Detection Systems for Biological Warfare Agents, Present and Future
生物戰劑偵檢系統之現在與未來(譯)

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On 9 December 1979 the World Health Organization (WHO) declared the world free of smallpox. The last known naturally-occurring case was identified in Somalia in 1977 (in 1978 a laboratory technician working with smallpox died of the disease in England). All known stocks of the virus were destroyed except those held by the Centers for Disease Control and Health Promotion (CDC) in the United States and the State Research Center of Virology and Biotechnology (VECTOR) in Koltsovo, Russia. An international debate ensued concerning destruction of these remaining stocks until 2002, when WHO determined it best to retain, for research purposes, this vestige of a by-gone scourge. While the debate took place, the Soviet Union secretly weaponized smallpox virus and packaged tons in missile warheads. Smallpox, an extremely contagious disease with a mortality rate as high as 50 percent for some strains, remains a biological weapon threat to this day.

1979 年 12 月 9 日，世界衛生組織（WHO）宣布全世界已免除天花疾病。最後已知的自然案例發生於 1977 年的索馬利亞（1978 年則有一位英國天花病毒的實驗室技術員死於此疾病）。除了美國的疾病控制與預防中心以及俄羅斯Koltsovo的State Research Center of Virology and Biotechnology的病毒庫所保存之外，所有已知的庫存皆已摧毀。國際間對於是否摧毀此剩餘庫存引發辯論，直到 2002 年WHO決定最好保留此項過去災害的遺跡以供研究。當辯論正進行時，蘇聯秘密地將天花病毒武器化、成噸地包裝於導彈彈頭。[1]天花，一種傳染性極高、部份病毒株有高達 50%的致死率，在今日仍是一項生物武器威脅。

[1] Alibek, K. *Biohazard*; Random House: New York, 1999.

Biological warfare is nearly as old as organized war itself. As early as the 6th century BCE the Assyrians used ergot (a fungus) to poison the drinking water of their adversaries. Hannibal is reputed to have used clay pots filled with poisonous snakes against his enemies in a naval battle with the Emperor of Pergamene in 184 BCE, and the Mongol's use of human carcasses catapulted over castle walls at the siege of Kaffa (Theodosia, Ukraine) is believed to be one source for the spread of Black Plague or "Black Death" in Europe during the 14th century. During the French-Indian war the British attempted to infect the Delaware Indians besieging Fort Pitt by giving them blankets contaminated with smallpox virus; the efficacy of this tactic is subject to debate.

生物戰幾乎與組織戰本身一樣古老。早在西元前 6 世紀亞述人（the Assyrians）使用麥角菌（ergot）（一種真菌）對敵人飲水下毒。漢尼拔（Hannibal）據說在西元前 184 年對抗 Pergamene 皇帝的海戰中，用裝有毒蛇的陶壺作戰。蒙古人在進攻卡法（Kaffa）（現烏克蘭城市 Feodosia）時，投射人類死屍進入城牆內，此舉被相信是 14 世紀歐洲黑死病的來源之一。在法印戰爭中，英國把染有天花病毒的毛毯丟給包圍皮特堡（Fort Pitt）的德拉瓦族印地安人（Delaware Indians），使他們受感染，此項戰術的功效至今仍受到辯論。

In modern times germ warfare has seen limited use. During WW I, the Germans attempted to infect allied horses with Burkholderia mallei, the bacterial species that causes Glanders. The effort was spearheaded by Dr. Anton Dilger, an American expatriot serving in the German army, while he was living in Baltimore prior to official U.S. involvement in the conflict. Although generally thought to be ineffective, these efforts marked the beginning of agricultural biowarfare.

在現代，生物戰運用有限。第一次世界大戰中德意志帝國嘗試用Burkholderia mallei－馬鼻疽（Glanders）的病原菌去感染協約國馬匹。這項嘗試由一位為德意志帝國陸軍工作的美國移民Dr. Anton Dilger主導。他在美國涉入衝突前曾居住在巴爾的摩。雖然總體而言，這策略並沒有實效，但這些嘗試開啟了農業生物戰的開端。[2]

In the lead-up to the siege of Stalingrad thousands of German and Soviet soldiers developed pneumonic tularemia (which is caused by the bacterium Francisella tularensis). Dr. Ken Alibek later speculated that the Soviets had utilized the bacterial pathogen for activities leading to the siege of Stalingrad. As the implied consequence, thousands of German and Soviet soldiers succumbed to pneumonic tularemia.

在史達林格勒（Stalingrad）戰役的前期，成千納粹德國與蘇聯士兵罹患由 Francisella tularensis 造成的肺炎型兔熱病。Ken Alibek 後來推測，蘇聯已在進入史達林格勒戰役的行動中使用該細菌。可想而知結果是成千上萬的納粹德國與蘇聯士兵病死於肺炎型兔熱病。

Probably the most widespread employment of biological warfare in World War II

[2] Sidell, F. R.; Takafuji, E. T.; Franz, D. R. *Medical Aspects of Chemical and Biological Warfare*; United States Government Printing: Washington D.C., 1997; Vol. 3.

was promulgated by Japan's infamous Unit 731 commanded by General Shiro Ishii. Allegations still exist that the Japanese deliberately infected thousands of Chinese with Yersinia pestis,2 the bacterium that causes the plague. As in nearly all allegations of biological warfare, the actual employment is shrouded in secrecy and the effects typically obfuscated by the possibility of a natural-disease outbreak. Nevertheless, it is well known that Unit 731 engaged in human experimentation with biowarfare agents and could count its victims in the thousands.

第二次世界大戰中，最大規模的生物戰運用者或許是日本的石井四郎將軍所指揮的惡名昭彰的 731 部隊。日本仍被指控蓄意以黑死病的病原菌-鼠疫桿菌（Yersinia pestis）感染成千上萬的中國人。幾乎像所有生物戰的指控一樣，它實際的運用情形被秘密隱藏掩蔽、且其作用往往與自然發生的疾病相混淆。儘管如此，眾所皆知的是 731 部隊進行了生物戰劑的人體試驗，其犧牲者達數以千計。

Since WW II, there are no confirmed large-scale employments of biological weapons. However, the Soviets are suspected of using fungal mycotoxins (trichothecenes) against Hmong tribesmen in Southeast Asia during the Vietnam War. Although plausible, there is insufficient evidence to substantiate this activity. In recent years the threat and practice of biological attacks has come largely from non-state sponsored terrorists. In September 1984, for instance, the Rajneesh religious cult attempted to sway local elections in The Dalles, Oregon, by poisoning the salad bars in several popular local restaurants with Salmonella typhimurium. The hope was that with a significant portion of the local population ill, the Rajneeshes could capture key political positions in an up-coming election. Although there were no fatalities, 751 individuals were infected and 45 required hospitalization. Only after two years and serious in-fighting among the membership of the Rajneesh organization, which resulted in law suits, was the etiological basis for these food poisoning outbreaks determined.

二次世界後，未被確認的生物武器被大規模使用。越戰時，蘇聯被懷疑在東南亞使用單端孢霉烯族真菌毒素（trichothecene mycotoxin）對抗苗族人（Hmong）。此說法雖然看似有理，卻無充分證據可證實這項行動。近年來生物攻擊的威脅與行動主要來自非政府資助的恐怖份子(或組織)。例如在 1984 年 9 月 Rajneesh 教徒使用沙門氏菌（Salmonella typhimurium）對當地幾個有名的餐館的沙拉吧下毒，欲藉此影響奧利岡州 The Dalles 的地區選舉。他們期望當地人大量生病後，Rajneesh 教徒可在即將到來的選舉獲得關鍵性的政治地位。雖然並未造成死亡，但仍有 751 人被感染、45 人須住院治療。只有在 2 年後 Rajneesh 教徒間產生嚴重內鬥並對簿公堂後，這起大量食物中毒的起因才被確定。

The Aum Shinrikyo sect, notorious for the Tokyo subway nerve agent attacks, attempted biological attacks on several occasions during the 1990s using botulinum toxin (a highly potent neurotoxin) and Bacillus anthracis spores (the causative agent of anthrax). In October 2001, shortly after the 9/11 terrorist attack, probably the most widely publicized act of bioterrorism in history was perpetrated through the U.S. Postal Service with mailings of Bacillus anthracis spores to various media outlets and

the U.S. Senate offices of Patrick Leahy and Thomas Daschle. The spores were carefully prepared in a fashion that allowed them to disseminate profusely through the air when the envelopes were opened. In addition, the particles were a size that allowed them to efficiently enter the lungs, which was the primary site of infection. Consequently, these attacks killed five people and infected at least 22 in addition to causing widespread panic. Less well publicized was a second bioterror attack in 2004 in which ricin, a plant toxin, was mailed to Senator Bill Frist. Although no one was injured, Senate offices were closed for several days. For the “anthrax letters” case after almost seven years of investigation, one suspect was identified and subsequently committed suicide. These events illustrate the dire consequences we can face when microbial pathogens are employed for nefarious activities.

因東京地鐵神經毒氣攻擊事件而惡名遠播的奧姆真理教徒，在 1990 年代曾於數個場合嘗試過使用肉毒桿菌毒素（botulinum toxin）（這是一種高強效神經性毒素）與炭疽桿菌孢子（Bacillus anthracis）進行生物攻擊。在 2001 年 911 恐怖攻擊過後到 10 月間，發生了歷史上最受關切的生物恐怖攻擊事件。炭疽桿菌孢子經由美國郵政被寄往各種媒體與 Patrick Leathy 及 Thomas Daschle 兩位參議員的辦公室。孢子被謹慎地製備完成，當信封打開時便可輕易由空氣散播。此外粒子的體積小到足以有效進入肺部，造成感染。結果這些攻擊殺死 5 人、感染至少 22 人、並造成大眾恐慌。2004 年發生的第 2 起生物恐怖攻擊較未受到關注，其中蓖麻毒素（ricin）被寄給參議員 Bill First。雖然無人受傷，參議員辦公室因此關閉了幾日。在將近 7 年的調查之後，查出一名嫌犯涉嫌製作“炭疽信”，但不久他自殺了。這些事件顯示了當微生物被用來進行惡毒行動時，我們會面臨的可怕後果。

From this brief history it should be clear that the threat of biological attack is of grave concern. A glance at world news should convince anyone that the terrorist threat, especially that from Islamic jihadist, has no bounds; they will take any measure to inflict suffering on their perceived enemies.

上述簡單歷史應可使我們明白生物攻擊的威脅值得嚴正關切。一窺世界新聞便可使任何人相信恐怖攻擊－尤其是來自伊斯蘭聖戰－是沒有邊界、無所不在的；他們將採取任何可能的手段，製造他們心目中的敵人的痛苦。

At some point, terrorists or adversary states could use biological pathogens to attack U.S. forces or civilians. Consequently, there is a dire need for defense capability that would avert or remediate such an attack. Central to biological defense is technology for detection or identification of biological agents released to critical sites of the battlefield or civilian populations. To be reliable, detection of biological threats must be effective for agents that could be released in a variety of sites, including air spaces, surfaces, water supplies, and food. Ideally, detection systems should provide results in time to prevent infection (detect-to-warn) or, lacking that, the capability to control spread of the biological agent (detect-to-treat). A key factor for reliable detection is its accuracy with respect to a “signal” being generated only when a biological threat is present. Conversely, the system should have no “signal” when the biological threat is not present. This article will describe the fundamental

technologies currently available for bioagent detection and the prospects for future improvements.

在某些情況下恐怖分子或敵國組織會使用病原體攻擊美國部隊或平民。因此迫切需要可避免或因應此類攻擊的防禦戰力。生物防禦的核心是偵測或辨識撒布於戰場重要地區或平民之生物製劑的技術。要可靠的偵測生物威脅必須確認釋放於各地的製劑有效，包括空氣、地表、水源與食物。理想情況下偵測系統應即時提供測量結果並提出警告以避免感染（偵測-警告 detect-to-warn）。其次，要能提供控制生物製劑擴散的能力（偵測-治療（detect-to-treat））。其中最關鍵的因素是可靠偵測的準度，只有具生物威脅才會有“信號”出現，否則系統應不會出現“信號”。本文將描述目前生物製劑偵測的基本技術與未來改進之前景。

Sample Collection and Recovery of Biological Material for Detection

The first task for bioagent detection is to recover target microorganisms from samples collected from the attack site or site of concern. In a scenario such as the “anthrax letters” this is a relatively easy task since there would likely be a sufficient amount of material and a major component of the material would be Bacillus anthracis spores. However, with respect to all the possible avenues for a biological attack, sample collection is usually a formidable task. To devise an effective sampling strategy, allowing reliable detection, a variety of factors must be addressed. For example, the concentration of the target biological agent in a sample is critical to the ability to detect the agent. Because any given detection system requires a certain number of organisms to generate a detection signal (i.e. detection sensitivity), a sample with a low density of target organisms requires a larger sample to be collected. With air samples, it may be possible simply to sample the air for a longer period to collect a larger volume, hence a larger number of organisms. In contrast, with complex samples such as blood, muddy water, or food, recovery and concentration of target microorganisms can require complex, cumbersome, and time-consuming procedures. Even then, other components may interfere with the detection process. In particular, many materials which are commonly present in clinical specimens (heme of red blood cells), environment samples (clay, tannins, humic acids, and metals), and foods (lipids) are reported to inhibit Deoxynucleic Acid (DNA) based detection.

樣本採集與復原生物材料以供偵測

生物偵測的第一項任務是從攻擊地或關注地收集的樣本中復原目標微生物。在“炭疽信”之類的情況下這是較容易的任務，因為材料數量充足且主要成分是炭疽桿菌孢子。然而就生物攻擊的所有可能途徑而言，樣本採集通常是艱鉅的任務。為了擬定有效採樣策略，實現有效偵測，必須顧及各種因素。例如，樣本中目標生物戰劑之濃度，對於能否偵測該戰劑是至關重要的。由於任何偵測系統需要一定數量的生物體，才能產生偵測信號（即偵測靈敏度），樣本中目標生物體濃度低將導致需要收集更大量樣本。對氣態樣本而言，或許可以加長收集時間以得到較大體積氣體、進而較大量生物體。相反地對於複雜樣本如血液、泥水、或食物，復原並濃化目標微生物需要複雜、繁瑣、又費時的程

序。除此之外，其他成分可能干擾偵測程序。尤其是常見於臨床樣本（紅血球的原血紅素）、環境樣本（黏土、單寧酸、腐植酸、金屬）、與食物（油脂）的許多物質已被報告會抑制基於 DNA 的偵測。

A variety of sampling systems have been developed to recover microorganisms from air, water, solid surfaces, and clinical specimens. Some systems are highly complex, especially those with automated sample preparation. Because aerosol delivery represents one of the likely scenarios for biological attack, this article will describe basic systems employed to sample air. As for any type of sampling, aerosol sampling strategies must address design and operational considerations to meet military requirements. For example, air sampling devices must operate over an array of environmental conditions including extreme temperatures, extreme levels of humidity, dust and fog. For most detection systems, transfer of the biological agent to a liquid phase is necessary. Hence, collection of sub-freezing air must be accomplished without freezing the liquid phase. The power requirements for aerosol collection can be impressive.

已有各種採樣系統發展出來從空氣、水、固態表面、與臨床標本復原微生物。有些系統高度複雜，尤其是有自動化樣本製備（automated sample preparation）的系統。由於氣膠（aersol—或譯為氣懸微粒）傳送是生物攻擊可能途徑的代表之一，下文將描述氣體取樣的基本系統。就任何類型的採樣而言，氣膠採樣策略必須顧及設計與作業考量以滿足軍事需求。例如，空氣取樣裝置必須在一系列環境條件下運作，包括極端的溫度、極端的濕度、沙塵、霧。大部份偵測系統需要將生物製劑送入液體，因此必須能收集低於冰點的空氣而不凍結液體。氣膠採集可能有很可觀的能源需求。

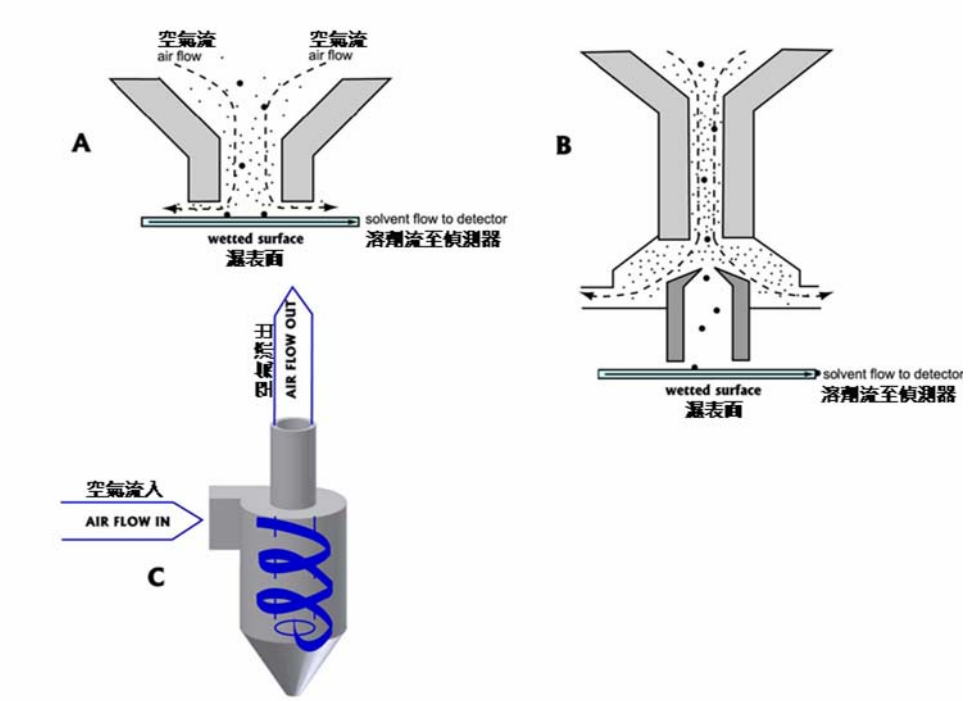

Figure 1. Bioaerosol Collectors.(A) Impact collector (B) Virtual impact collector (C) Cyclone type collector.

圖 1。生物氣膠採集器。(A)衝擊型採集器(B)虛擬衝擊型採集器(C)旋風型採集器。

Aerosol collectors can be classified into two general types: cyclone and impactor (Figure 1). The cyclone design involves a cylindrical chamber, usually with a cone shaped bottom. The inlet is on the side and the outlet is formed by an interior tube with its opening near the bottom of the chamber. Thus, incoming air is forced into a vortex, creating a centrifugal force on any entrained particles; the heavier ones being forced to the outer walls of the chamber. In some applications the walls of the cyclone chamber are “wetted” with a solvent or aqueous solution to facilitate an aerosol-to-hydrosol transfer stage (AHTS).

氣膠採集器可分為兩基本類型：旋風型（cyclone）與衝擊型（impactor）(如圖 1)。旋風型的設計有一個錐形底部的圓柱型室。氣體入口在側面，內有一管形成氣體出口，其開口靠近圓柱型室底部。進入氣體因此被迫形成旋渦，對夾帶粒子產生離心力，較重粒子被推向圓柱型室壁。在某些應用中，圓柱型室壁沾有溶劑或水溶液以便利氣膠轉移至水溶膠（aerosol-to-hydrosol transfer stage, AHTS）。

Impactor collectors work on a somewhat different principle analogous to the way that heavy, wet snow impacts on the windshield of a moving automobile while lighter snowflakes are diverted into the main airflow around the windshield. Impactors rely on the momentum of heavier, denser particles to maintain their trajectory onto a collecting surface while lighter particles are swept away into the effluent airstream. Virtual impactors are an adaptation where the impact surface is replaced by another collecting tube in which air flow is highly restricted. Thus the heavier particles are swept into a collecting tube for transport to an AHTS. There are practical limitations to each of these technologies. The cyclone collectors generally have a larger dynamic range for particle size but, especially for use in sub-freezing environments, can have excessive power requirements to avoid the problem of freezing. Because of the lower air flow rates at the AHTS, virtual impactors do not suffer this liability and relatively low power input can effectively prevent freezing. But the orifice sizes necessary to capture smaller diameter particles may restrict their ability to capture larger particles without becoming clogged. Limiting the size range of particles collected can facilitate collection efficiency. Particles from 1 to 10 μm are considered most important due to enhanced deposition in lung tissue.

衝擊型採集器根據不同的原理運作，類似汽車行進時重濕的雪撞擊擋風玻璃、而輕雪花被車周圍的氣流引導開來。衝擊型有賴重密粒子的動量來維持其移往採集面的軌跡、較輕粒子被外洩氣流帶走。虛擬衝擊型（virtual impactor）是一項變型，其中衝擊面換成採集管，進入管中的氣流被嚴格限制。因此重粒子被掃入採集管以送往 AHTS。這些每項技術皆有實際侷限。旋風型採集的粒子大小一般有較大動態範圍，但尤其在低於冰點的環境下可能需求特大能源以防止凍結。虛擬衝擊型由於在 AHTS 的氣體流速低故不受此困擾，相對低的能源輸入可有效防止凍結。但捕捉較小粒子所需的孔洞大小，可能限制其捕捉較大粒子且不堵塞之能力。限制採集的粒子大小範圍可增進採集效率。介於 1 到 10

微米的粒子由於在肺組織有較強的沉積故被認為是最重要的。

Aerosol samplers are available that are capable of collecting in excess of 1,000 liters of air per minute and concentrating the particulate matter to a relatively small volume (approximately 1 milliliter).

目前已有氣膠採集器可以每分鐘採集1000公升以上空氣並將微粒濃縮至相對小體積（約1毫升）。

Detection Technologies

One of the hallmarks of bioweapons is the extremely small amount of agent required to produce casualties. This, coupled with the complex chemical makeup of biological agents, precludes many of the technologies used for detection of chemical agents. The principle chemical components of biological particles are, in terms of physicochemical properties, rather uniform. These components consist of nucleic acid polymers (DNA and RNA), amino acid polymers (proteins) and lipids (molecules soluble in non-polar solvent and largely composed of hydrocarbons with specific functional groups). On a gross level these classes of molecules are quite similar and cannot be used to differentiate pathogenic and non-pathogenic organisms. But, as will be addressed below, they can be exploited, at a molecular level, to provide quite specific identification of essentially any microorganism.

偵測技術

生物武器其中一項標誌是極小劑量足可引發傷亡。這一特點，再加上生物製劑的複雜化學結構，排除了許多用於檢測化學製劑的技術。生物粒子的主要化學成分，就物化性質而言，是相當一致的。這些成分包括核酸聚合物（DNA與RNA）、胺基酸聚合物（蛋白質）、與脂質（可溶解於非極性溶劑之分子且主要由碳氫化合物與特定官能基構成）。就大體而言這些分子類別頗為相似、無法用來區別病原與非病原。但是，正如下文將討論的，在分子層級上他們可用來提供基本上對任何微生物相當特異的的辨識。

There are four basic detection technologies currently available. These are based on: (1) nucleic acid sequence, (2) molecular structure, (3) chemical property and (4) function.3 In this section the rationale of each of these is examined and their relative merits and weaknesses are explored.

目前有四種基本偵測技術，分別根據：（1）核酸序列（2）分子結構（3）化學性質（4）功能。[3]以下段落將檢視這四種原理、並探討其相對優缺點。

[3] Vitko, J. J.; Franz, D. R.; Alper, M.; Biggins, P. D. E.; Brandt, L. D.; Burge, H. A.; Ediger, R.; Hollis, M. A.; Laughlin, L. L.; Mariella, R. P. J.; McFarland, A. R.; Schaudies, R. P. "Sensor systems for biological agent attacks: Protecting buildings and military bases," National Academies of Science, 2005.

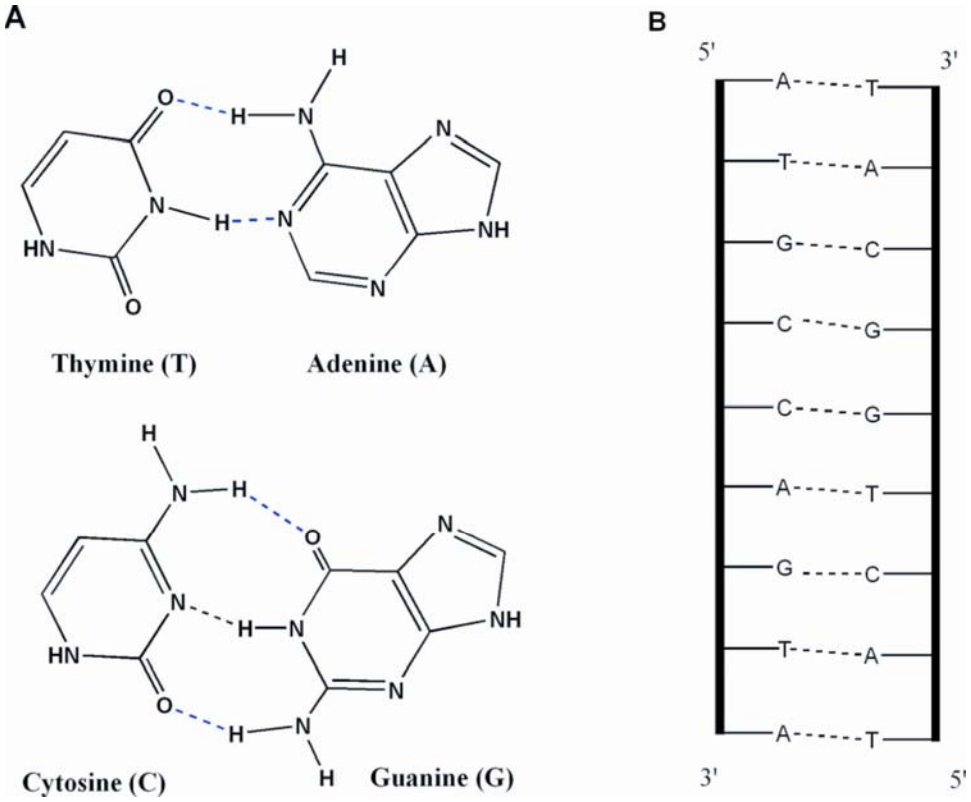

Figure 2. (DNA). (A) Chemical structures of the four bases responsible for complementary binding in DNA. Dashed lines represent hydrogen bonding between the base pairs. (B) Two anti-parallel strands of DNA showing the specific A:T and C:G complementation.

圖 2　DNA。(A)DNA 互補鍵結之原因在於四種鹼基的化學結構，虛線代表鹼基間的氫鍵。(B)雙股 DNA 顯示 A:T 與 C:G 互補之特異性。

Nucleic Acid Sequence

In all living organisms the genetic code is inscribed by the sequence of bases in the nucleic acid polymers, deoxynucleic acid (DNA) (some viruses employ the chemically less stable ribonucleic acid (RNA)). DNA contains four distinct heterocyclic bases: adenine (A), cytosine (C), guanine (G), and thymine (T). It is the particular sequence of these bases as they are arranged in genetic material that dictates all the characteristics of any living organism. These four bases are linked into a linear polymer by sugar and phosphate molecules to form a long single strand of DNA. DNA, then, consists of two single-stranded polymers joined together side-by-side in a spiral staircase fashion, commonly referred to as a “double helix.” The two single strands are held together by hydrogen bonds between complementary bases, with A’s and T’s always bonding with each other and C’s and G’s always bonding with each other (see Figure 2). Thus, each linear polymer will always bind to a complementary polymer to form double-stranded DNA. Moreover, these hydrogen bonds are weak and can be disrupted by relatively mild temperatures, increases in salt concentrations, pH changes or combinations of these parameters. The fidelity of DNA sequence complementation can be exploited in numerous ways to identify specific DNA sequences and thus the organism from which they originate.

核酸序列

所有生物體的遺傳訊息是由在 DNA 這種核酸聚合物裏的鹼基序列所註記（有些病毒使用化學穩定性較差的 RNA）。DNA 含有 4 種不同雜環鹼基：腺嘌呤（adenine, A）、胞嘧啶（cytosine, C）、鳥嘌呤（guanine, G）、胸腺嘧啶（thymine, T）。正是這些鹼基在遺傳物質中排成的特定序列支配了生物體的所有特徵。此 4 種鹼基由醣與磷酸分子作為連結構成線性聚合物，即 DNA 的一個單股。接著 DNA 由兩個並肩鍵結在一起的的單股聚合物構成並形成如螺旋樓梯的形狀，一般稱之“雙螺旋”。兩個單股由互補鹼基間的氫鍵結合在一起，其中永遠是 A 與 T 鍵結、C 與 G 鍵結（如圖 2）。所以各個線性聚合物將總是與互補聚合物鍵結以構成雙股 DNA。此外，這些氫鍵是脆弱的，可由相對溫和的溫度、提升鹽類濃度、改變 pH 值、或這些的組合加以打斷。DNA 互補的專一性可用來辨識特定 DNA 序列及其來源生物體。

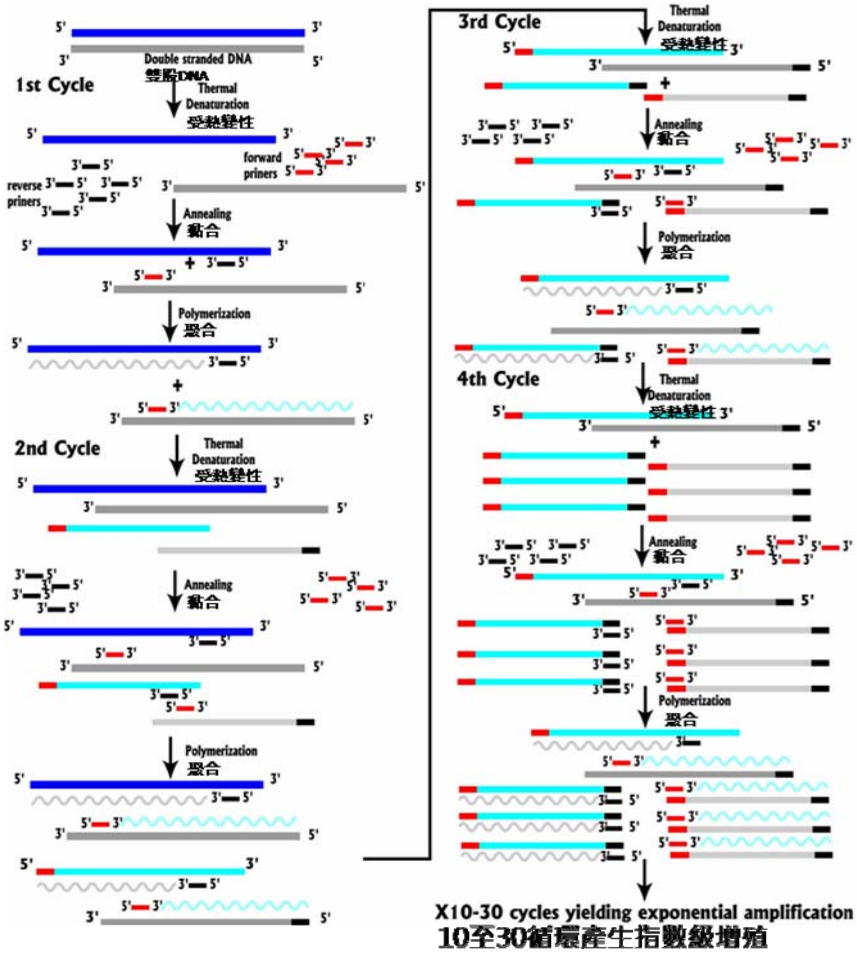

Figure 3. Polymerase Chain Reaction (PCR). A sample of DNA serves as template for amplification of a target segment. Short nucleic acid oligomers with target specific sequence serve as primers. The reaction mixture also contains a thermally-stable DNA polymerase and a supply of individual nucleotides. The original template DNA (solid blue and gray) is only depicted in cycle 1 and 2.

圖 3　聚合酶連鎖反應。以 DNA 樣本作為增殖目標序列之模板，短核酸寡聚物作為引子，反應混合物亦包含熱穩定 DNA 聚合酶與足量的個別核苷酸，原始模板 DNA（深藍與灰）只在循環 1 與 2 畫出。

One tool to employ DNA sequence for detection is the polymerase chain reaction (PCR), arguably the most significant development in biochemical technology in the twentieth century. This technique relies on the thermal lability of DNA complementation as well as the discovery of certain DNA polymerases that withstand the temperature regimes necessary to denature the double-stranded DNA. DNA polymerases are enzymes (proteins) that biosynthesize linear strands of DNA from the individual nucleic acids, given a complementary strand and a short segment (10 to 25 nucleotides) of double-stranded DNA (Figure 3). By combining pairs of short DNA oligomers ("primers") which complement a segment of DNA on opposite strands and distal to one another, with a template of target DNA, along with thermally-stable DNA polymerase in a solution of individual nucleotides in a thermocycler (a device to alternatively heat and cool the solution to prescribed temperatures for specific time periods) it is possible to amplify the target DNA exponentially.

要用 DNA 序列來偵測，其中一項工具是聚合酶連鎖反應（polymerase chain reaction, PCR），這可說是生物科技的最重大進展。這項技術有賴於 DNA 互補的受熱不穩定、以及發現某些 DNA 聚合酶在一定溫度範圍下可以使雙股 DNA 保持變性（denature）功用。DNA 聚合酶是指在給定互補股與雙股 DNA 的短片段（10 至 25 核苷酸）後，能由各個核酸合成 DNA 的線性股的酵素（蛋白質，如圖 3）。在熱循環器（以預定的時間週期交替加熱冷卻溶液至預定溫度的儀器）中，置入個別核苷酸之溶液與熱穩定 DNA 聚合酶後，藉由將"引子（primer）"（與對應股互補且相隔一段距離的成對 DNA 寡聚物）與目標 DNA 模板結合，目標 DNA 得以指數級增殖。

If a sequence of DNA in an organism is known it is quite simple and inexpensive to synthesize short DNA primers of a prescribed sequence. Complete gene sequences for an ever expanding library of organisms are being determined and literally millions of partial sequences are now known and readily available from public databases on the internet. By alternately raising the solution temperature to the point that the double-stranded DNA denatures into two complementary single strands, then lowering the reaction temperature to a point that allows complementary DNA strands to re-anneal, some of the primers will spontaneously bind to their complementary segments on the single-stranded DNA. These short segments of double-stranded DNA serve to initiate polymerization, through the action of DNA polymerase, which fill out the complementary strand until the reaction vessel is re-heated and again denatures the double strands into single strands. Because DNA strands have directionality (the ends are termed 5' and 3') and DNA polymerase only works by extending the DNA polymer in the 5' to 3' direction, and because the primers are designed to complement the double-stranded DNA on opposite strands, only the intervening sequence is amplified during a single thermal cycle. As the reaction vessel is subjected to repeated cycles of high and low temperatures the intervening sequence is doubled with each round. Hence, with X thermocycles 2X copies of the original sequence are generated. Thermocyclers are now available that can complete 3 to 4 cycles per minute. In addition, clever use of fluorescent labels for the generated

amplicons (amplified segments of DNA) has substantially improved detection levels. Other nucleic acid hybridization techniques are also being exploited for the identification of biowarfare agents.4

如果一個生物體的DNA序列已知，合成給定序列的短DNA引子是相當容易且便宜的。越來越多生物體的完整基因序列正被定序，且目前已有百萬計的部分序列已知（可從網路上的公共資料庫取得）。藉由交替提升溶液溫度使雙股DNA變性成兩互補單股，接著降低反應溫度使互補的股重新黏合（anneal），某些引子將自動與單股DNA上的互補片段鍵結。這些雙股DNA短片段提供聚合的開頭，DNA聚合酶的作用則是填滿互補股，直到反應器重新加熱而雙股再變成單股。由於DNA股有方向性（末端記為 5'與 3'），而DNA聚合酶只由 5’至 3’方向延長聚合物，且引子是設計來與對應股上的雙股DNA互補，在單一熱循環中只有間隔序列被增殖。隨著反應器重複高低溫循環，每一回合間隔序列成為兩倍多，所以X個熱循環產生 2X個原始序列。目前已有熱循環器可每分鐘完成 3 到 4 個循環。此外，對於被增殖的DNA片段（amplicon）可巧妙使用螢光標籤大大增進它的偵測靈敏度。其它核酸雜交（DNA hybridization）技術亦可用來辨識生物戰劑。[4]

Currently, detection methods based on DNA hybridization are generally considered the most specific. Unfortunately, while the hybridization of specific DNA sequences to target DNA can and does occur in a milieu of competing DNA sequences, interference by other substances or DNA polymerase inhibitors, such as those found in crude extracts of environmental samples, can be severely limiting. Preventing “carry-over” of tar-get DNA from one analysis to another, resulting in false-positive signals, must also be avoided. Employment of fluorescent-labeled hybridization probes for the nascent amplified sequences can substantially lower the amount of PCR product required for detection. Although fairly rapid thermal cycling instruments have been developed, even the best PCR detectors require tens of minutes to amplify detectable levels of target sequence.

目前基於 DNA 雜交的偵測方法被認為是最特異的。不幸地，雖然雜交特定 DNA 序列至目標 DNA，可以也確實存在充滿競爭 DNA 環境中，來自其它物質或 DNA 聚合酶抑制劑的干擾－例如在環境樣本之原始萃取物中發現者－可以造成嚴重的限制。避免目標 DNA 從一項分析帶入另一項，導致假陽性信號亦必須顧慮。對新增殖序列運用有螢光標籤之雜交探針可大為降低偵測所需的 PCR 產物數量。雖然已開發出相當迅速的熱循環器，但即使最好的 PCR 偵測器亦須要幾十分鐘，才能增殖目標序列至可偵測程度。

[4] Ivnitski, D.; O'Neil, D. J.; Gattuso, A.; Schlicht, R.; Calidonna, M.; Fisher, R. *BioTechniques* 2003, *35*, 862-869.

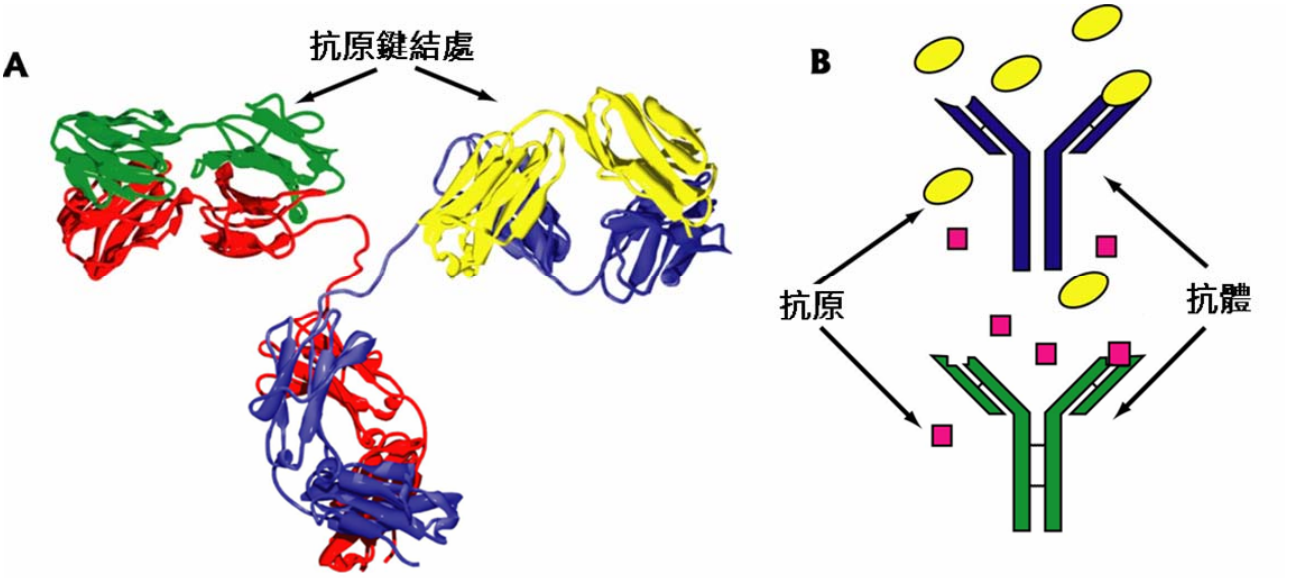

Figure 4. Antibody Molecules. (A) A ribbon structure depicting the protein molecules composing an antibody. (B) Stylistic representation of antibody molecules.

圖 4　抗體分子。(A) 緞帶結構圖描畫組成抗體的蛋白質分子，(B) 抗體分子示意圖。

Molecular Structure-Based Techniques

Immunization is a concept familiar to most people. One of the cornerstones of the immune response is the generation of antibodies. Antibodies are proteins expressed on the surface of and secreted into the blood stream by white blood cells (leukocytes) termed B-cells. These proteins form, in essence, a Y-shaped structure with the tips of the Y convoluted into a three-dimensional configuration with pockets shaped to accommodate the three-dimensional shape of surface molecules on the invading organism (termed antigens) like a hand fits into a glove. In addition to the complementation of the antigen-antibody three-dimensional configuration, chemical and electrostatic interactions between the bound antigen and the antibody are also possible (Figure 4). Recent advances in biotechnology have allowed the production of clones of individual B-cells, i.e. a collection of cells from a single parent, expressing a specific antibody, in artificial culture. Indeed, it is now even possible to generate specific antibodies in genetically engineered yeast cells. Mammals may be able to produce antibodies to more than a billion unique antigens. In addition there are well developed methods to chemically modify antibody molecules, allowing them to be attached to other molecules such as fluorescent dyes or to other surfaces like polymeric beads.

基於分子結構的技術

免疫對大多數人而言是熟悉的概念。免疫反應的根基之一是抗體的產生，抗體是由白血球（leukocyte）中的 B 細胞呈現在表面及釋放到血液中的蛋白質。這些蛋白質基本上形成 Y 型結構，Y 的頂端捲曲成有口袋形的三維組態，以容納入侵生物體（稱為抗原）表面分子的三維形狀，如同手套與手的吻合。除了抗原抗體三維組態的互補外，鍵結抗原抗體間亦可能有化學與靜電作用（如圖 4）。近來生物科技的進展已可在培養皿中生產個別 B 細胞株（clones）－即來自

單母細胞的一群細胞，並呈現特定抗體。事實上目前甚至可能用基因改造過的酵母菌來生產特定抗體，哺乳類可能有能力對超過十億種獨特抗原產生抗體。此外，有完善的化學方法來修飾抗體分子使其可連接其他分子，例如螢光染劑或其它表面聚合物顆粒。

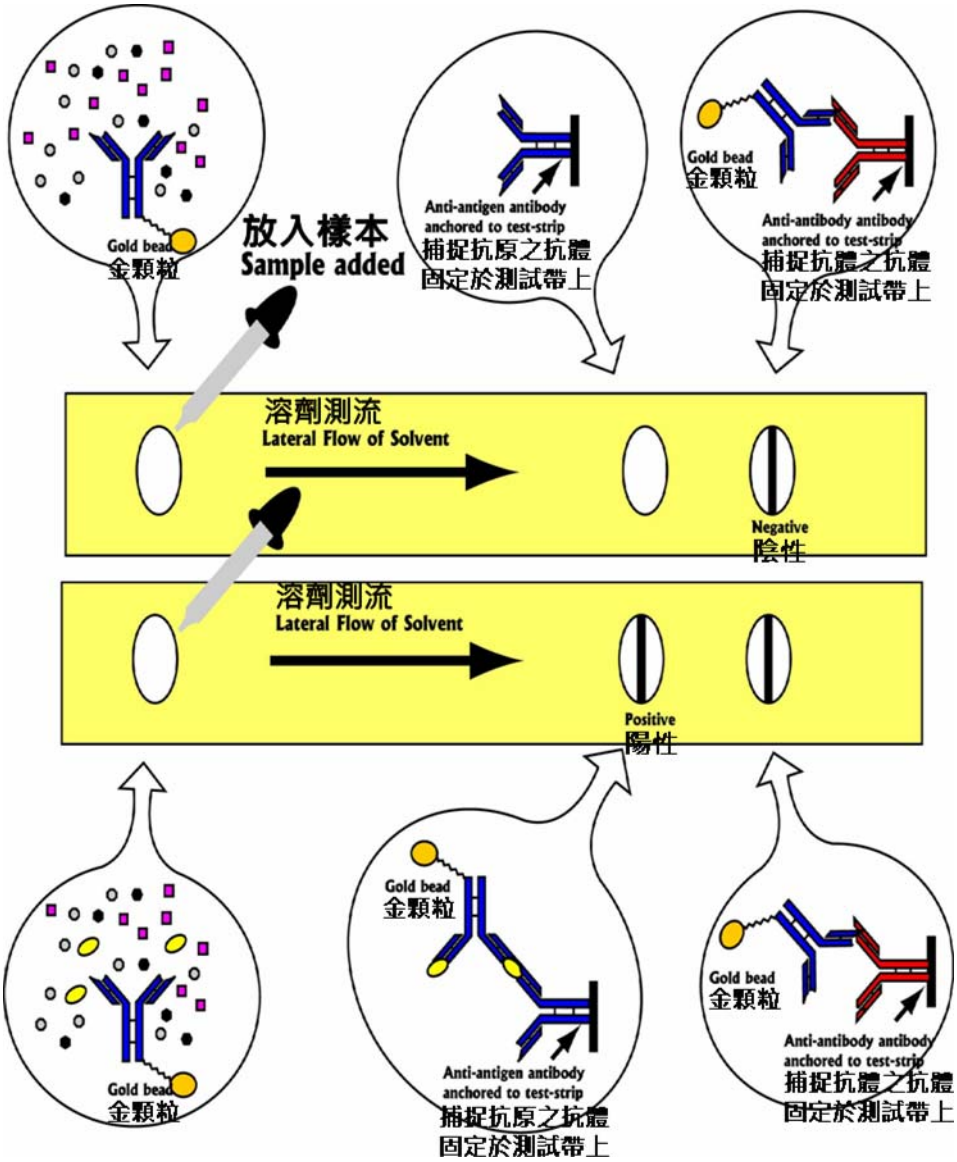

Figure 5. Lateral Flow Immunoassay. A sample solution is loaded into the well containing free, labeled antigen-specific antibodies. The antibodies, bound and/or unbound to antigen, are wicked up the chamber where they encounter a line of antigen specific antibodies and a line of anti-antibody antibodies. A similar technology is employed with the Smart Ticket® and the JPBDS.

圖 5　側流免疫層析。樣本溶液被滴入含有自由標記抗體的測試帶，鍵結/或未鍵結之抗體累積在捕捉抗原之抗體線上與捕捉抗體之抗體線上，類似技術被用在 Smart Ticket®與 JPBDS。

Exploitation of the high specificity of the antibody-antigen interaction for identification of biological molecules has led to numerous bioassay techniques. 5 One familiar example is the over-the-counter pregnancy test. Similar “enzyme linked immunosorbant assays” (ELISA) are now available for biowarfare agent detection.

The Hand-Held Immunochromatographic Assays (HHA) and SMART® tickets are examples of immunoassay-based detectors (Figure 5). Immunochemistry-based detection systems can be quite rapid (seconds to minutes) and fairly specific but, because there is no inherent amplification of the target molecules, they cannot rival PCR-based detection for sensitivity. There are, however, numerous mechanisms for signal transfer from antibody-based detectors that portend their utility, especially in detect-to-warn systems where response time is a critical consideration.

利用抗體抗原反應的高特異性來辨識生物分子導致眾多生物檢定技術。[5]一個大家熟悉的例子是市售的懷孕檢驗。類似的酵素連結免疫吸附法（enzyme linked immunosorbant assays, ELISA）目前可用來偵測生物戰劑。手提式免疫層析生物分析儀（Hand-Held Immunochromatographic Assays, HHA）與SMART® tickets是基於免疫分析的偵測器的例子（如圖 5）。基於免疫化學的偵測系統可以相當迅速（幾秒到幾分鐘）且特異，但由於本質上無目標分子的增殖，其靈敏度無法媲美基於PCR的偵測。然而有許多來自於以抗體為基礎的偵測器的信號轉換機制預示了他的用途，尤其在反應時間是關鍵因素的偵測警告型系統。

One approach, for example, is a system developed by Luminex Corporation that uses styrene beads coated with bioagent-specific antibodies. The beads are impregnated with a fluorescent dye that can be illuminated by a laser. The agent specific beads are thus “color coded” to indicate the particular bioagent detected. Analysis by flow cytometry, i.e. passing the fluorescent-antibody-labeled cells or beads through a narrow tube allowing only one bead at a time and illuminated by a laser coupled with a light detector to measure the fluorescence, allows identification of a biohazard. This device is incorporated into the Autonomous Pathogen Detection System (APDS), developed by Lawrence Livermore National Laboratory and currently used by the Department of Homeland Security.

舉例而言，由 Luminex Corporation 發展的系統是一個方法。此系統使用生物製劑抗體包覆苯乙烯顆粒，顆粒浸過可由雷射顯色的螢光染劑，具製劑特異性之顆粒因此被編上色彩，以表示偵測到特定生物製劑。藉由流式細胞技術（flow cytometry）分析，即將有螢光抗體標記的細胞或顆粒一次一顆通過狹窄管，並由雷射顯色加上光偵測器以測量螢光，如此便得以辨識生物災害。此裝置被整合到勞倫斯利弗莫爾國家實驗室（Lawrence Livermore National Laboratory, LLNL）所發展的全自動致病原偵測系統（Autonomous Pathogen Detection System, APDS），且目前被國土安全部使用。

Chemical and Physical Methods

As indicated earlier, biological agents are composed of certain classes of chemicals. In general terms these are encompassed by: proteins (polymers of the 20 common amino acids), carbohydrates (sugar molecules), nucleic acids, lipids (metabolites soluble in non-polar solvents, typically variations of hydrocarbons with associated functional groups) and relatively low molecular weight metabolites. Although the bulk properties of these classes of compounds are indistinguishable

[5] Peruski, A. H.; Peruski, L. F. J. *Clinical and Diagnostic Laboratory Immunology* 2003, *10*, 506-513.

between organisms, certain aspects of their occurrence can be exploited to identify individual agents. We have already seen how nucleic acid sequence information can be used. Proteins, likewise, have specific sequences. Indeed the central dogma of biology describes the relationship between the DNA sequence and the amino acid sequence of proteins. Unfortunately, the hybridization approach used for DNA sequencing is not possible to determine protein sequences. Nevertheless, methods are available to allow protein sequence determination. One of these is through mass spectrometry.

化學與物理方法

如前所述，生物製劑是由一定種類之化學物質組成。總體而言這些包括蛋白質（20 種常見胺基酸的聚合物）、碳水化合物（醣分子）、核酸、脂質（可溶於非極性溶劑的代謝物，通常是碳水化合物與相連官能基的衍生物）、與分子量相對低的代謝物。雖然在生物體間，這些化合物大部份的性質是無法區別的，它們出現的某些形態卻可用來辨識個別製劑。我們已經知道核酸序列訊息如何使用，蛋白質也有同樣的特定序列。事實上，生物學中心法則描述的是 DNA 序列與蛋白質的胺基酸序列關係。不幸地，用於 DNA 定序的雜交方法不可能決定蛋白質序列。儘管如此，已有方法可以決定蛋白質序列，質譜分析便是其中之一。

The principal of mass spectrometry is really quite simple. A particle moving through a force field will, due to inertia, be more or less affected as a consequence of its mass. Early models of mass spectrometers employed large magnetic sectors to influence the path of charged molecules and had substantial space requirements. In the past few decades far more compact mass selectors have been designed. Based on the use of radiofrequency alternating voltage as well as direct current electric fields to manipulate the trajectory of ions, these instruments are configured as either quadrupole mass filters or ion-traps. Quadrupole mass spectrometers are small and physically robust, permitting use in mobile laboratories such as the M93A1 FOX chemical reconnaissance vehicle. Early designs for the E31 Biological Integrated Detection System (BIDS) included a mass spectrometer but they are not included in the currently fielded version.

質譜分析的原理相當簡單。由於慣性，粒子移動經過一個力場，將根據其質量受到或多或少的影響。早期質譜儀模型乃運用大磁扇形影響帶電分子的路徑，故需要大量空間，近幾十年較為精緻的質量分析器被設計出來。基於使用射頻交流電壓與直流電場操縱離子軌跡，這些儀器被設置成四極棒質量分析器或離子井。四極棒質譜儀小且堅固，可用於機動實驗室如 M93A1 狐式化學偵檢車。E31 生物整合偵測系統（Biological Integrated Detection System, BIDS）的早期設計也部置質譜儀，但目前的版本則否。

Typically the quadrupole or ion-trap is interfaced with a gas or liquid chromatography system that allows separation of complex solutions into individual components. As the individual analytes elute from the chromatographic column they are ionized (electrically charged). In the case of liquid chromatography the process is called electrospray ionization (ESI). The charged particles can then be subjected to

mass spectral analysis. Another format for mass spectral analysis particularly useful for analysis of biological material is termed Matrix Associated Laser Desorption-Time of Flight (MALDI-TOF). This approach uses a light absorbing chemical matrix to embed the analyte of interest, which is then subjected to a pulse of high intensity laser light. The solid matrix volatilizes almost instantly and at the same time imparts an electrical charge on the analyte. The charged analyte is subjected to a voltage potential in a vacuum where it is accelerated to a detector. The lower molecular weight analytes are accelerated more rapidly than the heavier ones; thus molecular weights can be determined by “time-of-flight”. Using sophisticated computer algorithms it is now possible to determine protein sequences from mass spectral data obtained by either ESI-quadrupole mass spectrometry or MALDI-TOF. While the sizes of the quadrupole-type mass spectrometers are comparable to a large coffee cup (TOF instruments are somewhat larger), they both require high vacuums, usually supplied through a rotary vane rough pump and a diffusion or turbomolecular pump. These hardware requirements substantially increase the size, weight, and power requirements of the system. Mass spectrometry is also useful for identification of lipids, carbohydrates, and small metabolites.

通常四極棒或離子井可串連氣相或液相層析系統將複雜容液分離為個別成分。個別分析物從層析柱流出時便被離子化（帶有電荷），就液相層析而言，這個過程稱為電噴灑離子化（electrospray ionization, ESI），接著帶電粒子可接受質譜分析。對分析生物物質特別有用的另一種質譜分析是基質輔助雷射誘發脫附離子化－飛行時間（Matrix Associated Laser Desorption/Ionization-Time of Flight, MALDI-TOF）。此方法使用吸光化學基質包覆目標分析物，接著接受高強度雷射脈衝光，固態基質幾乎瞬間蒸發且同時將電荷賦予分析物。帶電分析物在真空中被施加電壓而加速移往偵測器，較低分子量分析物比重者加速更快，因此分子量可由“飛行時間”決定。運用複雜電腦程式，目前是可能從 ESI-四極棒質譜分析或 MALDI-TOF 得到的質譜資訊來決定蛋白質序列。儘管四極棒型質譜儀大小可比擬一個大咖啡杯（TOF 儀器較大），它們皆需要高度真空，通常由旋轉翼片粗抽泵浦與擴散或渦輪分子泵浦提供。這些硬體需求大為提升系統的體積、重量與能源需求。質譜分析對脂質、碳水化合物、與小代謝物的辨識亦有用。

Another common analysis system is gas chromatography. This instrument is limited to analysis of volatile compounds such as lipids (molecules soluble in non-polar organic solvents) or chemically derivatized lipids. The typical laboratory gas chromatograph is about the size of a large microwave oven and requires external gas tanks to provide a “carrier” gas to carry the volatilized analytes through a long, narrow tubular column. By heating the column as the mixture of particles passes through, advantage can be taken of the differing affinities of the sample admixture for the material coating the wall of the column as well as their different volatilities. Various components can be separated and, with a detector to determine retention time, an indication of the analyte’s identification is obtained. Additional information is afforded depending on the detection method used. Quadrupole mass spectrometers

are commonly employed; thus the chromatographic characteristic as well as a mass spectrum is obtained providing substantial evidence for identification of a chemical component. Microorganisms often produce unique chemical signatures allowing presumptive identification. For the purposes of bioagent detection the inlet to the GC can be interfaced with a pyrolysis chamber. A system that utilizes aerosol pyrolysis (thermal decomposition without oxygen) with gas chromatography and ion-mobility spectrometry is currently under development at the U.S. Army Edgewood Chemical Biological Center (ECBC). 6 Ion-mobility is analogous to a TOF-MS; however, the charged particle traverses an air filled chamber (rather than through a vacuum) and the time of transition under defined voltage conditions provides some indication of the analyte's identity. Although not nearly as definitive as mass spectrometry, this methodology is considerably less expensive and far more compatible to field application. The currently fielded M41 chemical agent alarm uses this technology.

另一常見的分析系統是氣相層析（gas chromatography, GC）。此儀器只限於分析揮發性化合物，例如脂質（可溶於非極性有機溶劑分子）或化學衍生脂質。典型實驗室氣相層析儀大小約同一個微波爐，且須外部氣體筒以攜帶揮發性分析物通過一長窄型管柱。當粒子混合物經過時加熱管柱，利用樣本混合物對附在柱壁物質的不同親和力及其不同的揮發性，分離各種化合物，並且使用偵測器測定滯留時間後，可獲得鑑定分析物的指標，額外訊息可根據使用的偵測方法獲得。四極棒質譜儀常被用來獲得層析特徵與質譜圖，提供大量證據以鑑定化學組成，因為微生物常產生獨特化學標誌，因此可進行推測性鑑定。對生物製劑偵測而言，GC的入口可接到熱裂解室。目前美國陸軍Edgewood Chemical Biological Center正著手研發一個利用氣膠熱裂解（無氧熱分解）及氣相層析與離子遷移質譜分析之系統。[6]離子遷移與TOF質譜儀分析方法類似，然而帶電粒子通過充滿空氣而非真空的腔室，在給定電壓條件下的移動時間提供一些指標以鑑定分析物。雖然不如質譜儀分析結果明確，此法卻廉價的多，而且與更能與戰場應用相結合，目前配賦的M41 化學戰劑警報器便使用這項技術。

Surface Active Sensors

Optical and acoustic waveguide technology is being employed for bioagent detection. Waveguides are simply structures that propagate electromagnetic (e.g. light) or acoustic waves along a specific path. Fiber optics is a familiar application of waveguide technology. Waveguide technology is more accurately termed a signal transduction mechanism. Optical and acoustic waveguides are typically linked to one of four biological recognition elements: enzymatic, immunochemical, nucleic acid, or whole-cell sensors.7 The advantages of waveguide signal transduction are the rapidity with which the signal is transmitted, the relatively simple design of the detectors and potentially low costs and durability. Signal initiation can be generated by a variety of mechanisms, e.g. enzyme reaction with a substrate to generate an

[6] Snyder, A. P.; Maswadeh, W. M.; Wick, C. H.; Dworzanski, J. P.; Tripathi, A. "Correlation of mass spectrometry identified bacterial biomarkers from a fielded pyrolysis-gas chromatography ion mobility spectrometry biodetector with the microbiological gram stain classification scheme," Edgewood Chemical Biological Center, 2005.

optically active (light absorbing or fluorescent) product that alters the incident light to the detector. For example the system could involve linkage to a bioluminescent reaction catalyzed by luciferease (an enzyme that yields light as a product) or to green fluorescent protein. Alternately, single-stranded DNA molecules might be employed to attract a complementary sequence which in turn binds, through a down-stream DNA sequence, to another complementary sequence covalently linked to an optically active probe.

表面作用感測器

光學與波導技術正被運用於生物製劑偵測。波導是將電磁波（例如光）或聲波沿特定路徑傳播的結構。光纖是波導技術的熟悉應用。波導技術更準確地稱為信號換能機制。光學與聲學波導通常連接四種生物感測元件：酵素型、免疫化學型、核酸型、或全細胞感測器。[7]波導信號傳遞的優點是傳遞快速、相對簡單的偵測器設計、潛在低的成本與耐用。信號之引發可由各種機制產生，例如酵素受體反應產生光學活性產物，後者將入射光轉換至偵測器。舉例而言系統可牽涉由冷光酶催化的生物冷光反應或綠螢光蛋白。或者單股DNA分子可能用來吸引互補序列，後者的下游DNA序列鍵結另一共價連接光學活性探針的互補序列。

The basic principle of waveguide transducers involves a core fiber, suitable for transmission of a light or acoustic wave that is surrounded by a cladding with different optical or acoustic properties (e.g. refractive index). Light or sound waves are transmitted by reflectance between the two surfaces with little or no attenuation. Some detector designs incorporate an analyte binding molecule, e.g. an antibody or DNA probe, into the cladding, resulting in an alteration in the optical or acoustic properties of the cladding material upon binding of the target. This, in turn, results in changes in signal transfer efficiency that can be determined electronically.

波導信號換能器的基本原理包含一個適合光或聲傳遞的光纖核心，外層由不同光學或聲學性質（例如折射率）包覆。光波或聲波藉由兩介面間折射傳遞，且幾乎無衰減。有些偵測器設計可將分析物鍵結之分子(例如抗體或 DNA 探針)整合到外層組織，當它與目標鍵結時會導致外層物質光學或聲學性質改變，接著導致訊息傳遞效率改變，這些均可由電子儀器測定得知。

[7] Monk, D. J.; Walt, D. R. *Analytical and Bioanalytical Chemistry* 2004, *379*, 931-945.

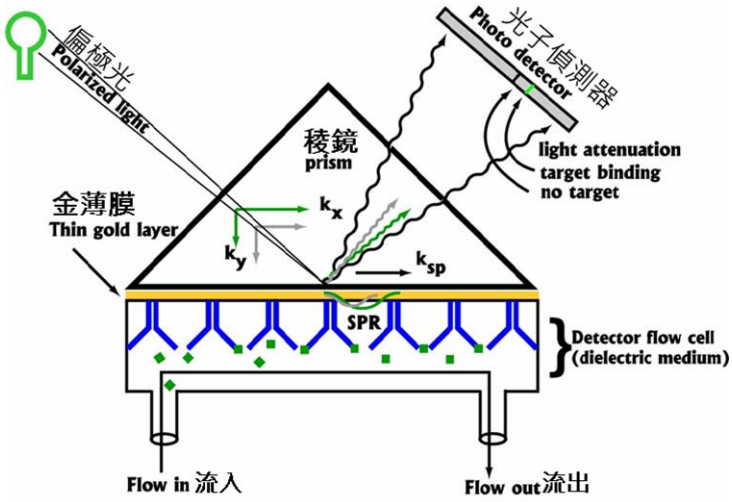

Figure 6. Surface Plasmon Resonance. At the angle where the incident light energy vector (ky) coincides with the charge density wavelength along the metal-dielectric interface, the adsorbed energy results in a sharp decrease in reflected light.

圖 6　表面電漿共振。當入射光能量向量（ky）與沿金屬－電介質介面的電荷密度波長吻合時，能量吸收導致反射光明顯衰減。

A similar type of biosensor that uses refractive index to generate a signal is termed surface plasmon resonance (SPR). When plane polarized light of a particular wavelength impinges at a specific angle on a thin (50 to 100 nm) metal film (e.g. gold) interfaced on one side with a dielectric medium, some of the energy can be adsorbed through interaction with the metal lattice electrons in the form of a charge density wave or plasmon. This adsorbed energy results in a reduction of intensity of the reflected light, which can be measured with high precision. The precise incidence angle at which the surface plasmon occurs is a function of several factors, one being the refractive index of the underlying (dielectric) surface. By including a biomolecular recognition element, e.g. an antibody or DNA probe, at or near the surface of the metal, the reflective index will be altered upon binding of the target (Figure 6). The advantage of SPR is that no fluorescent or otherwise labeled molecules are needed for signal elicitation. This technology also lends itself to miniaturization and offers the potential for detect-to-warn bioagent detection.3,8

利用折射率產生信號的類生物感測器是利用表面電漿共振（surface plasmon resonance, SPR）。當特定波長之平面偏極光由特定角度射向一面貼著電介質的金屬（例如金）薄膜（50 至 150 奈米）時，部分能量經由與金屬晶格電子的作用，以電荷密度波或電漿之形式吸收。能量的吸收導致反射光的強度衰減可精確量測。表面電漿發生的精確入射角度是幾個因素的函數，其一是電介質介面的折射率。將生物分子辨識元素(例如抗體或DNA探針)融入或貼附於金屬表面，當與目標鍵結後折射率將改變（如圖 6）。SPR的優點是不需要螢光或其他分子來標識。這項技術已微型化並可提供偵測警告型生物戰劑偵測器運用。[8]

[8] Homola, J. *Chemical Reviews* 2008, *108*, 462-493.

Currently Fielded Systems

The Joint Biological Point Detection System (JBPDS) is the principal biological agent detection system fielded by the DOD. This system employs a Biological Agent Warning Sensor (BAWS) as a triggering device. Developed by the MIT Lincoln Laboratory and manufactured by Intellitec Products LLC, the BAWS constantly draws environmental air and monitors for a surge in biological material. Using a laser beam to stimulate fluorescence of the particles in the air stream, photomultiplier tubes measure the fluorescent and back-scattered light at particular wavelengths. An algorithm translates these light signatures and interprets whether they derive from biological material; a sudden increase in particle count triggers the JBPDS cyclone aerosol collector to begin operation. The aerosol is transferred through an AHTS and streamed into a immunochemistry based detector. Aliquots are simultaneously collected and stored for follow-up confirmatory analysis. This system is currently capable of detecting 10 biowarfare agents and provides results in less than 30 minutes. The JBPDS is the detection system employed in the Biological Integrated Detection System (BIDS) used by the U.S. Army, Navy, and Air Force.

目前部署系統

聯合生物點偵測系統（JBPDS）是美國國防部(Department of Defense, DOD)部署的主要生物戰劑偵測系統。此系統運用生物戰劑警報器(Biological Agent Warning Sensor, BAWS)作為觸發裝置，由麻省理工學院林肯實驗室研發，由 Intellitec Products LLC 公司製造，BAWS 持續監偵環境空氣及生物物質突增。使用雷射光激發氣流中粒子的螢光，利用光電倍增管來測量特定波長的螢光與反散射光，電腦程式翻譯這些光記號並解釋它們是否來自生物物質，粒子數的突增觸使 JBPDS 旋風型氣膠採集器開始運作。氣膠經過 AHTS 並蒸發進入免疫化學偵測器，部份同時被收集儲存以供後續確認分析。此系統目前能偵測 10 種生物戰劑，並可在 30 分鐘內提供檢測結果。JBPDS 是美國陸海空軍使用的生物整合偵測系統(BIDS)上所用的偵測系統。

A PCR-based detection device called the Joint Biological Agent Identification and Detection System (JBAIDS) is also in the DOD inventory. This instrument evolved from the Ruggedized Advanced Pathogen Identification Device (R.A.P.I.D.® System) developed by Idaho Technology, Inc. The R.A.P.I.D.® System weighs about 50 pounds (23 kg) and measures 19 x 14 x 10 inches (49 x 36 x 27 cm); it is man portable and battery operated. The PCR instrument's detection signal is termed "real-time," meaning it uses fluorescent probes to signal PCR amplification of a target DNA sequence enabling electronic, hence real-time, detection of the amplification. The reagents employed in the JBAIDS are freeze-dried for improved shelf-life and are reported to be stable for 12 months at 28˚C or 2 months at 45˚C. Many of the National Guard Civil Support Teams (CSTs) and other first responders as well as the U.S. Army 1st and 9th Area Medical Laboratories (AMLs) employ the JBAIDS. As the "next generation" R.A.P.I.D.® System, the RAZOR® was recently developed by Idaho Technologies, Inc., for simpler operation using a plastic pouch for easier sample handling.

因 PCR 偵測系統的運用，聯合生物戰劑偵檢辨識系統(Joint Biological Agent Identification and Detection System, JBAIDS)亦在美國國防部作業庫中。此儀器從 Idaho Technology 所研發的 RAPID® System 病原菌辨識器(Ruggedized Advanced Pathogen Identification Device, RAPID)演變而來。RAPID® System 重約 50 磅(23 公斤）大小為 19x14x10 吋（49x36x27 公分），可攜行且以電池供電。PCR 裝置的偵測信號號稱"即時"，意指它使用螢光探針來標示目標 DNA 序列的 PCR 增殖，即可藉電子儀器即時偵測增殖情形。JBAIDS 運用的反應物被冷凍乾燥以延長保存期限，據報告在 20℃環境下可穩定保持 12 個月，在 45℃下則有 2 個月。許多民事支援小組（Civil Support Teams, CST）與其他第一線應援人員，以及美國陸軍第一、第九區醫學研究所（Army Medical Laboratory, AML）都使用 JBAIDS。新一代的 RAPID® System--RAZOR®正由 Idaho Technologies, Inc.研發，期使操作更簡易，它使用塑膠小袋來處理樣本。

A similar device, the Hand-Held Advanced Nucleic Acid Analyzer (HANAA), was developed largely at the Lawrence Livermore National Laboratory. Smiths Detection, a United Kingdom-based technology company, has commercially developed this instrument with the trade name BioSeeqTM. The HANAA marketed by Smiths Detection is a compact (13 x 7 x 4 inches or 34 x 19 x 10 cm), light-weight (approximately 5 pounds or 2.6 kg), battery operated, field-deployable PCR instrument and costs about $35K. This device can monitor for Bacillus anthracis, Francisella tularensis, Yersinia pestis and orthopox virus simultaneously and provide identification in less than 30 minutes. The machine is robust and requires relatively little training. These systems require user sample preparation but little technical expertise. As the "next generation" unit, the BioSeeqTM PLUS was developed as a fully field-deployable detector that is more amenable to hand-held operation.

類似的裝置如手提式高等核酸分析儀（Hand-Held Advanced Nucleic Acid Analyzer, HANAA）主要由勞倫斯利弗莫爾國家實驗室(LLNL)研發。一家英國科技公司 Smith Detection 已將此儀器商業化發展，並申請商標「Bio-SeeqTM」。Smith Detection 的 HANAA 是一組較小（34x19x10 公分）、較輕（2.6 公斤），可以電池供電，可野戰部署（field-deployable）的 PCR 儀器，且其成本約為美金 35,000 元。此裝置可同時監偵炭疽桿菌、兔熱病桿菌、鼠疫桿菌與痘病毒，並可在 30 分鐘內提供辨識結果，機器堅固而且幾乎不須特別操作訓練，惟須人工樣本製備，但幾乎不須具備專業知識。Bio-Seeq TM PLUS 預訂研發下一代裝置為完全可供野戰運用的偵測器且更適合手持操作。

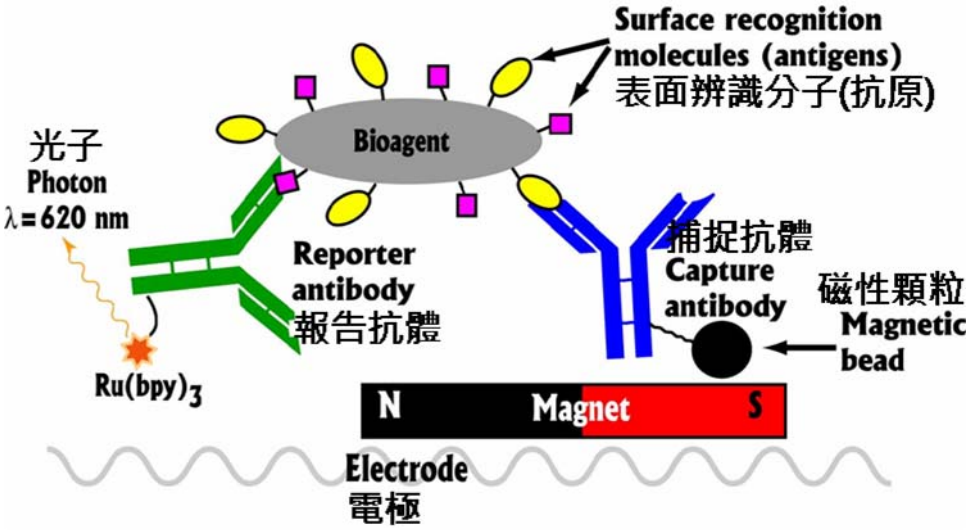

Figure 7. Electrochemiluminescence (ECL). One antibody is bound to a magnetic bead facilitating transport of the complex to the proximity of an electrode. The other antibody is linked to ruthenium tribispyridine (Ru(byp)3) which yields a photon of light upon stimulus through a reduction-oxidation reaction with tripropylamine (TPA) in the presence of a charged anode. This technology is employed with the BioVeris M1M.

圖 7　電化學發光。一抗體連接磁性顆粒，以利將複合物輸送至電極末端。另一抗體連接 Ru(byp)3，與 TPA 在施加電壓後進行氧化還原反應而放出光子。此技術被用在 BioVeris M1M。

Complementing the JBAIDS on many CSTs and the AMLs is the BioVeris M1M electrochemiluminescence (ECL) detector. ECL allows substantially lower limits of detection than conventional immunoassay techniques. This is accomplished through a light signal generated by the antigen-antibody complex. Basically this instrument employs ELISA technology in which one antigen specific antibody is linked to a magnetic bead and a second antigen specific antibody is bound with ruthenium-tri 2,2'-bipyridine (Ru(bpy)3). When both antibodies bind the target antigen (a bioagent or toxin) a magnet attracts the complex to the proximity of an electrode in a solution of tripropylamine (TPA). Application of an electric potential oxidizes both the TPA and the Ru(bpy)3 complex. These two chemical species interact resulting in the Ru(bpy)3 emitting a photon, which can be detected with a photomultiplier tube and converted to an electric current. By rapid alteration of the electrode potential multiple photons are emitted from each of the bound Ru(bpy)3 labeled antibodies (Figure 7). This advent allows exquisite sensitivity; femtomolar levels (10-15 moles per liter) of analyte can be detected.

與眾多的民事支援小組（CST）及陸軍醫學研究所(AML)互補的是，JBAIDS 的 Bio Veris M1M 是電化學發光（electrochemiluminescence, ECL）偵測器。比起傳統免疫分析技術，ECL 由抗原抗體複合物產生的光信號使得偵測極限大為降低。基本上此儀器運用酵素連結免疫吸附技術（ELISA），其中一個捕捉抗原之抗體連接磁性顆粒，另一個捕捉抗原之抗體連接 ruthenium-tri 2,2'-bipyridine（Ru(bpy)3）。當兩抗體與目標抗原（生物製劑或毒素）在三丙基胺（tripropylamine,

TPA）溶液中鍵結後，磁鐵吸引複合物至電極末端。施加電壓使 TPA 與 Ru(bpy)3 複合物皆氧化，此二化學物質交互作用導致 Ru(bpy)3 放出一光子，這可用光電倍增管偵測並轉化為電流，藉由快速改變電極電壓，從各鍵結的 Ru(bpy)3 標記抗體可放出多個光子（如圖 7）。此技術出現使精密靈敏度成為可能，飛莫耳濃度（femtomolar）等級（每公升 10-15 莫耳）的分析物可被偵測到。

Future Directions

The “anthrax letter” attacks in the aftermath of 9/11 clearly illustrate our nation’s vulnerability to biological terrorism. As safeguards to provide homeland defense against future biological attacks, bioagent detection systems, like those described in this article, are employed to monitor key government buildings, transportation hubs, and special events. Systems are also employed to protect military installations and the warfighter on the battlefield. While these systems provide reliable detection capability, they are costly and require operators with specialized training. In order to field a more comprehensive, responsive detection capability, we need small, inexpensive, autonomous, and highly sensitive bioagent detectors a concept similar to today’s “smoke detector”. Such devices could be mounted in multiple locations or worn by soldiers, left un-attended or under remote control, and would be relatively inexpensive to maintain.

未來方向

在 911 餘波中的炭疽信攻擊，清楚顯示美國對生物恐怖主義的脆弱性。作為提供對抗未來生物攻擊的國土防衛機制，像本文描述的生物戰劑偵測系統被用來監偵重要政府建物、交通樞紐與特別活動，這些系統亦用來保護軍事設施與戰場上的戰士。儘管這些系統能提供可靠的偵測能力，但它們造價昂貴且須有專門訓練的操作者。為了部署更全面的、反應迅速的偵測戰力，我們需要更小、更廉價、更自動且高度靈敏的生物製劑偵測器，類似今日的煙霧偵測器的概念。此種裝置可安裝於多個地點或由士兵隨身配戴，不須特別關注或由遠端遙控，更重要的是它的維護費用會相對便宜。

Nearly all the biorecognition chemistries described in this article necessarily occur in solution. Transfer of aerosolized bioagents to solution at detectable concentrations is a major technological hurdle. At present there are numerous biodetection systems available. A 2007 survey of systems with utility for homeland security catalogs over 100 bioagent detection devices commercially available.9 However, they all suffer serious shortcomings, especially in the area of cost, speed of operation, and maintenance requirements. The JBPDS, for example, has a fairly rapid response time and requires little on-site operation, but system size, power requirements and capital as well as operating costs limit its employment. A U.S. Army BIDS company, for example, is a Corps-level asset consisting of 35 mobile units. The APDS, which combines automated sampling with automated immunochemical detection, sample preparation and PCR confirmation, is an impressive system in terms of autonomous operation, but also carries an impressive price tag and it occupies the space of a three-drawer file cabinet.

幾乎所有本文所列述的生物辨識化學裝置均須在溶液中作用。如何將氣膠

化生物製劑以可偵測濃度轉移至溶液是主要的技術障礙，目前有許多生物偵測系統可用。2007 年執行了一項有利於國土防衛之系統調查，編列超過 100 項生物製劑偵測器商品[9]，然而它們全有某些缺點，尤其在成本、運作速度、與維護需求方面。例如JBPDS反應時間相當快速，且幾乎不須要現場操作，但因系統大小、能源需求與資金及運作成本而限制其採用。美國陸軍BIDS連屬軍團等級，由 35 個機動單位組成。APDS結合自動採樣、自動免疫化學偵測、樣本製備與PCR確認，就自動化作業而言是令人印象深刻的系統，但也有著可觀的價格，並且體積占據一個三層抽屜文件櫃的大小。

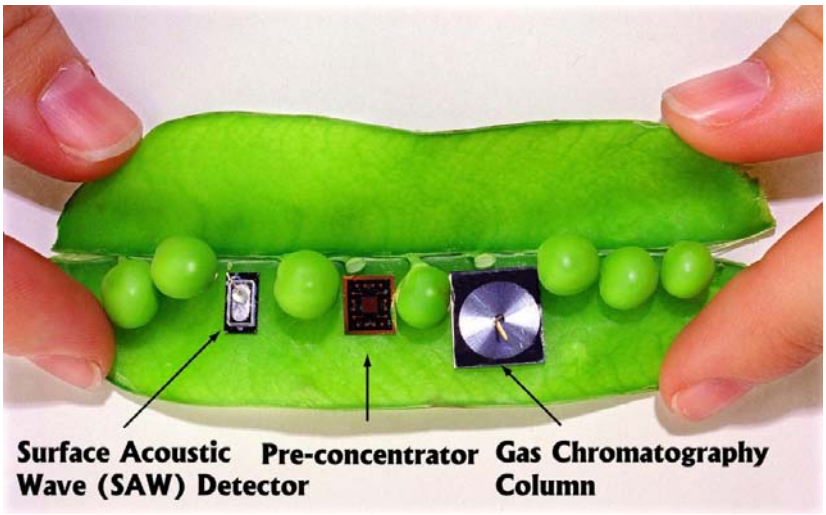

Figure 8. Key components of Sandia's ChemLab compared to "peas in a pod". Advances in micro-fabrication portend rapid development in the field of micro total analysis (or "lab-on-a-chip") instrumentation.

圖 8　聖地亞的 ChemLab 的主要元件與英中豌豆比較。微製造之進展預示了微全分析系統（或實驗室晶片）領域的迅速開發。

Nevertheless, tremendous strides are being taken in the field of biodetection. For example, the MicroChem station, in development by Sandia National Laboratory since 1996, was originally designed as a chemical agent and explosives detector. This device employs micro-fabrication technology to miniaturized chromatography and detection components. Early models intended for chemical agent detection included a gas-chromatography column the size of a nickel (Figure 8). Recent developments have incorporated a liquid chromatographic capability, with a working volume of picoliters (10-12 liter), which allows detection of non-volatile biological material including bacteria, viruses and toxins. Separation of complex mixtures is accomplished by electrokinetic mobility of extremely small volumes of ionic solvents through semiporous media under the influence of a small electric field. Although presently of limited applicability for the detection of biological warfare agents, this system illustrates some of the remarkable advances being made in miniaturization of biosensors, the socalled "lab-on-a-chip" or micro total analysis systems. It has been likened to the "tricorder" used by Bones, the pixilated ship's doctor in the Star Trek

[9] Fatah, A. A.; Barrett, J. A.; Arcilesi, R. D. J.; Ewing, K. J.; Lattin, C. H.; Moshier, T. F.; Justice, D. O., Ed., 2001; National Institute of Justice Guide 101-00; pp 41.

television series of the 1960s.

儘管如此，生物偵測領域正有重大進展。例如，1996 年由聖地亞國家實驗室（Sandia National Laboratory）開發的微型化學站(Micro-Chem station) ，此裝置運用微製造技術，以微型化層析與偵測元件，原本是為化學製劑與爆裂物偵測而設計。早期用作化學製劑偵測的模型包含五分錢幣大小的氣相層析柱（如圖 8）。最近已完成液相層析能力發展整合，具有 picoliter（10-12 公升）的運作體積，可偵測非揮發性生物物質，包括細菌、病毒與毒素。複雜混合物的分離藉由極小體積之分子溶劑在小電場作用下，通過 semi-porous 介質的電動遷移率來達成。雖然目前生物戰劑偵測之應用有限，此系統展現了部分在生物感測器微型化方面的顯著進展，即所謂“實驗室晶片”或“微全分析系統”。它已被比擬為 1960 年代 Star Trek 電視影集中太空艦上 Bones 醫生所使用的“tricorder”。

Since 2001 the U.S. Federal Government has provided over $40 billion for biodefense, a significant portion going to research and acquisition of biodetection devices.10 While there has been legitimate criticism for these expenditures,11 we need still more investment to counter the emerging bioterrorism threat. Furthermore, like the advances in computer technology and materials science resulting from our national space program, research on bioagent detection technology is also having an impact outside the arena of biodefense, including medicine, forensics, and food safety. The DOD should help guide future investment in the field to expedite improvements in bioagent detection systems. Strategies to advance biodetection capability must address development of systems that not only provide reliable, accurate and sensitive detection (essential features) but also focus on minimal cost, operation speed and simplicity, minimal operator burden, field utility, and effective sample handling.

自 2001 年起美國聯邦政府在生物防禦上已提供超過 400 億美元，其中為數可觀的一部分用於研發與獲取生物偵測裝置。[10]雖然這些支出的正當性受到批評[11]，我們仍需要更多投資來反擊浮現中的生物恐怖攻擊威脅。如同電腦科技與材料科學的進展來自國家太空計畫，生物製劑偵測技術之研究亦在生物防禦領域之外有重大影響，包括醫學、法醫學與食物安全。美國國防部應協助指導在此領域的投資，以促進生物製劑偵測系統之進展。推進生物偵測戰力之策略，必須滿足發展出來的偵測系統不只可靠、準確、靈敏（必要性質），亦著眼於成本最小、作業簡單迅速、操作負擔最小、方便緊急使用與有效率的樣本處理。

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