th Annual General Meeting. Pensacola Beach, Florida, USA.
28 Aug – 1 Sep 2016	The International Association of Forensic Toxicologists (TIAFT) 54th Annual Meeting. Brisbane, Queensland, Australia.
26 Sep – 29 Sep 2016	27th International Symposium on Human Identification (ISHI). Minneapolis, Minnesota, USA.
16 Oct – 21 Oct 2016	Society of Forensic Toxicologists (SOFT) Annual Meeting.