# The use of rapid diagnostic kits for the rapid presumptive identification of bioterrorist agents

The events of 11 September 2001 and the subsequent US anthrax mail attacks placed enormous pressure on emergency response agencies to be able to rapidly assess the potential risk of an incident as a possible case of bioterrorism. In response to this perceived need to rapidly identify bioterrorist agents in the field, a number of hand-held 'tickets' appeared in the market and were promoted heavily to emergency response personnel. These included products from Tetracore (Guardian Bio-Threat Alert System), Alexis, Sigma-Aldrich (BADD – Biowarfare Agent Detection Device) and RAMP. This brief discussion will examine the current knowledge on such devices and their applications for the detection of bioterrorist agents.

***John Bates***

Chief Scientist
Public Health Microbiology
Queensland Health Scientific Services
39 Kessels Road, Coopers Plains QLD 4108
Tel: (07) 3274 9101
Fax: (07) 3274 9175
E-mail: John_Bates@health.qld.gov.au

Hand-held lateral flow devices rely on the diffusion of the test solution from a starting well up an analyte-specific immunochromatographic strip by thin-layer chromatography. The strip has a test band consisting of fluorescent-dyed latex particles coated with antigen-specific antibodies against the target organism/toxin, and an internal control band, which must produce a positive result for the test result to be valid. When the target antigen reaches the detection line, the antigen-antibody complex is visualised by the development of a red stripe across the device. A positive result should have two red stripes, one for the control and one for the positive test result.

There were a number of problems associated with these lateral flow devices from the outset. The one characteristic in common with all these products, apart from the technology, was the high individual price per test. This has made evaluation of these products in a fully-equipped laboratory prohibitively expensive, as frequently a whole box of tickets would need to be purchased. In addition, despite claims that they had been tested against a wide range of bacterial genera and were quite specific for their target agent, in Australia at least it was difficult to verify such claims due to a lack of positive control material for the exotic agents (plague and tularaemia) in most jurisdictions. In a number of cases in the US, false positive results were recorded in the field that had far-reaching implications before analysis by a proper laboratory could prove the initial result false.

At a Laboratory Response Network meeting in New Orleans in 2005, staff from the New York State Health Department outlined the major problems associated with field use of hand-held tickets. One incident in particular caused considerable embarrassment for officials, and the effects of this and similar incidents were summarised as follows:

- When a positive result was recorded in the field, the emergency response personnel would often retest to verify the initial result. Frequently there was not a lot of powder to test initially, and the end result was that, on a number of occasions, there was insufficient material left for the laboratory to confirm the field result.
- Every time a test was performed in the field, it increased the time taken to get the sample to a proper laboratory for confirmation. Laboratories found themselves being blamed for the considerable delay in providing a result when in fact the result was directly related to the time of receipt of the sample.

In recognition of these problems, the CDC issued a statement on the use of hand-held devices for the detection of bio agents, published in MMWR [1], which strongly discouraged their use in the field by first responders. The Public Health Laboratory Network (PHLN), an affiliation of public health laboratories around Australia, issued a similar statement in Australia discouraging emergency response personnel from using such devices in the field.

In 2005, the Commonwealth Department of Health and Ageing provided agent-specific RAMP kits to all the PHLN laboratories in Australia, following considerable research into their efficacy. By providing the kits to laboratories, the PHLN sent out a clear message that the use of such kits should be supported by laboratory resources to enable accurate confirmation of any positives. Testing of the anthrax RAMP kits to date has proved their worth for the investigation of white powders (they seem to be quite specific for *Bacillus anthracis* spores). The kits have also received AOAC approval, demonstrating their efficacy in multi-laboratory trials. However, these kits do not work on vegetative cells and therefore are not suitable for clinical use. In addition, they are unable to differentiate between virulent and avirulent anthrax spores, further emphasising the need for laboratory backup to test for specific virulence genes.

The Ricin RAMP kits detect ricin in a crude castor bean extract (independently verified by PCR on the extract), thereby providing screening technology to each jurisdictional laboratory for this toxin for the first time. However, their efficacy for the rapid identification of botulinum toxin appears to be quite unsatisfactory, based on testing to date. The botulinum kits were tested against ATCC strains of culture filtrates of *Clostridium botulinum* types A, B, C, D, E and F. Type A extract was the only extract that produced a positive reaction with the RAMP kit. Further testing on an isolate from a clinical case of infant botulism (confirmed as type A by mouse bioassay) failed to elicit a positive response in the RAMP kit.

Whilst rapid diagnostic kits are marketed for the rapid presumptive identification of bioterrorist agents from environmental samples, to date only the RAMP kits have been shown to provide reliable presumptive results for anthrax spores and ricin. It is important that, when used in the field, a positive RAMP result is regarded as a presumptive positive, and that this is independently verified in a laboratory that has appropriate levels of quality assurance and controls to confirm any positive results, before a final report is issued.

## Reference


1. Use of onsite technologies for rapidly assessing environmental *Bacillus anthracis* contamination on surfaces in buildings. *MMWR* 2001; 50:1087.


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Managing Director
ams Laboratories Pty Ltd
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