出國報告（出國類別：國外會議）

參加 2018 年第 70 屆美國鑑識科學學會年度會議報告書

服務機關：法務部法醫研究所
姓名職稱：徐竹君助理研究員
派赴國家：美國
出國期間：107 年 2 月 18 日至 107 年 2 月 25 日
報告日期：107 年 4 月 30 日

摘要

本所派員參加第 70 屆美國鑑識科學學會（American Academy of Forensic Sciences, AAFS）年度會議，本屆年會會議期間為 107 年 2 月 19 日至 24 日，於美國西雅圖華盛頓州會議中心舉行，本次大會主題為”Science Matters”(科學很重要)。美國鑑識科學學會成立至今已 70 年，有超過 6700 個會員，本研討會議主題大致可再細分成 11 個領域，涵括人類學（Anthropology）、犯罪學（Criminalistics）、數位及多媒體科學（Digital & Multimedia Sciences）、工程學（Engineering Sciences）、一般法醫刑事（General）、法律裁判學（Jurisprudence）、法醫齒科學（Odontology）、病理/生物學（Pathology/Biology）、精神及行為科學（Psychiatry & Behavioral Science）、問題文書（Questioned Documents）及毒物學（Toxicology）等。參加本次年會有分別來自美國、加拿大等 70 餘國之實務鑑識人員、教學或研究學者。該學會廣邀各領域知名專家學者發表約 7 場專題演講（breakfast seminars and luncheon seminars），並開設約 24 場不同領域之專題討論（workshops），參加演講及專題討論並可獲得美國刑事犯罪協會核可之繼續教育學分，提供鑑識領域工作者進修的機會。許多學術、產業及實務單位亦透過年會進行最新科技技術交流，除了於年會中展示研究成果或進行口頭演說，廠商攤位也展示許多先進技術商品並與參加人員互動，使整個年會更為熱絡。

本所亦利用此次機會，於年會中發表研究論文 1 篇：「結合微流道晶片及差異萃取法提昇精液斑混合檢體檢測技術之研究」（Enhance the Power of Discrimination of Semen Identification by Combination of Microfluidic Chips and Erase Kits），此研究有效檢出證物含有男性精子細胞存在，並將結果與傳統差異萃取法相互比較，可以改善精斑鑑定的鑑別能力。

感謝法務部每年支持本所參與此項國際會議，參與國際會議除了可拓展視野以及對多元鑑識領域的認識更深入之外，更可提升本國於國際上之能見度。

目次

目的

美國鑑識科學學會（American Academy of Forensic Sciences）成立於 1948 年，總部位於科羅拉多泉(Colorado Springs, Colorado State)。每年 2 月都會在美國不同城市舉辦年度會議，每次會議約聚集了世界上大約 5,000 位知名學者，除了發表他們各自專業領域的最新發現，也傳遞各領域的當期資訊及對鑑識結果的通用解讀。

年會中每一篇研究報告及每一場演講皆為各自領域的專家經年累月研究成果。藉由參加年度會議可汲取這些寶貴的知識及經驗，了解國際上鑑識研究最新發展並與國外專家學者交流；此外，也可作為與會人員之在職訓練，溫故知新。因此，本所每年編列預算派員參與年會，旨在於提升本所鑑識能力及鑑驗技術。而為了與國際交流互動並提升國際能見度，本所早於 2017 年即投稿該學會，經該學會評審團認可後，將研究成果發表於 2018 年第 70 屆年會中。

本所於年會中發表之研究係比較傳統差異萃取法及結合微流道/核酸酶改良式差異萃取法，以此兩種方法分離混合溶液中上皮細胞及精子細胞 DNA，比較兩種方法之優劣。本研究先以口腔上皮細胞及精子細胞以不同比例混合，放置於室溫下 1 至 14 天不等時間，模擬新鮮及陳舊陰道棉棒檢體，以體染色體上短片段重複序列(Short tandem repeat, STR)以及 Y 染色體上短片段重複序列(Y-STR)之檢出率，評估傳統方法及微流道/核酸酶改良式差異萃取法。不僅如此，本研究更應用於法醫實際案例，效果優異。本研究從提出構想、實驗設計、製作微流道晶片及後續方法評估耗時數年，最終有了具體研究成果。在此次會議展出時，亦有幾位鑑識領域研究者現場熱烈討論本研究結果。更有來自加拿大的團隊於會議結束後向本所索取研究內容，充分發揮本次出國交流效果。

此次參加國際會議，除希望能於國際會議中得到相關的寶貴意見及回饋，更期待透過與國際上經驗豐富之專家學者進行交流，使本所研究能獲得啟發與更加精闢深入，對於成果之應用能更多元廣泛。

過程

107 年 02 月 18 日 抵達美國西雅圖

107 年 02 月 19 日 於會場辦理報到手續

參加「Proposed Revisions to the Federal Bureau of Investigation(FBI) Quality Assurance Standards-DNA」及「Moving from the Combined Probability of Inclusion (CPI) to Probabilistic Genotyping for DNA mixture Interpretation」專題研討會（workshop）

107 年 02 月 20 日 參加「An Introduction to Lean Fundamentals and Six Sigma Operational Improvement」專題研討會（workshop）

107 年 02 月 21 日 研究成果海報展示、研究成果口頭報告、專家演講、新穎試劑耗材、儀器及相關鑑識書籍展覽

107 年 02 月 22 日 發表本所研究成果「Enhance the Power of Discrimination of Semen Identification by Combination of Microfluidic Chips and Erase Kits」

研究成果海報展示、研究成果口頭報告、專家演講、新穎試劑耗材、儀器及相關鑑識書籍展覽

107 年 02 月 23 日 研究成果海報展示、研究成果口頭報告、專家演講、新穎試劑耗材、儀器及相關鑑識書籍展覽

107 年 02 月 24 日 研究成果海報展示、研究成果口頭報告、專家演講、新穎試劑耗材、儀器及相關鑑識書籍展覽

107 年 02 月 25 日 搭機返國

研討會專題內容

一、 專題研討會議題：「Proposed Revisions to the Federal Bureau of Investigation(FBI) Quality Assurance Standards-DNA」

本研討會目地為提案修改 FBI DNA 實驗室品質保證標準文件。DNA 鑑識的品質控管步驟最早在 1988 年發表於 Crime Laboratory Digest 期刊上，後來經過多次修正，於 1998 年始有初稿，2000 年經 FBI 審核後發行第一版。2000 年至今已經過兩次的修訂，本次提案針對 FBI DNA 實驗室品質保證標準文件進行修正、新增、刪除、澄清與調整條文順序，使此標準文件更符合實驗室需要以及更符合邏輯性。

FBI DNA 實驗室品質保證標準文件內部條文涵括：適用範圍、名詞定義、案例重審、組織管理、人員資格、教育訓練、工作能力試驗、對過去數據的重新解讀、設施及證據保存、方法確效、分析步驟、數據結果比較及結論、設備、報告、外部能力試驗、錯誤更正政策、內部及外部稽核、人員繼續教育、外包所有權等。

主要修訂幅度較大者為新增教育訓練相關標準，針對人員分別進行新進人員訓練、能力試驗方式、能力評估及在職教育等。刪除了原有實驗室操作汙染性或化學性物質之安全衛生等章節。

FBI DNA 實驗室品質保證標準文件與 TAF 認證標準所界定之大方向相仿，惟 FBI DNA 實驗室品質保證標準文件針對 DNA 鑑驗實驗室，因此規範較 TAF 一般校正實驗室使用的 17025 較為具體容易了解，認證之 DNA 實驗室可參考此規範及 17025 條文以作為對照。

本研討會一開始先請學員們針對成員背景進行線上調查，接著隨著課程，再進行不同問題的線上投票。此研討會主要參加者為美國來自各地方政府 DNA 鑑識實驗室成員，約莫一半參加者來自 20 人以上實驗室，其他參加者多半來自 10 人以上實驗室，實驗室 10 人以下者為非常少數。比較美國地方

政府 DNA 鑑識實驗室人數，本所 DNA 實驗室成員人數相對較少，正式編制人員含組長僅 5 名，足見本所人力之困窘。又國外各項鑑識人員針對不同鑑驗實驗，訓練期皆有數個月之久，針對不同實驗室在職訓練要求的內容亦不相同，培養專業領域鑑驗人員耗費。法醫鑑定具有證據效果，鑑驗過程必須非常嚴謹及小心，然而本所人力不足，又需撥出人力從事研究、行政及認證業務等工作，處境實為艱難。

二、專題研討會議題：「Moving from the Combined Probability of Inclusion (CPI) to Probabilistic Genotyping for DNA mixture Interpretation」專題研討會（workshop）

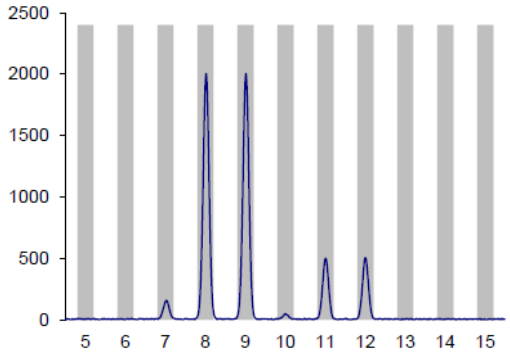
混合型 DNA 實驗數據判讀一直是鑑識領域相當困難的部分，一般現代鑑識科學常用累績親子指數(CPI, 或 Random man not excluded)、隨機相符頻率(Random match probability)或似然比(Likelihood Ratio)進行計算。

針對鑑別混合型別 DNA，1998 年 Clayton 發表了一個鑑定流程。步驟 1：先判斷實驗數據是否顯示為混合型；步驟 2：辨別真實型別與偽型別；步驟 3：定義造成型別的來源數量(人數)；步驟 4：定義混合 DNA 的混合比例；步驟 5：評估各種基因型組合的可能；步驟 6：與參考型別做比較；步驟 7：做混合型統計計算。

犯案人別或親緣計算，多是用似然比(Likelihood ratio, LR)來表示在特定情況下產生某特定結果機率以及非特定情況下產生某特定結果機率的機會比值，表示發生這個特定結果的證據能力。跟據貝氏定理，除了似然比 LR，還要考慮事前機率(prior odds)及事後機率(posterior odds)。事前機率(prior odds)表示發生特定情況的可能性，與其他因素完全獨立。事後機率(posterior odds)表示在特定情況成立下，我們會得到特定結果的機率。

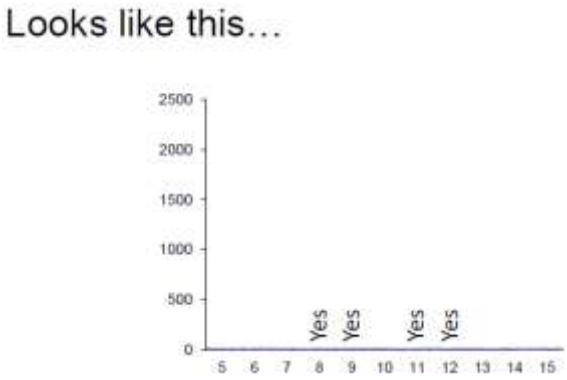
本課程以二元計算(binary approaches)解釋混合型別 DNA 的限制，也介紹了以概率性計算(probabilistic approaches)解釋混合型 DNA 的好處。二元

計算將發生機率很小的基因型別組合定義為 0，發生機率較高的基因型別組合定義為 1，是一種很粗略的計算方式。而概率性計算包括了半連續性(semi-continuous)及連續性(continuous)計算模式。半連續性計算模式不考慮圖譜基因型峰高(peak height)，只考慮圖譜上出現那些基因型別，如圖一及圖二所示：



圖一：

實際圖譜有 4 個"高低不同"的基因型別：8, 9, 11 及 12。基因型別 8, 9 峰高較高而基因型別 11, 12 峰高較低



圖二：

但在半連續性計算模式只被視作 4 個出現的基因型別：8, 9, 11 及 12。

簡而言之，在有嫌疑人及被害者 DNA 參考型別的情況下，半連續性計算模式的重點在於，嫌疑人在圖譜上出現的型別"沒有 drop-out"機率及嫌疑人在圖譜上沒有出現的型別"drop-out"機率以及整個圖譜"有 drop-in"或"沒有 drop-in"相乘後的結果。

連續性計算模式則以圖譜上基因型別的峰高，考慮基因型別各種組合方式可能的機率。以圖一基因型別來說，基因型別組合是(8, 9)加(11, 12)的機率(權重)有 0.8；基因型別組合是(8, 11)加(9, 12)的機率(權重)有 0.05；基因型別組合是(8,12)加(9, 11)的機率(權重)有 0.05，將所有可能的組合列出並考慮

各種不同影響因素才計算各種組合機率。而各種組合權重比例計算可以由 Markov chain Monte Carlo 方法計算出。

因為連續性計算混合型相當複雜，需考慮相當多影響因子，連續性計算混合型別目前使用的應用程式，例如 TrueAllele、STRmix 及 DNA-View Mixture solution。DNA mixtures 及 EuroforMix，目前為開放資源(open-source)的線上應用軟體。關於混合型別計算，國際上並沒有一定的標準或計算方法，學者認為最好的計算方法為使用連續性計算模式，至於要使用哪種軟體，並沒有特別建議，建議國內各鑑驗單位可以測試其中幾個軟體，找出最適用於混合型計算的方法。

本課程為 Coble 博士所講授，Coble 博士是 DNA 親緣計算與混合型別的專家，Coble 博士在 2016 年也曾受內政部警政署刑事警察局之邀，來台講授課程。Coble 博士在台灣上課時，有特地將語速放慢，是個相當貼心的老師。由於參加 AAFS 的專家及學員多以英語為母語，Coble 博士在課程中用一般講話速度授課，和之前來台灣時不太一樣。博士在每講解一個概念後也要求學員計算例題，務必讓學員了解所有分析模式的原理概念，因此本堂課收獲甚多。

三、專題研討會議題：「An Introduction to Lean Fundamentals and Six Sigma Operational Improvement」專題研討會（workshop）

Six sigma，譯成六西格瑪或六標準差，是商業管理策略之一，最初於 1986 年由摩托羅拉創立，後來由通用電器執行長傑克·偉爾奇所推廣而廣泛應用於許多行業。核心概念為藉由持續改進，並透過測量、分析及控制進行改善品質控制及品質管理過程。

Lean six sigma，精益六標準差是精益生產與六標準差管理的結合，精益六標準差目標在於改善生產流程，減少浪費與提高產量。本課程藉由精益六標準差方法管理實驗室，改善實驗室流程。達成方法有 4 個主要步驟。步驟

1：定義所出現的問題。步驟 2：審慎檢查目前運作流程，這個運作流程有多好?可以更好嗎?什麼阻止了他更好?要如何改善?步驟 3：收集各項供分析的數據後，做出改善流程的決定(data-based decision)。步驟 4：在各項流程置入控制方法，確保持續改善流程。

商業管理有 3.8 標準差與 6 標準差兩種不同管理方法，3.8 標準差必須有 99%商品符合品質要求，而 6 標準差必須有 99.99966%的商品達到品質要求，企業以客戶為導向，可選擇 3.8 或 6 標準差管理方法。課程以美國產業舉例，醫療業若使用 3.8 標準差管理，則每週有 5000 個不良手術；若使用 6 標準差管理則可達成每周僅剩 1.7 個不良手術。

精益生產是衍生自豐田汽車生產方式的一種管理方法，由 4P 模型(4P model)及 14 條原則所構成。4P 模型是三角形模型由底層到尖端分別為企業理念(Philosophy)、生產流程(Process)、人力資源管理(People and Partners)及解決問題(Problem Solving)。企業理念原則包括一個長遠的核心理念與目標；生產流程各項原則及核心思想則均以消除浪費為主；在人力資源管理方面的核心思想則是尊重員工、挑戰員工及栽培員工使他們成長；透過持續的改進與學習解決企業發生的問題。

精益六標準差需要大量熟悉實驗運作的人員來找出問題、分析問題並思考如何加入控制組來改善流程，並營造實驗室共同的核心思想。找出問題需要大量數據支持，分析這些數據需要大量人力，改善流程需要的手段更需要具有豐富經驗的員工。美國單一實驗室內部又區分為不同團隊，鑑驗團隊負責案件、研究團隊負責研究、訓練團隊負責新進人員及在職教育訓練以及管理團隊等等，因此可以如此運作，台灣實驗室人力不足，每個人又需處理完全不同的工作項目，身兼數職，因此要找出問題、分析數據並據以改善流程並不是小規模實驗室可以達成的。

心得與建議

這次有機會奉派出國，實屬難得。參加的每一堂課程均有小組討論及隨堂計算等，學員互動熱絡，除了專業知識的累積，對於當地生活也有初步觀察。西雅圖當地人看起來相當勤奮，清晨五、六點飯店對面的健身房就已經有人在運動、市區路邊建造工程也開始出現工作人員，還有許多住家的燈都慢慢亮了起來，準備一天的工作。當地也相當注重環保，在超市購物，紙袋需額外收取費用，購買食物不提供餐具，旅館針對不需每天清潔服務的客房也提供實質回饋。甚至本次會議期間付費課程完全沒有提供紙本資料，必須仰賴自己攜帶的平板或電腦先行將課程講義下載，大多數學員沒有自行準備平板或電腦，因此課程中詢問筆者要如何下載講義。

本次年會期間適逢農曆春節，訂於農曆初三晚間出發，於初一值班結束後，抓緊時間整理 9 天會議期間所需行囊。安頓好 2 個年幼子女後，匆匆趕赴桃園機場，搭機前往美國西雅圖，抵達後，調整時差，稍作休息，接著參加行程緊湊的研討會，本次出國時差不時困擾著筆者，著實讓人感受到年紀的增長，與歲月的無情。

本所目前人力及經費相當困窘，於鑑驗案件業務壓力之下，每年均須向科技部爭取科技經費，以提昇本所現有技術。在此感謝本所涂所長達人對外極力爭取經費，對內鼎力栽培部屬外，也感謝法務部與科技部每年均支持本所發展最新鑑驗技術，於國際會議場，與世界各國專家進行交流，除了可拓展視野外，更可提升本國於國際上之能見度，著實獲益良多，創造多贏局面。

本出國報告已濃縮成一篇約 1000 字心得與見聞，已於 107 年 4 月間投稿於「法務通訊」，期待刊登，以分享更多有興趣讀者。

附錄

一、 研究內容投稿摘要

Enhance the Power of Discrimination of Semen Identification by Combination of Microfluidic Chips and Erase Kits

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It is the key point of forensic examination how to effectively check out sperms in evidences in sexual assault cases. Practically, the differential extraction method was applied to isolate male DNA from the mixtures of sperms and epithelial cells. However, it is a time-consuming and less effective process. Nowadays, the Erase Sperm Isolation kit, a commercial available reagent, hydrolyzes cell-free female DNA before extraction of sperm DNA to reduce contamination of female DNA in isolated sperm DNA. Additionally, the microfluidic chip technique can separate sperms from the mixture of sperms and epithelial cells according to the differences of density, size, and settling rate between sperms and epithelial cells. The aim of this study was to overcome the problems of the differential extraction method by using the microfluidic chip technique combined with the Erase Sperm Isolation kit (combination method). We collected 5 semen samples and 2 buccal swabs from adult men and women, respectively, and then prepared 2 mixtures with the ratios of sperms and epithelial cells of 1:1 and 1:3, respectively. These mixtures were stored at room temperature for 1, 3, 7, and 14 days, and then their DNA profiles were analyzed. The combination method presented excellent results that the ratios of complete STR DNA profiles (15 loci, without female DNA interference) of sperm DNA were around 80% and 60% in the 3 and 14 days, respectively; whereas the results using differential extraction method were less than 30%, accompanying over 60% of interference of female DNA, in all time periods. These data indicated that the combination method can greatly decrease the female DNA interference in STR DNA profiles. We also examined the effectiveness of the combination method with 9 forensic specimens which were positive semen stains. The data of STR DNA profiles showed that interference of female DNA was observed just in one case. However, only 2 cases were obtained the complete STR DNA profiles. The possible reasons may that the amounts of isolated sperm DNA were too little to get the complete STR DNA profiles. In contrast, sperm DNA isolated by the differential extraction method presented high interference of female DNA (7 cases), and only one case of STR DNA profile was complete (15 loci, without female DNA interference). Taken together, these results suggested that the combination method can greatly reduce the interference of female DNA in the isolated sperm DNA, and enhance the power of discrimination in the semen identification.

Keywords: Semen identification, Microfluidic chip, Differential extraction, Power of discrimination

二、 張貼研究海報全文

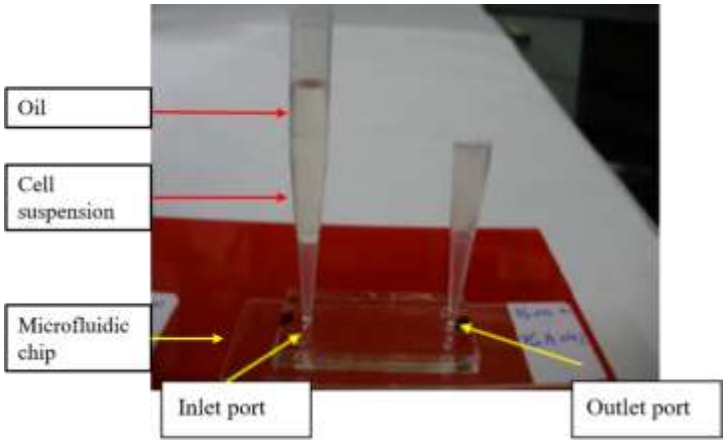
Vaginal swabs are specimens commonly collected in cases of sexual assault; these swabs mostly consist of a mixture of vaginal epithelial cells and seminal fluid that contain the suspect's spermatozoa. Because the number of epithelial cells far exceeds the number of spermatozoa in the vaginal swab, it is difficult to obtain a definite autosomal STR profile from the spermatozoa[1]. To eliminate excessive epithelial DNA interference, forensic laboratories use differential extraction methods to separate epithelial cells and DNA from spermatozoa. Recently, a modified differential extraction method, the Erase sperm isolation kit, has been adapted in our lab. A microfluidic chip can also be employed as an additional epithelial cell-spermatozoa separation step prior to the Erase sperm isolation kit[2,3,4,5]. Our study evaluated the differential extraction method and the combination of the microfluidic chip with the Erase kit by examining spermatozoa STR profiles from fresh and aged specimens.

Materials and Methods

Sample preparation

Five semen samples and two female buccal swabs were collected. Sperm and epithelial cells were enumerated using a hemocytometer in order to obtain 1:1 and 1:3 mixtures with a total cell count of 24,000 and 48,000, respectively. These mixtures were stored at ambient temperature (15 to 25 °C) for 1, 3, 7, and 14 days, mimicking fresh and aged specimens. The mixtures were then processed using either the standard differential extraction method or the combination method consisting of a microfluidic system and the Erase isolation kit.

Microfluidic chips



The microfluidic chips are comprised of polydimethylsiloxane (PDMS) and 0.2 wt% Triton X-100 (Merck, Darmstadt, Germany). The microchannel is 100 μm in diameter and 3 cm in length. Phosphate buffered saline (PBS) is introduced into the inlet port using a pipette, ensuring that there are no bubbles inside the microchannel. The inlet tip is filled with 300 μL of the sperm/epithelial cell mixture and the outlet tip is filled with an equal volume of PBS. The cells are allowed to settle for 10 minutes and

then the outlet PBS is removed. Next, a layer of oil is applied to the inlet tip to create a gravity-driven flow during the cell collection process. Cell flow-through is collected every 30 minutes and the separation efficiency of each flow-through sample is determined by microscopic examination.

Forensic cases

Nine forensic vaginal swabs with both ACP (acid phosphatase) and PSA (prostate specific antigen) positive results were collected to evaluate the microfluidic/Erase combination method and the differential extraction method.

Erase sperm isolation kit

The cell flow-through can be further separated using the Erase sperm isolation kit (Paternity Testing Corporation, Columbia, MO, USA). Briefly, 400 μL Extraction buffer and 7 μL proteinase K are added to the cell flow-through and incubated at 56 °C for 1 hour to lyse the remaining epithelial cells. Next, the spermatozoa are pelleted by centrifugation at 12,000 ×*g*, the supernatant is removed, and solutions 1 and 2 are added to the pellet and incubated at 37 °C for 15 minutes to digest the epithelial DNA. Solution 3 is then added to the pellet and incubated at 37 °C for another 15 minutes to lyse the sperm cells. Both supernatant and pellet fractions are then processed using a silica-based DNA extraction method (PureLink Genomic DNA Kits).

Differential Extraction

Differential extraction was performed as follows: 500 μL TNE1 buffer (Tris-NaCl-EDTA [10 mM Tris-HCl, 100 mM NaCl, and 2 mM EDTA] with 1% SDS and 100 μg/mL proteinase K) are added to the sperm/epithelial cell mixture and incubated at 56 °C for 30 minutes. The sperm cells are then pelleted by centrifugation, the supernatant is removed, and the pellet is washed twice with 500 μL TNE1 buffer and centrifugation. Next, 500 μL TNE2 buffer (TNE1 buffer with 0.04 M DTT) are added to lyse the sperm cells. Both the supernatant and pellet fractions are then processed using a silica-based DNA extraction method.

DNA quantification

DNA quantification is performed using the Quantifiler Duo DNA Quantification Kit and ViiA 7 real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA).

STR analysis of spermatozoa and vaginal epithelial cells

Sperm and epithelial DNA were analyzed using the AmpFlSTR Identifiler PCR Amplification kit with a 3500xL genetic analyzer (Thermo Fisher Scientific), according to the manufacturer's instructions.

Results

The STR profiles of the mixtures indicate that the combination method provided better recovery and separation than the differential extraction method; see Tables 1 and 2 for details.

Table 1. STR profiles of spermatozoa isolated using the microfluidic/Erase combination method

	Storage time							
	1-day		3-day		7-day		14-day	
	Sperm STR profile	Epithelial interference	Sperm STR profile	Epithelial interference	Sperm STR profile	Epithelial interference	Sperm STR profile	Epithelial interference
1:1								
mixture 1	Δ	—	○	—	Δ	—	Δ	—
mixture 2	○	—	Δ	—	○	—	○	—
mixture 3	○	—	○	—	Δ	+	Δ	+
mixture 4	○	—	○	—	○	—	○	—
mixture 5	○	—	○	—	○	—	○	—
1:3								
mixture 1	○	—	○	—	○	—	○	—
mixture 2	○	—	○	—	Δ	—	Δ	—
mixture 3	Δ	—	Δ	—	Δ	+	Δ	+
mixture 4	○	—	○	—	○	—	○	—
mixture 5	○	—	○	—	○	—	○	—
Full STR profile (%)	80%		80%		60%		60%	

Time course experiment for sperm and epithelial cell mixtures using the microfluidic chip and Erase sperm isolation kit. Sperm and epithelial cell mixtures at a 1:1 and 1:3 ratio were tested; each experiment included five sample mixtures. STR profile assessment to evaluate the recovery and effectiveness of the method. "○" indicates a full profile, 15 STR loci were determined; "Δ" represents a partial profile, 8 to 14 STR loci were determined; "—" represents no epithelial interference in the STR profile; and "+" represents a mixed DNA profile with epithelial DNA interference. The full STR profile identification rates were 80% (8 out of 10) for 1-day and 3-day mixture storage and 60% for 7-day and 14-day mixture storage.

Table 2. STR profile of spermatozoa isolated using the differential extraction method

	Storage time							
	1-day		3-day		7-day		14-day	
	Sperm STR profile	Epithelial interference	Sperm STR profile	Epithelial interference	Sperm STR profile	Epithelial interference	Sperm STR profile	Epithelial interference
1:1								
mixture 1	Δ	+	Δ	+	×	—	Δ	+
mixture 2	Δ	+	Δ	+	Δ	+	×	+
mixture 3	Δ	+	Δ	+	○	—	Δ	+
mixture 4	Δ	+	○	—	○	—	○	—
mixture 5	×	+	×	+	Δ	+	Δ	+
1:3								
mixture 1	×	+	×	+	Δ	+	Δ	+
mixture 2	×	+	×	+	Δ	+	Δ	+
mixture 3	Δ	+	Δ	+	○	—	○	—
mixture 4	×	+	Δ	+	Δ	+	○	—
mixture 5	×	+	×	+	○	+	Δ	+
Full STR profile (%)	0%		10%		40%		30%	

Time course experiment for sperm and epithelial cell mixtures using the differential extraction method. Sperm and epithelial cell mixtures at a ratio of 1:1 and 1:3 were examined; each experiment included five sample mixtures. STR profile assessment to evaluate the recovery and effectiveness of the method. "○" indicates a full profile, 15 STR loci were determined; "Δ" represents a partial profile, 8 to 14 STR loci were determined; "×" represents <8 STR loci were determined; "—" represents no epithelial interference in the STR profiles; and "+" represents a mixed DNA profile with epithelial DNA interference. The full STR profile identification rates were 0%, 10%, 40%, and 30% for 1-day, 3-day, 7-day, and 14-day mixture storage, respectively.

Table 3. Y-STR profile of spermatozoa isolated using the microfluidic/Erase combination method

	Storage time							
	1-day		3-day		7-day		14-day	
	Microfluidic /Erase	Differential extraction	Microfluidic /Erase	Differential extraction	Microfluidic /Erase	Differential extraction	Microfluidic /Erase	Differential extraction
1:1								
mixture 1	△	○	○	○	×	○	○	○
mixture 2	○	○	○	○	△	○	○	○
mixture 3	○	○	○	○	△	○	○	○
mixture 4	○	○	○	○	○	△	○	○
mixture 5	○	○	○	○	○	○	○	○
1:3								
mixture 1	○	○	△	○	○	○	○	○
mixture 2	○	○	○	○	△	○	○	○
mixture 3	△	○	△	○	△	○	△	○
mixture 4	○	○	○	○	○	△	○	○
mixture 5	○	○	○	○	○	○	○	○
Full Y-STR profile (%)	80%	100%	80%	100%	50%	80%	60%	100%

Time course experiment for sperm and epithelial cells mixtures using the microfluidic/Erase combination method and differential extraction method; each experiment included five sample mixtures. Y-STR profiles were performed to evaluate the recovery of sperm cell DNA. "○" indicates a full Y-STR profile, 17 Y-STR loci were determined; and "△" represents a partial profile, 8 to 17 Y-STR loci were determined.

Forensic cases

Nine forensic vaginal swabs were evaluated using these two separation methods. When the combination method was used, female DNA interference was evident in a single STR profile; however, a complete STR DNA profile was obtained in only two samples. These results might be due to low recovery caused by the two-step isolation of sperm DNA, which results in a low yield of sperm DNA. In contrast, seven cases of sperm DNA isolated by differential extraction exhibited female DNA interference and only one case provided a full STR profile.

Discussions

The combination of the microfluidic chip and the Erase method yielded excellent results. The rate of complete sperm STR DNA profiles was approximately 80% and 60% at 1 to 3 days and 7 to14 days, respectively; whereas that of the differential extraction method rate was <30%, accompanied by >60% female DNA interference. Full Y-STR DNA profiles of sperm DNA were detected in approximately 50-80% of samples using the microfluidic/Erase method, compared to 80-100% using the differential extraction method. Although the combination method demonstrates good separation of sperm and epithelial cells, it can be time-consuming. Moreover, the cotton fiber of the specimen swab or bodily secretions can clog the microchannel and the recovery of sperm cell DNA is lower than with the differential extraction method.

Conclusions

The differential extraction method provides better DNA recovery, but lower sperm cell DNA purity. Our study demonstrates that the combination of the microfluidic chip and Erase isolation method offers good results. Moreover, the most efficient time period for collecting spermatozoa using our microfluidic chip is 30 to 120 minutes after the mixture is flowed into the microchannel. Cell sorting on chips provides several advantages over conventional methods; this technique has been shown to be a powerful tool for isolating circulating tumor cells from blood samples in which the number of blood cells far exceeds that of circulating tumor cells[6]. Further developments in 3D-printed technology and an automated microfluidic platform would increase the applicability of microfluidic systems in the field of forensics, allowing on-site cell-type-specific isolation or even single cell manipulation in a crime scene investigation.

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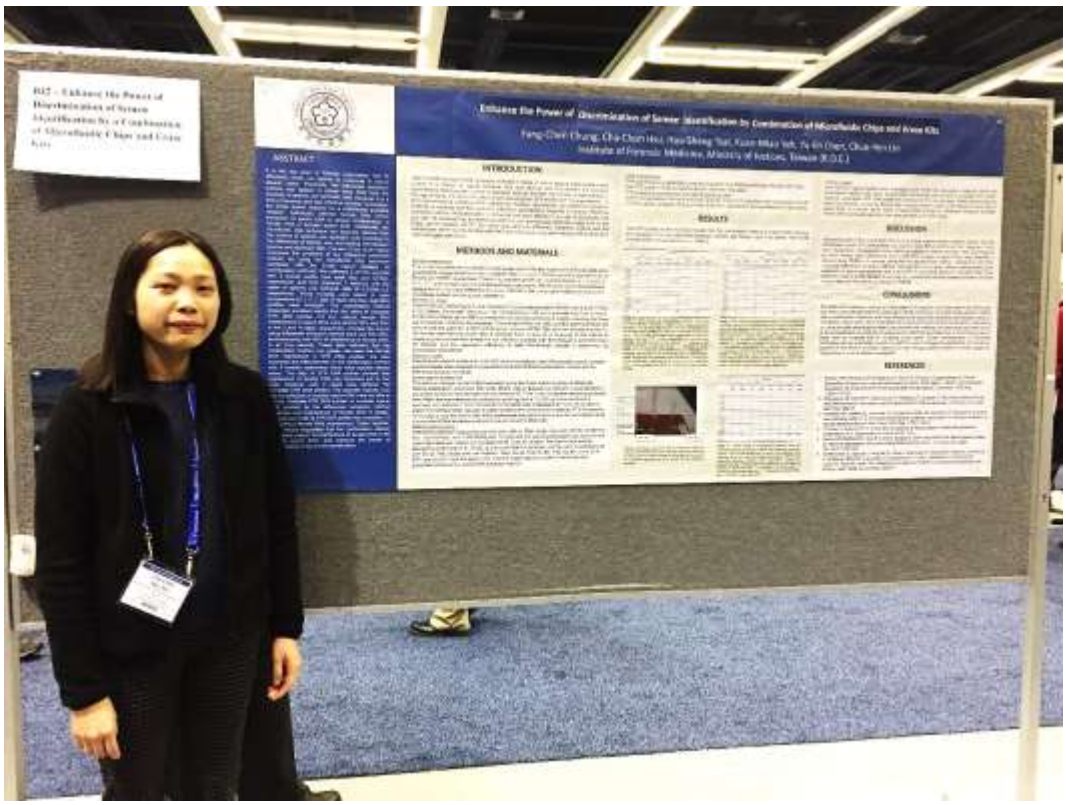
三、 本所發表研究成果之海報展示現場

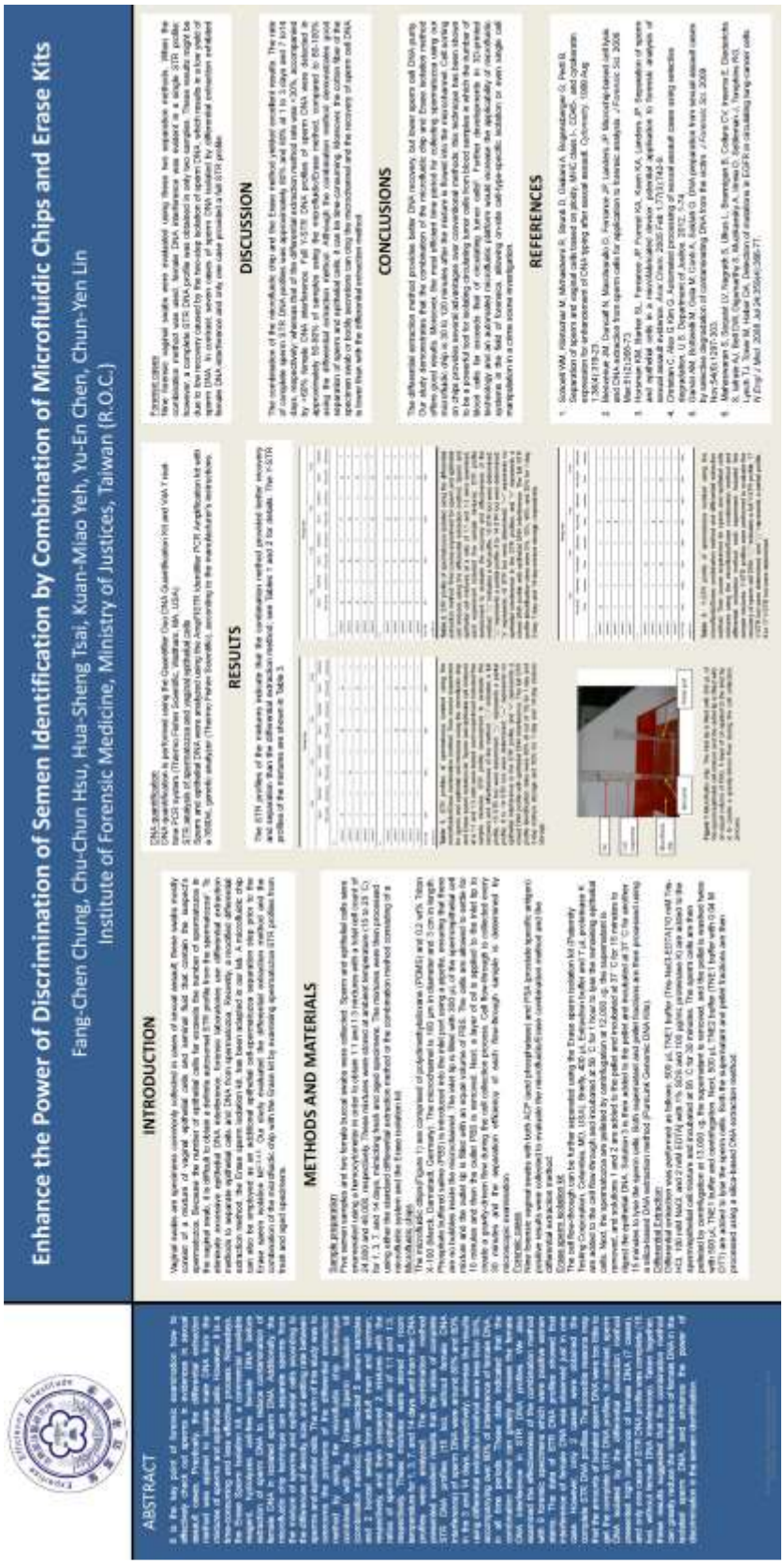


●筆者與本所研究論文海報合影

●會場一隅，各國鑑識領域專家學者與展出者熱烈討論。

●展覽會場-華盛頓會議中心花園，會議期間為冬天，花園覆蓋一層薄薄積雪。

四、 本所研究成果海報



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