Forensic and Scientific Services

POCT and Rapid Testing Technology for Emerging Diseases and Biothreat Agents

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Overview

- POCT – Strengths and weaknesses
- Types of POCT
- The Regulatory Environment
  - PHLN
  - ABLN
  - LRN
  - The SSBA Regulatory Scheme
- POCT Biothreat Tests
- BioFire FilmArray
- RCPA Biosecurity QAP

POCT – Strength and Weaknesses

- Offer a much earlier time point in outbreak detection
- Costs per sample
- Biosafety levels required for sample preparation
- Complexity of equipment
- Infrastructure required
- Complexity of sample tracking
- Levels of training required to conduct tests
- Quality control

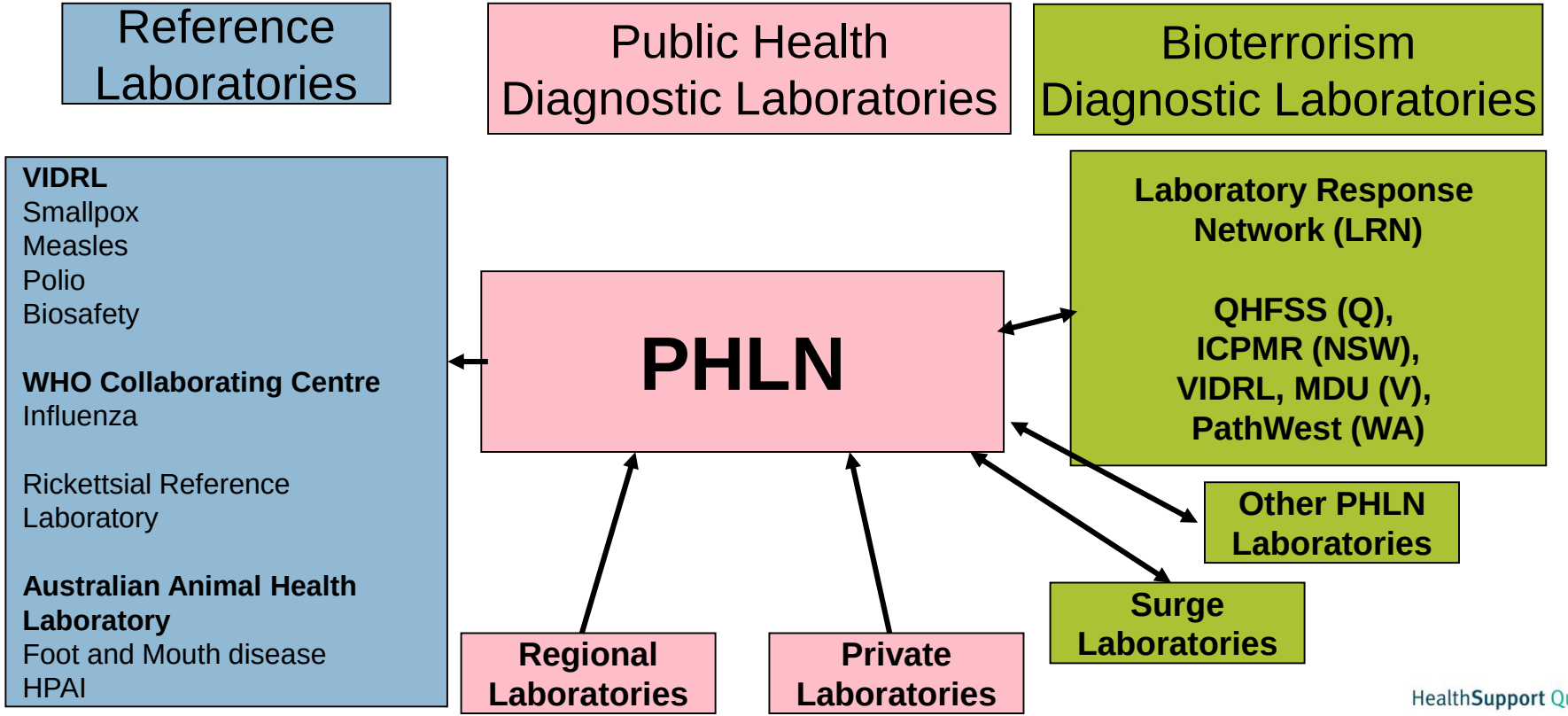
POCT – Tests for Emerging Viral Pathogens

- An ideal diagnostic would combine the sensitivity, specificity, and flexibility of nucleic acid diagnostics with the speed and ease of use of antigen-based tests.
- A suitable diagnostic test should be able to be rapidly deployed for emerging viral outbreaks in the field as well as being of sufficient calibre to be used for routine clinical use.
- One such test is the Cas13-based nucleic acid detection platform SHERLOCK (specific high-sensitivity enzymatic reporter unlocking)
- Combines isothermal amplification via recombinase polymerase amplification with highly specific Cas13-based detection.
- To bypass the need for nucleic acid extraction, a technique called HUDSON (heating unextracted diagnostic samples to obliterate nucleases) was developed.
- These techniques combined reliably differentiate DENV, ZIKV and YFV, as well as differentiating the 4 DENV serotypes and detecting SNPs among ZIKV isolates

Types of POCT

- Lateral flow devices - Tetracore
- Lateral flow devices that require a reader (eg. RAMP)
- Molecular tests (loop isothermal amplification) – being developed by DST for biothreat agents. RT-LAMP applied to ZIKV outbreak in Central & South America
- Fluorescent excitation
- Field-deployable viral diagnostics using CRISPR-Cas13 – DENV, ZIKV, YFV
- Nanopore Sequencing (MinION) – used for EBV
- Mobile phone microplate readers for viral serology & RT-LAMP (ZIKV, DENV & CHIKV)
- Other molecular platforms, eg. GeneXpert, Biofire FilmArray

Diagnostic Laboratory Networks in Australia



HealthSupport Queensland

Australian (Counter) Bioterrorism Laboratory Network

- The Australian (counter) Bioterrorism Laboratory Network had its first meeting on 20th June 2008
- This is a network of the Australian LRN labs (and PHLN labs who are not members), AFP, NSW Police Forensic Services group, Aust. Animal Health Lab & DST
- Department provides secretariat and policy support

Role of the ABLN

- To establish, maintain and expand collaborative links between public health and law enforcement agencies at a technical level to advise on issues relating to detection and analysis of security sensitive biological agents (SSBA).
- The ABLN provides the final confirmation of BT agents and toxins in Australia.

Functions of the ABLN

- Advise on laboratory procedures used to:

  - detect, identify and further characterise biological threat agents

  - otherwise support the accurate diagnosis of infectious disease in order to improve the scientific integrity of evidence supplied for forensic investigation and subsequent prosecution of biocrime.
- Provide technical advice on the detection of biological agents including training of scientific staff in public health laboratories and law enforcement agencies.
- Collaboration with, and provision of strategic advice to, PHLN and the Chemical Warfare Agent Laboratory Network (CWALN), to ensure optimal use of relevant laboratory resources during incidents involving suspected and/or confirmed use of biological agents.
- Enhance collaboration and facilitate dialogue between public health and law enforcement agencies in the States/Territories, including better coordination of responses and joint investigation.
- Identify research opportunities and gap analysis for biological agents as required.

Relationship with the LRN

- The Laboratory Response Network is a select group of public health labs (usually State) in the US.
- Since its creation, more labs have been added to the membership in the US
- It has now become international, with labs in the UK, Canada, and Australia, invited to join.
- All labs have to meet certain requirements in terms of capability, facilities and biosecurity

Role of the Laboratory Response Network

- To promote a common methodology for use by all member labs using validated protocols
- To supply reagents for the identification of bioterrorist agents
- To develop new tests and protocols for the rapid identification of bioterrorist agents
- To provide a secure conduit for the rapid dissemination of information related to potential bioterrorist attacks

Capabilities provided by LRN Membership

- Diagnosis of anthrax, plague, tularaemia, brucella, botulism, Burkholderia, staphylococcal enterotoxin, ricin, Q-fever and smallpox, Zika, MERS, Ebola
- Utilises a range of technologies, including DFA, TRF, Gamma-phage, and PCR
- In addition, we could access a number of non-virulent control organisms and positive control material – this is exceedingly important!!
- A key component of this membership is participation in quality assurance exercises to test our capability and ability to provide accurate diagnoses of agents.

Genesis of SSBA Regulations

- COAG Review of Hazardous Materials
  - Ammonium nitrate (completed 2005)
  - Biological (completed 2007)
  - Chemical (completed 2008)
  - Radiological (completed 2007)
- January 2006—Banks Report—national consistency in regulation, build on existing arrangements, cost effectiveness, minimal impact regulation

COAG Biological Review

- 2007—recommended a regulatory scheme for security-sensitive biological agents (SSBAs)
- Purpose:
  - prevent the deliberate use of biological agents that may cause harm to both human health and the economy
- Review acknowledged there are few controls currently covering security of biological agents in Australia

Regulatory Scheme

- Commenced 31 January 2009 with regulation of Tier 1 SSBAs
- NHS Act 2007
- NHS Regulations 2008
- SSBA Standards
- National Register supported by mandatory reporting
- Education and awareness raising
- Training

The List of SSBA

Tier 1

- Abrin toxin
- *Bacillus anthracis* (Anthrax—virulent forms)
- Botulinum toxins
- *Ebolavirus*
- Foot and mouth disease virus
- Highly pathogenic influenza virus, infecting humans
- *Marburgvirus*
- Ricin toxins
- Rinderpest
- SARS coronavirus
- *Variolavirus* (Smallpox)
- *Yersinia pestis* (Plague)

Tier 2

- African swine fever
- *Capripoxvirus* (Sheep pox virus & goat pox virus)
- Classical swine fever virus
- *Clostridium botulinum* (Botulism; toxin-producing strains)
- *Francisella tularensis* (Tularemia)
- Lumpy skin disease virus
- Peste des petits ruminants virus
- Yellow fever virus (non-vaccine strains)

DoH OHP funded PHLN/ABLN Outputs

- Rapid Analyte Measurement Platform (RAMP) equipment and kits to all jurisdictions
- Time Resolved Fluorometry (TRF) equipment and training to QHFSS and MDU
- Laboratory capacity planning for Avian Influenza A H5N1, Pandemic Influenza, SARS, biosecurity
- AB 7500 FAST DX units to replace the Light Cyclers originally purchased

RAMP

- Supplied by DoH
- Strips for anthrax, ricin and Bot tox and Smallpox
- Assay read in dedicated reader
- Results in 15 minutes
- Technology has reached its useful lifetime now
- Bot tox strip totally unreliable
- Used by First Responders in Australia

Fig.1 – Leitor RAMP portátil [2]

Fig.2 – "Ramp Ricin Test cartridge" [3]

RAMP Pox strip

- RAMP® POX

- The RAMP® Pox test is used to screen for the presence of variola virus, the causative agent of smallpox (Pox). The test also recognizes monkey pox and cow pox, the only other orthopox viruses in addition to variola and vaccinia, known to cause human infection. Suspect environmental samples may include liquids or powders. A positive test result indicates the presence of variola virus at or above the detectable concentration of 100,000 plaque-forming units (pfu) or more pox viruses (equivalent to 50,000 pfu delivered to the test cartridge).

RAID Biothreat strips from Alexeter Technologies

Tetracore Biothreat Lateral Flow Strips

- Recently rated #1 by an independently conducted FBI/CDC study, the BioThreat Alert strip was proven to be the most accurate and reliable product in the evaluation.
- Simple, easy-to-use test strip system for rapid, on-site analysis of unknown biological samples ... specific test for Anthrax (no cross-reaction to other bacteria).
- The breakthrough test for Anthrax is the first, antibody-based anthrax test demonstrated anywhere in the world that does not cross-react with commonly-available bacterial strains.
- The BioThreat Alert™ Test Strip for Anthrax is the first commercial antibody test demonstrated world-wide with such specificity and is now available for sale to qualified emergency material handlers and reference laboratory facilities.
- In addition, offer other highly specific tests which test for ricin, botulinum toxin, staphylococcal enterotoxin B (SEB), plague, tularemia and brucella. Strips for additional biological agents (such as B. thuringiensis) are expected to be available for delivery in the next few months.

Tetracore Targets

- Anthrax (Cat. # S-1001) Bacillus anthracis - Highly specific. Does not cross-react with other near-neighbor bacterial strains. According to a recent FBI/CDC study, the highest-rated anthrax test strip in America!
- Ricin (Cat. # S-1002) Ricinus communis - Derived from the common castor bean, Ricin is one of the most poisonous naturally occurring substances known.
- Botulinum Toxin (Cat. # S-1003) Derived from: Clostridium botulinum - Derived from the bacteria Clostridium botulinum, botulinum toxin is the second most toxic substance known to man after plutonium.
- SEB (Cat. # S-1004) Derived from: Staphylococcus aureus - Derived from the bacteria Staphylococcus aureaus and also known as Staphylococcal Enterotoxin B, its toxins can induce illness within hours after exposure.
- Plague (Cat. # S-1005) Yersinia pestis - This gram-negative coccobacillus is a zoonotic disease that can be used in a biological warfare scenario & cause many harmful symptoms, the worst being circulatory collapse, respiratory failure and death.
- Tularemia (Cat. # S-1006) Francisella tularensis - From a gram-negative bacillus, known as Francisella tularensis, tularemia is a zoonotic disease.
- Brucella (Cat. # S-1007) Derived from: Brucella genus - Brucellosis is an infectious disease caused by the bacteria of the genus Brucella. These bacteria are primarily passed among animals, and they cause disease in many different vertebrates.
- Orthopox (Cat. # S-1008)

Where’s the Development?

- Given that it’s now 16 years since the anthrax mail attacks, there has been an absolute dearth of new rapid detection technologies in that time (hence our continued reliance on the RAMP platform).
- Whilst a number of products have come to market, many are beset with cross-reaction problems which makes them unreliable for field use (given the nature of the agents being tested for, false positives can have dramatic side effects!)
- ABLN have always maintained that any field result needs to be validated in an ABLN laboratory for this reason.

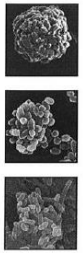
The BioFire FilmArray Biothreat Pouches

- Product is sold by EPE, who operate out of Brisbane. It is not available through Biomerieux.
- It comes with a simple flowchart rather than detailed instructions
- It is classed as a research only application
- The BioFire rep had to install additional software on our PC to accommodate this kit.

FilmArray® BioThreat Panel

Information Sheet

1 Test. 16 BioThreat Pathogens/26 Targets. All in about an hour.

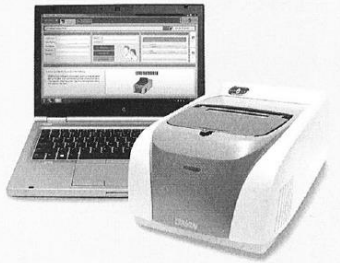


- *Bacillus anthracis*, 3 Targets
- *Brucella* species, 2 Targets
- *Burkholderia mallei / pseudomallei*
- Botulinum toxin gene
- *Coxiella burnetii*, 2 Targets
- Ebola virus
- EEE virus
- *Francisella tularensis*, 2 Targets
- Marburg virus, 2 Targets
- Ricin toxin gene
- *Rickettsia*, 2 Targets
- Variola virus
- VEE virus, 2 Targets
- WEE virus
- *Yersinia pestis*, 2 Targets
- Orthopox genus viruses, 2 Targets

26 Targets

Comprehensive BioThreat Detection

- **Multi-Use:** Used for BioThreat Detection and Pandemic BioSurveillance.
- **Easy-to-Use:** 2 minutes of hands-on time.
- **Fully Automated:** Sample prep, amplification, identification, and reporting.
- **Single Instrument Integration:** Minimal equipment and consumables.
- **Freeze-dried Reagents:** Room temperature stable.
- **More Sample Types:** Integerated sample prep removes PCR inhibitors and allows BioThreat detection in challenging environmental sample types.



If you are interested in a free, no obligation demonstration of the FilmArray in your lab visit www.BioFireDefense.com or call 1-801-266-3592

FREE Demo!

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FilmArray
BioThreat Panel

BIOFIRE

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Run Summary

Sample ID:	EXO206 Validation	**Run Date:**	20 Mar 2018 12:13 PM
Detected:	*Francisella tularensis*	**Controls:**	Passed

Result Summary

	Bacteria	
	Not Detected	*Bacillus anthracis*
	Not Detected	*Brucella melitensis*
	Not Detected	*Burkholderia mallei/pseudomallei*
	Not Detected	*Coxiella burnetii*
✓	Detected	*Francisella tularensis*
	Not Detected	*Rickettsia prowazekii*
	Not Detected	*Yersinia pestis*
	Viruses	
	Not Detected	Ebola Zaire
	Not Detected	Marburg virus
	Not Detected	Orthopox genus virus
	Not Detected	Variola virus
	Not Detected	EEE virus
	Not Detected	VEE virus
	Not Detected	WEE virus
	Toxins	
	Not Detected	*Clostridium botulinum*
	Not Detected	*Ricinus communis*

Run Details

Pouch:	BioThread Panel v2.5	**Protocol:**	BT PBS v3.0
Run Status:	Completed	**Operator:**	Brett Heron (Brett)
Serial No.:	D01238564	**Instrument:**	2FA05351
Lot No.:	171108		

The BioFire Challenge

- We purchased our BioFire specifically to provide rapid detection of faecal pathogens during the Commonwealth Games.
- This meant that the unit had to be housed in the PC2 Lab.
- How to inoculate the biothreat pouches in a safe environment?
- The pouches couldn't be inoculated directly in PC3 because:
  - A. It would result in the removal of a live agent from the PC3 lab
  - B. There was no way of decontaminating the pouch to bring it out
- We decided to trial inactivation of the agents in PC3 (as we do normally for our PCR tests), followed by decontamination of the vial.
- The DNA preparation was then used to inoculate the biothreat pouch, rather than a suspension of live bacteria.

Inactivation Procedure

- Culture resuspended in 400uL of TE in a 1.5 mL tube is sprayed down with 1% sodium hypochlorite and removed from BSC CII to be placed in 100 degree Celsius heat block for 30 minutes. The final volume is then filtered through a 0.1uM filter unit by centrifugation (8000xg 2 minutes). Tube is then placed in a glad bag, sealed and sprayed down with 1% sodium hypochlorite and removed from the PC3 laboratory.
- Toxin preparations are resuspended in sterile PBS in a 1.5mL or 2mL tube sprayed down with 1% sodium hypochlorite and removed from BSC CII to be placed in 100 degree Celsius heat block for 60 minutes. The final volume is then filtered through a 0.1uM filter unit by centrifugation (8000xg 2 minutes). Tube is then placed in a glad bag, sealed and sprayed down with 1% sodium hypochlorite and removed from the PC3 laboratory.
- This risk assessment was based on information around moist heat treatment times for B. anthracis vegetative cells and spores (Spotts Whitney et al, 2003. Inactivation of Bacillus anthracis spores. Emerging Infect Dis. Vol 9(6) 623 and Carrera et al. 2007. J Appl Microbiol. 102(2):303-12) and CDC guidelines around ricin inactivation by heat treatment (CDC Biosafety in Microbiological and Biomedical Laboratories

Inactivation QC Results

- Viability plating: Bacteria solutions were prepared and incubated for 5 days. There was no growth from the treated solutions for *Bacillus anthracis*, *Brucella spp*., *Francisella tularensis* and *Yersinia pestis* indicating that the organisms were inactivated. Therefore solutions were considered suitable for removal from PC3.

RCPA Biosecurity Proficiency Testing SSBA

Rationale for the program

- Biological weapons and bioterrorism, biowarfare - increasingly secular
- Natural infectious disease outbreaks
- Laboratory safety and security
- 

RCPA Biosecurity Proficiency Testing SSBA

- From its humble beginnings which set about providing a QAP for anthrax and its near neighbours, the program has expanded considerably to encompass a range of emerging threat agents
- Some of the modules on offer this year include Plague, Anthrax, Smallpox, Ebola, Abrin, Tularaemia, Yellow Fever virus, Marburg virus
- This includes online learning modules and specimen modules (the viruses are all synthetic).
- The challenge for the RCPA is that they cannot send out a real SSBA, so they have to be cunning in how they prepare their samples.
- These modules, at least on the bacterial side, are becoming increasingly sophisticated with the inclusion of confounding organisms to make the exercises more challenging.