Crime scene investigation: can we detect anthrax??

An act of bioterrorism refers to both the threat to conduct as well as an actual incident involving the use of a biological agent against the civilian population. The detection of potential biological pathogens and toxins can now be conducted at the scene of a biological threat or release of a suspicious substance. Improvements in science and technology have enabled manufacturers of traditional laboratory based equipment to produce smaller, more basic models, enabling microbial testing to be conducted in the field.

Focused primarily at the first responders such as fire brigade and local police, these instruments can provide a basis for screening and evaluating the level of risk associated with the threat or suspect substance. Within Australia, the use of detection devices for chemical and radiological materials is vast and used widely within the emergency services. Biological detection is, on the other hand, relatively new to the market and its use in the field is restricted at present to specialised policing units such as the Forensic Counter Terrorism and DVI Unit (FCTDVI) of the NSW police. Our experience in using a range of biological detection equipment has allowed us to evaluate their effectiveness, re-design procedures and recognise the limitations associated with their environmental use.

Currently the technology being incorporated into these hand-held detection devices can be categorised into three major types: immunochromatographic/ immunoassay technology; polymerase chain reaction/ real time assay; and infra-red spectrometry (Table 1).

Rebecca Hoile

Forensic Microbiologist
NSW Police – Forensic Counter Terrorism & Disaster Victim Identification Unit
Police Headquarters Parramatta NSW 2150
Tel: (02) 9375 8930
Fax: (02) 9375 8914
E-mail: hoil1reb@police.nsw.gov.au

Immunochromatographic/ immunoassay technology

Immunoassay based technology was the first generation of biological agent detection to hit the market and primarily focussed on detecting the select agent via visual identification of positive anti-body/ antigen complexes [1]. This first generation of immunoassay tickets has since been improved to incorporate fluorescently labelled antibodies, which, when combined with the antigen, form complexes which are detected via a fluorescent reader (such as the RAMP®). This improvement has seen a reduction in false positive results by eliminating the need for visual identification [unpublished observation].

Experience and evaluation of such instruments has demonstrated a high level of reliability and specificity [manuscript in preparation], yet only one agent can be tested at any one time and, while no false positives were recorded, false negatives can arise due to environmental contaminants and overloading of test cartridge.

Immunoassay technology can be a reliable and sensitive tool, provided the limit of detection is known and the results are interpreted within the context of the information at hand.

Polymerase chain reaction/ real time assay

For many years PCR based technology has been at the forefront of microbial testing, providing a rapid result based on detecting DNA specific to the target of choice. It would be virtually inconceivable to conduct routine PCR at a crime scene, although, with the development of real time PCR and the application of this technology to smaller based instruments, PCR has again become the weapon of choice in detecting potential biological agents in the field.

The conversion of laboratory based PCR into field based PCR has been made possible with the inclusion of pre-

Crime scene personnel conducting sample testing.

prepared, freeze dried (most cases) masters mixes. Pre-prepared mixes may need to be reconstituted, mixed and then added to the collection vessel. Problems arise when the collection vessel may be plastic or glass, sample clean up steps are not included and master mixes may not reconstitute or mix effectively in the field. Importantly, environmental samples vary in constitution and may contain substances which inhibit successful completion of the PCR reaction. All of these are likely to increase the incidents of false results [unpublished observation]. While PCR technology in the laboratory setting has proven to provide reliable test results with a degree of sensitivity unmatched by other technologies, this experience demonstrates the need for understanding the limitations of PCR instrumentation outside the laboratory setting.

Infra-red spectrometry

Infra-red spectrometry has been used for many years to identify powders and liquids primarily of a chemical nature, e.g. narcotics, hazardous chemicals and explosives. In principle, the powder or liquid is exposed to infrared radiation which is absorbed by the substance creating a unique spectrograph. Portable infra-red instruments form part of the identification process as they are sensitive, rapid, non-destructive and applicable to a broad range of samples. Pure substances have a spectrograph which is unique and therefore easily matched to spectrograph libraries; mixtures, however, can be difficult to correlate.

In the context of biological substances, infra-red spectrometry is not able to identify specific biological agents and is therefore used to rule out household products such as sugar and flour or identify chemical products.

Table 1. Comparison of biological detection technology.

Detection technology	Advantages	Disadvantages
Immunoassay	• Rapid – 15 minutes • Compact • Specific to select agent • Good sensitivity for spores and toxins	• Test cartridge may be overloaded • Ab/Ag complexes may not be unique to select agent • Some rely on visual result interpretation
PCR real time	• Fast – 40 minutes • Specific to DNA target • Highly sensitive for spores • Can detect more multiple targets in one test	• PCR reaction may be inhibited • Cannot detect pure toxin • Master mix problems due to reconstitution in field
Infra-red spectrometry	• Rapid – 2 minutes • Can identify a range of household and industry-based substances • Used to identify unknown powders/ liquids • Can indicate presence of protein	• Can not identify biological agents • Can not easily identify mixtures

Conclusion

The greatest challenge to both the manufacture and the operator of these hand-held instruments is the fact that we are applying laboratory based detection methods, primarily designed for clinical based testing, to environmental samples. We are now looking for Class III pathogens and toxins not within a public health setting but rather the incident site and potential crime scene.

A potential act of bioterrorism requires both a law enforcement and public health response. The priority lies in determining whether a biological threat agent has been used (non-credible threat), its subsequent identification and the preservation of the crime scene. By utilising specific detection instruments at the scene, presumptive identification of an agent may be provided, enabling information to be forwarded to other responding agencies such as public health [2]. This rapid assessment provides the best possible chance for all affected to be managed in the most appropriate way and within the most effective timeframe.

Hand-held detection devices are the way of the future, as technology improves and the need for information at a critical incident increases. While beneficial, the use of presumptive tests should only be used by scientifically trained operators as part of a layered detection approach.

References

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2. Kliemann W & Ruoff K. Bioterrorism: implications for the clinical microbiologist. *Clin Microbiol Rev* 2001; 14:364-81.