Commercial platforms for Security Sensitive Biological Agents testing

John Bates
Email: jhnrbates@outlook.com

Abstract. The rapid testing for Security Sensitive Biological Agents is carried out by Public Health laboratories. The commercial platforms for performing such tasks in Australia are described.

When the Anthrax Mail attacks struck the USA at the end of 2001, there was very little capability to detect Security Sensitive Biological Agents (SSBAs) in Australia. Forensic and Scientific Services in Queensland had developed an anthrax PCR based on a published paper[1] and this test methodology was shared with the major Public Health Laboratory Network (PHLN) laboratories in a workshop coordinated by the Commonwealth Department of Health so that all States could rapidly process environmental samples for anthrax, which were being received in large numbers across the country at the time. While the emphasis at this time in Australia was totally on the detection of anthrax spores, in the USA the focus quickly shifted to an all hazards approach and the Laboratory Response Network (LRN) laboratories there were tasked with the detection of multiple agents.

The technologies that have been developed fall into two main categories, namely immunological detection of SSBAs using handheld tickets impregnated with specific antibodies, and molecular detection methods including PCR and molecular arrays. While there has been a lot of development in this area, there are only a handful of products that have become commercially available. The biggest challenge for any test is the wide range of sample matrices that it needs to be able to analyse. This includes clinical isolates or samples, a wide range of white powders and swabs collected from environmental sites. The handheld tickets in particular have suffered from a lack of sensitivity and specificity, which has compromised their reliability for field testing. At best, they indicate which samples need to be re-tested in a NATA accredited laboratory. PCR reactions are also affected by sample matrices like faeces, fats from food samples and some white powders, so sample preparation is very important. The other major handicap of many of the tests on the market was that they could only test one agent at a time. This is particularly true for the handheld tickets. The development of microarray technology has greatly increased the laboratory's ability to screen for multiple agents at the same time.

There was a clear need for a rapid test that would detect anthrax spores in a white powder sample at the time of the anthrax mail attacks, and in consultation with the public health laboratories the Commonwealth purchased Rapid Analyte Measurement Platform (RAMP) machines and placed them in PHLN laboratories around the country. The RAMP involved a ticket that went through a 15-min incubation and the final result was then read in a reader to give a result. This provided detection of anthrax spores and using a separate ticket, ricin toxin. The botulinum ticket was unreliable for the detection of Australian strains of botulism. However, extensive testing in PHLN and police laboratories demonstrated that false positives could occur with some sample matrices, for example, urine.

In the USA, bioterrorism attracted big funding and gave birth to the LRN, which was then resourced to provide a response to a wide range of potential bioterrorist agents as well as anthrax. To further improve scanning of the mail network, environmental sampling units were placed in all major mail sorting areas. Over a 24-h period, a number of filters were used to filter the airflow through the sampler, and these were then transferred to a public health laboratory for PCR testing (the BioWatch program)[2]. Public data suggests that the PCR testing provided surveillance for anthrax, plague, tularaemia and smallpox. This system was also deployed in the US for air monitoring in major urban settings (first deployed for the Salt Lake City Olympics in 2002). Testing in LRN laboratories was conducted using the Cepheid GeneXpert system that allows for multiplex testing. This program of environmental surveillance was never adopted in Australia. A number of Australian laboratories purchased GeneXpert machines for clinical microbiology purposes, and were able to purchase

specific pouches for SSBAs, but these were quite expensive and required specific software and regular maintenance calibration of the machine, which involved sending the unit overseas.

Following the white powder incidents around Australia, the Commonwealth Department of Health approached the Centres for Disease Control in Atlanta, Georgia and as a result of those discussions, six laboratories in Australia were admitted to the LRN. Membership of LRN enabled access to LRN reagents and test protocols for a range of agents, including phage lysis assays, Direct Fluorescent antibody stains, phenotypic tests and specific PCRs. The reagents were all quality controlled and could be used with confidence by strict adherence to the LRN protocols. The original PCR assays were developed for the Light Cycler instrument, but the testing platform was subsequently migrated to the Applied Biosystems 7500 Fast DX real time instrument. The Commonwealth Department of Health agreed to fund the placement of an instrument in each Australian LRN laboratory to ensure continuity of testing.

Despite the ongoing need for rapid testing of both clinical and environmental samples in this area, technical developments were slow and there has not been an explosion of new commercial technologies in this field[3,4]. By 2017 it was becoming clear that the RAMP was at the end of its useful lifetime, but there was still a need for rapid diagnostics with environmental samples. Although there are a number of tickets on the market, some of them have proven unreliable (not something you want when you're potentially diagnosing a SSBA). The Tetracore tickets have proven to be the best on the market, with the added advantage that there are individual tickets for a range of SSBAs, including anthrax, ricin, abrin and botulinum toxin (the Tetracore product is the only commercial means by which abrin toxin can be detected, but in-house PCRs exist for detection of plant DNA). The main problem with these tickets is that they have limited lifetimes and they are very expensive (the abrin tickets are $1000 a box for 25 tests). There are also F1 antigen kits on the market for rapid diagnosis of *Yersinia pestis*, the causative agent of plague.

The most promising technological development to date has been the release of the BioFire FilmArray unit (Figure 1). While, like the GeneXpert, this has been designed for rapid diagnosis of clinical pathogens and includes pouches for respiratory and faecal pathogens, a company separate from BioFire developed a specific Biothreat pouch for SSBAs that provided 1 h detection of 24 separate agents (*Bacillus anthracis, Francisella tularensis, Yersinia pestis, Brucella sp., Burkholderia mallei/pseudomallei,* Botulinum toxin gene, Staphylococcal enterotoxin gene, Ricin toxin gene, *Coxiella burnetii,* Ebola virus, Eastern Equine Encephalitis virus, Western Equine Encephalitis virus, Venezuelan Equine Encephalitis virus, Marburg virus, Variola virus, Orthopox virus genes, *Rickettsia sp.*). This also required a special software update to the Biofire machine. Each test costs ~$300 and again the shelf life of the pouches is not great, but the PHLN laboratories doing this testing are encouraged to use the Biofire instrument on samples supplied as part of the RCPA Biosecurity Quality Assurance Program, so this helps in turning over available stock. In 2018 the Commonwealth Department of Health purchased a Biofire instrument for each PHLN laboratory doing SSBA work and provided a box (six panels per kit) of Biothreat panel per laboratory every year. To increase the usefulness of these instruments for routine clinical assays, many of them were sited in the PC2 laboratory rather than being a dedicated PC3 instrument. This created some problems because it necessitated the transfer of decontaminated and deactivated DNA from the PC3 laboratory to the PC2 laboratory. Dr Amy Jennison from Queensland Health Forensic and Scientific Services developed a protocol to enable this and demonstrated that the DNA from a culture in PC3 could be applied to the microarray kits (rather than a culture in solution) and still produce a detectable result.

Figure 1. The BioFire FilmArray System (reproduced with permission from BioFire Diagnostics).

So, there remains a place for both handheld tickets for rapid detection of anthrax spores, and ricin and abrin toxins (15 min), and the FilmArray technology for multi-agent detection on a single sample (1 h). Any positive result needs to be thoroughly supported by conventional tests using LRN protocols, including agent specific PCRs, preferably following successful culture of the agent in the PC3 laboratory.

References

1. Ramisse, V. *et al.* (1996) Identification and characterization of *Bacillus anthracis* by multiplex PCR analysis on sequences on plasmids pXO1 and pXO2 and chromosomal DNA. *FEMS Microbiol. Lett.* **145**, 9–16. doi:10.1111/j.1574-6968.1996.tb08548.x

2. Grundmann, O. (2014) The current state of bioterrorism attack surveillance and preparedness in the US. *Risk Manag. Healthc. Policy* **7**, 177–187. doi:10.2147/RMHP.S56047

3. Lim, D.V. *et al*. (2005) Current and developing technologies for monitoring agents of bioterrorism and biowarfare. *Clin. Microbiol. Rev.* **18**, 583–607. doi:10.1128/CMR.18.4.583-607.2005

4. Mirski, T. *et al*. (2014) Review of methods used for the identification of biothreat agents in environmental protection and human health aspects. *Ann. Agric. Environ. Med.* **21**, 224–234. doi:10.5604/1232-1966.1108581

Biography

Prior to retirement, **John Bates** specialised in the examination of micro-organisms of public health significance in clinical, food, water and environmental samples, including *Giardia*, *Cryptosporidium* and *Legionella*. Other areas of expertise included laboratory-based outbreak investigations, notifiable pathogens and bioterrorist agents referred to the laboratory for identification and typing. He ran a complex surveillance system for *Salmonella* infections across the State, and the tools of outbreak investigation and surveillance can be readily applied to intentional releases. Through his involvement with the Public Health Laboratory Network of Australia (which he has chaired twice), the Australian (counter) Bioterrorism Laboratory Network and the US Laboratory Response Network, he played a key role in the development of a capability within Queensland to identify bioterrorist bacterial agents and toxins in a timely manner. As a result, he provided expert advice to Queensland Health and to laboratory clients, as well as reports, based on the monitoring of trends of known diseases and the emergence of new diseases, to health authorities and clients.

A decade of RCPAQAP Biosecurity improving testing for biological threats in Australia





Katherine A Lau[A,C], *Torsten Theis*[A], *Alexa M Kaufer*[A], *Joanna L Gray*[A] *and William D Rawlinson*[B]

[A]Royal College of Pathologists of Australasia Quality Assurance Programs, Biosecurity Department, Sydney, NSW, Australia

[B]Serology and Virology Division (SAViD), NSW Health Pathology, SOMS and BABS, University of New South Wales, Sydney, NSW, Australia

[C]Email: Katherine.Lau@rcpaqap.com.au