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Immunoassays for the development and validation of Bioalloy Detection Prototypes

Final Report

C. Ruttan, Res. Sci.; T. Dickinson-Laing, RTM; and D.C.W. Mah, Projects Director
Canada West Biosciences Inc.

Contract Scientific Authority: W.E. Lee, DRDC Suffield

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Contract Report

DRDC Suffield CR 2009-155

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Contract Number: W7702-03R979/001/EDM

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Abstract

With the current concerns over potential biowarfare incidents, attention has turned to improving technologies for the rapid detection and identification of biowarfare agents. These agents include viruses, protists, bacteria, fungi, parasites and toxins and may be classified into three levels. Category A agents have high rates of transmission and/or fatalities. Category B agents can spread with moderate ease and cause moderate rates of disease with low death rates. Category C agents could be modified for dissemination due to availability or easy production and spreading thus potentially leading to high infection and/or death rates. Due to the variety of agents of interest, a single approach for detection and identification is not feasible.

This report provides an overview of detection platforms that are currently available or being investigated for pathogen detection. Although the original objective of this contract was to provide DRDC Suffield with an independent analysis of the breadboards and prototypes delivered by IatroQuest Corporation through the development of applicable comprehensive immunoassays such could not be completed without the delivery of the necessary hardware. Therefore it is through this review of present detection and identification platforms that an understanding of the objectives of the work is shown.

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Executive Summary

Introduction

The work in this contractor report was carried out through funding from CRTI project 0019TA. CRTI funded the proposal by IatroQuest Corporation to develop a Real-Time Measurement Device for field detection and identification. The proposed measurement device incorporated 'smart' nano-technology material termed Bio-Alloy^{TM}, materials that possessed inherent photoluminescent properties and were sensitive to changes in the chemical state of the surface. The smart Bio-Alloy^{TM} materials would contain bio-molecular recognition elements such as antibodies and would function as biosensors. The purpose of the contract, carried out by Canada West Biosciences (CWB), was to provide DRDC Suffield with independent test and evaluation analysis of the breadboards and prototypes delivered by IatroQuest.

Results and Significance

Canada West Biosciences undertook a thorough systems analysis of the IatroQuest biosenor technology in an attempt to create a test and evaluation procedure to determine the effectiveness of the Bio-Alloy^{TM} biosensor technology and its accompanying measurement system that incorporated the technology. However, the work was terminated mid-way through the contract by mutual agreement of CWB and DRDC Suffield as a result of IatroQuest Corporation withdrawing from the CRTI project 0019TA prior to being able to deliver any manufactured hardware. After the project was terminated by IatroQuest, the requirements for the statement of work then no longer existed. The work completed by CWB comprised of a comprehensive analysis of present biodetection platforms used for pathogen identification. No problems were ever experienced in the contract between CWB and DRDC Suffield.

Future plans

The CRTI project 0019TA was terminated by IatroQuest. No further work was undertaken and no further work was planned with respect to analysis platforms.

C. Ruttan, T. Dickinson-Laing, D.C.W. Mah 2008 Immunoassays for the development and validation of Bioalloy Detection Prototypes PWGS Canada W7702-03R979/001/EDM Canada West Biosciences

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Acknowledgements

The authors would like to thank Dr. William Lee and Dr. Thomas Sawyer .at DRDC Suffield for the invaluable advice and understanding in consideration of this contract.

Biodetector Platforms

1. Fatty acid methyl ester gas chromatography (FAME-GC)

Fatty acids are extracted from cells then are converted to fatty acid methyl esters before running them through a GC. The microbial identity is determined by matching the resulting profile to searchable databases created from reference strains grown under standardised conditions (www.midi-inc.com).

2. Substrate Utilisation

Cells are grown in the presence of specific metabolites or physiological conditions. Positive results may be determined by visual inspection or by spectrometry. For example, the GENIII (Figure 1) determines the ability of the cell to metabolise all major classes of biochemicals, as well as its response to pH, salt and lactic acid and its reducing power and chemical sensitivity in a 96-well plate (www.biolog.com). Degree of positivity equates to the intensity of purple in each well.

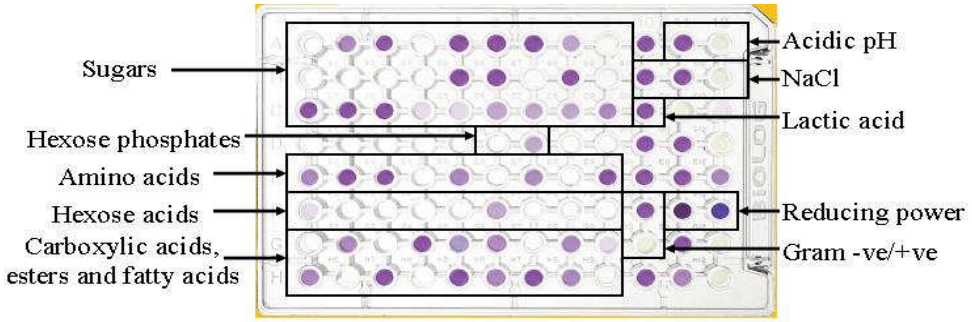

Figure 1: Substrate utilisation profile of Stenotrophomonas maltophilia (from www.biolog.com)

The VITEK 2 system (www.bioMerieux.com) uses a 64 well card to determine microbial identification and antibiotic susceptibility of a single sample. The card is inserted into an automated system to record test results. The integrated analysis software reports data.

3. Antibody-Based Identification

Antibody-based assays rely on the highly specific formation of antibody-antigen complexes. There are a wide variety of test methodologies and detection systems utilising antibody interactions. Several are listed below.

3.1 Microsphere Assay

X-Map (www.luminexcorp.com) uses polystyrene beads filled with a specific mixture of red and infrared fluorophores that are coated with fluorescent-labelled antibodies, receptors, oligonucleotides or peptides for different bioassays. A microfluidics system separates the beads then lasers excite both the internal and reagent fluorophores to give specific results on how the sample reacted to each.

3.2 Enzyme-linked immunosorbant assay (ELISA)

ELISAs are used to detect the presence of antigens or antibodies in a sample. In a direct ELISA, known antigens are fixed to a surface. Enzyme-conjugated primary antibodies are added followed by a substrate. To increase signal, the primary antibody can be biotinylated, allowing more substrate to be cleaved per antigen. An indirect ELISA is similar to the direct ELISA except that the enzyme is conjugated to a secondary antibody, which binds to the constant region of the primary antibodies. In a sandwich assay, primary antibodies are bound to the surface and antigens, if present in the sample, bind to these. A second set of primary antibodies is then applied to

sandwich the antigen between the two sets of antibodies. The secondary antibody and substrate are applied as above. Results for all types of ELISA are determined by spectrometry.

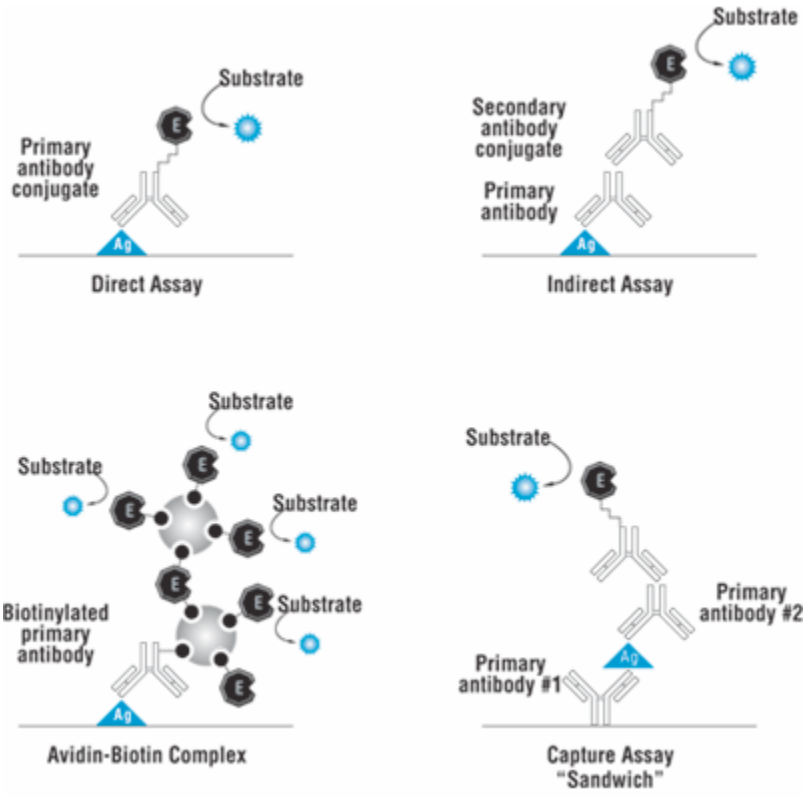

Figure 2: Types of ELISA (from www.piercenet.com/media/PD-Figure29.gif)

3.3 Protein Microarrays

Protein microarrays expand upon the complex formation concept of ELISAs, allowing the spotting of a combination of probe types (antibody, antigen, protein, peptide) on a single surface. Microwell and Nanowell chips have the added benefit that captured molecules may be recovered from the chip for further analysis.

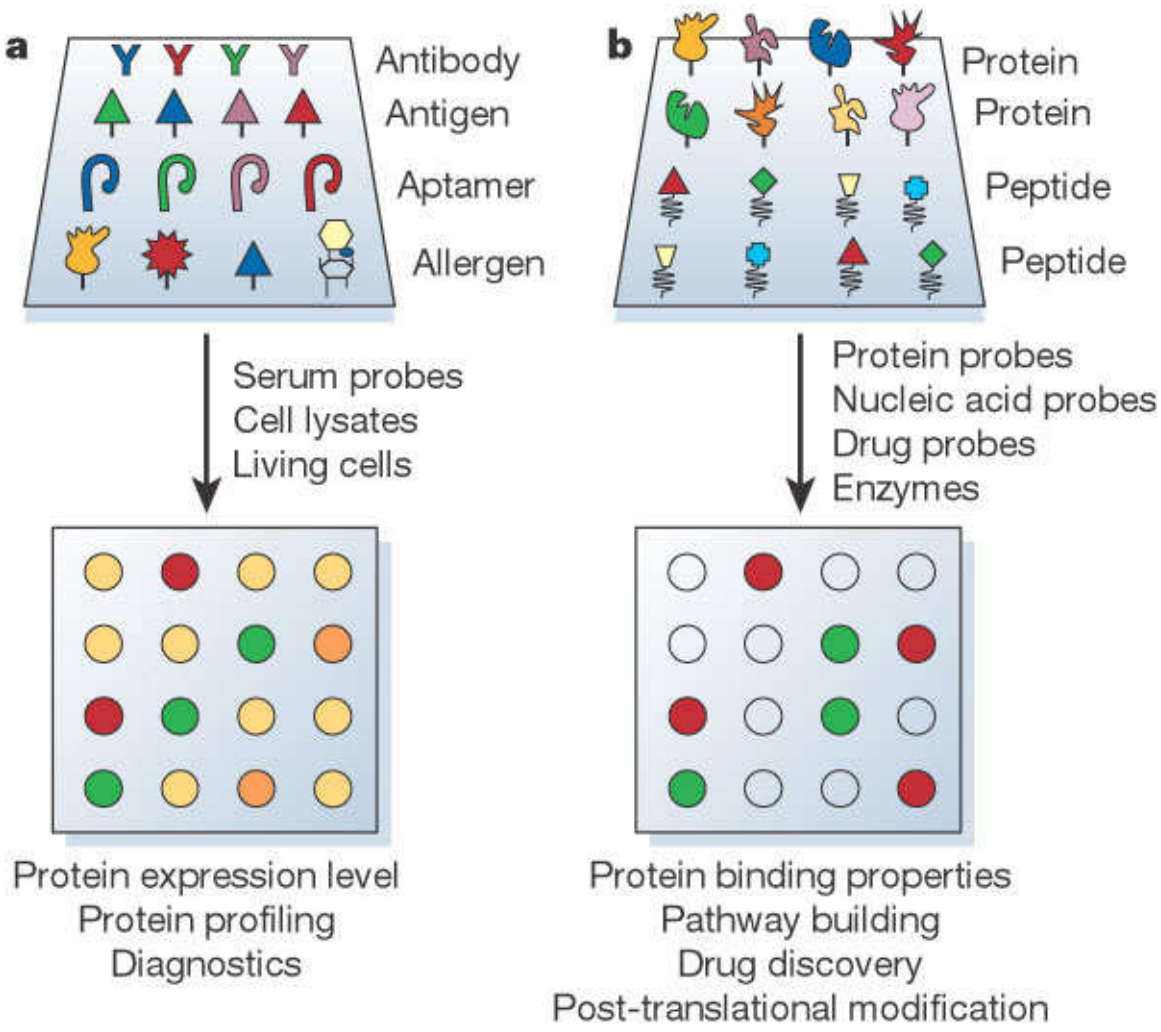

Figure 3: Protein microarray formats (from Phizicky et al., 2003)

a, Analytical protein microarray. Different types of ligands, including antibodies, antigens, DNA or RNA aptamers, carbohydrates or small molecules, with high affinity and specificity, are covalently spotted down onto a surface. These chips can be used for monitoring protein expression level, protein profiling and clinical diagnostics. Protein samples from two biological states are separately labelled with

red or green fluorescent dyes, mixed, and incubated with the chips. Spots in red or green colour identify an excess of proteins from one state over the other.

b, Functional protein microarray. Native proteins or peptides are individually purified or synthesised using high-throughput approaches and arrayed onto a suitable surface. These chips are used to analyse protein activities, binding properties and post-translational modifications and can be used to identify the substrates of enzymes of interest.

4. Nucleotide-Based Identification Methods

The tried and true polymerase chain reaction (PCR) is used to amplify small amount of DNA from laboratory cultures, field samples or tissues. Reverse transcription PCR is used for RNA amplification. Either sequence-specific or random primers may be used to amplify genomic material. In single step detection kits, only primers specific for the bacterial/viral agent of interest are used so that any amplification represents a positive result. There are several drawbacks of using PCR as a biodetector including the need for a thermocycler and detection equipment, the potential for sample contamination and the time required to produce results.

4.1 Real-Time PCR

Real-time PCR uses fluorescent-tagged reagents to monitor the accumulation of PCR product during a reaction. The Taq-man process uses probes with a fluorescent dye at the 5 end and a quencher at the 3 end. The dye emission is quenched when the probe is free in solution or hybridised to the target DNA. Signal is observed when the dye is cleaved from the probe during DNA polymeration (Figure 4).

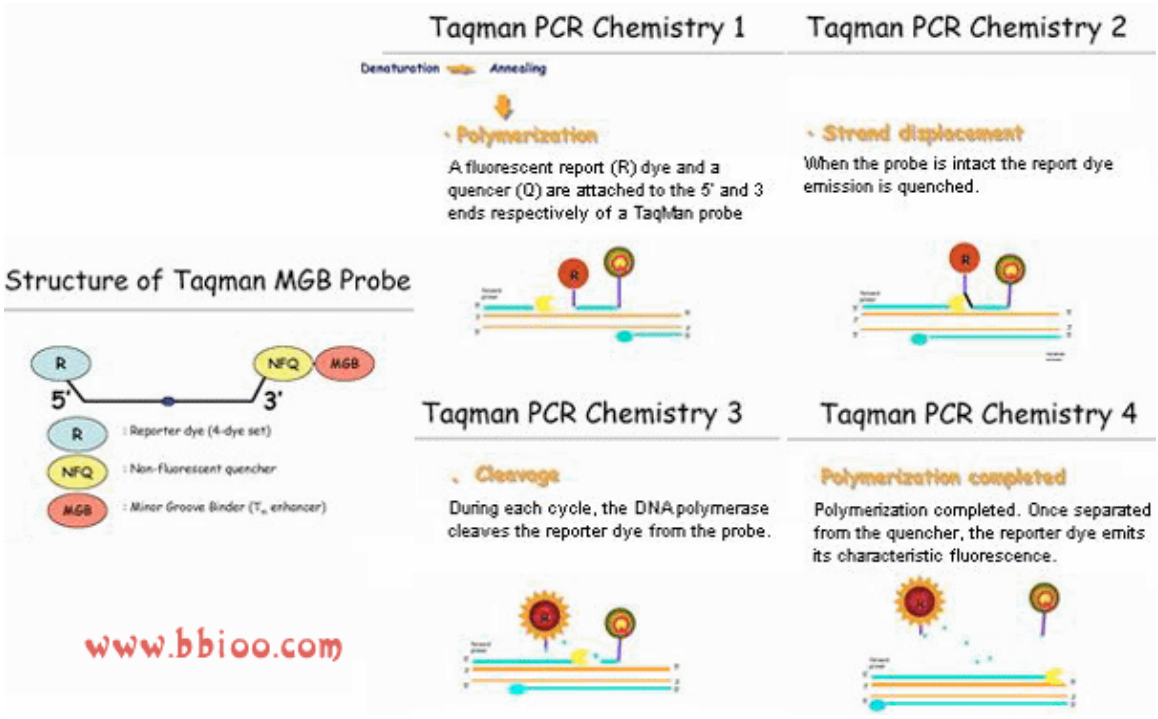

Figure 4: Taqman PCR (from http://www.51protocol.com/PCR/REAL-TIME/20070913/37182.html)

The MiniPCR instrument from Lawrence Livermore National Laboratory allows Taqman based PCR to be performed in the field. MiniPCR houses ten chambers each with a small, rapid thermocycler and optics. Pathogen-specific Taqman probes emit fluorescence when a target sequence is present in the sample and amplified. The optics read the increase in signal in near-real time providing a rapid and sensitive detection method for specific pathogens.

The LightCycler, (Idaho Technology) uses a pair of single-stranded fluorescently-labelled oligonucleotide probes for hybridisation. One probe is 3 labeled with a donor fluorophore, usually fluorescein while the acceptor probe is 5 labeled with one of four available fluorophores (red 610, 640, 670 or 705). A phosphate group is added

to the free 3' hydroxyl group of the acceptor probe to prevent strand extension. During annealing to the target sequence, the donor and acceptor dyes enter close proximity. Upon excitation by the instrument□s laser, energy is transferred from donor to acceptor dye by fluorescence resonance energy transfer (FRET) and light is emitted. The intensity of the emitted signal is directly proportional to the amount of accumulating target DNA.

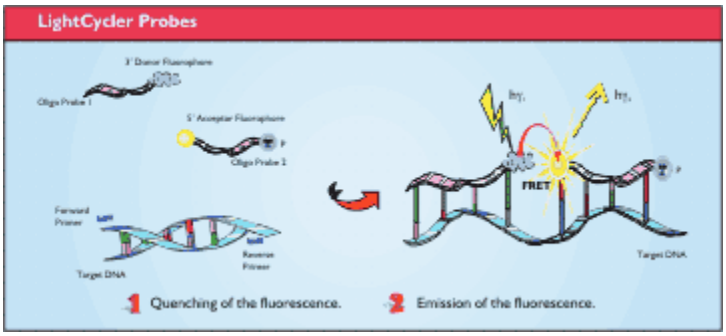

Figure 5: LightCycler Probes (from http://www.sigmaaldrich.com/Brands/Sigma_ Genosys/DNA_Probes/Product_Lines/Fluorescent_Probes/LightCycler_Probes.html)

LightCycler technology has been incorporated into a portable platform with the introduction of RAPID (Ruggedized Advanced Pathogen Identification Device) from Idaho Technology. RAPID can run a reaction and provide results in less than 30 min. The integrated software package enables users to process samples without risk of exposure to potentially dangerous specimens.

4.2 Molecular Beacons

Molecular beacons are single-stranded hairpin probes composed of a sequence-specific loop and fluorophore/quencher ends (Figure 6a). In solution, the probe is dark due to the proximity of the dye and quencher. In the presence of complimentary

target sequence, the probe hybridises and linearises, thus separating the dye and quencher (Figure 5b). Once separated, the dye emits a visible wavelength allowing rapid detection of positive samples. Due to the large number of available fluorophores/colours, multiple sequences may be interrogated in a single reaction tube (Figure 7).

. b.

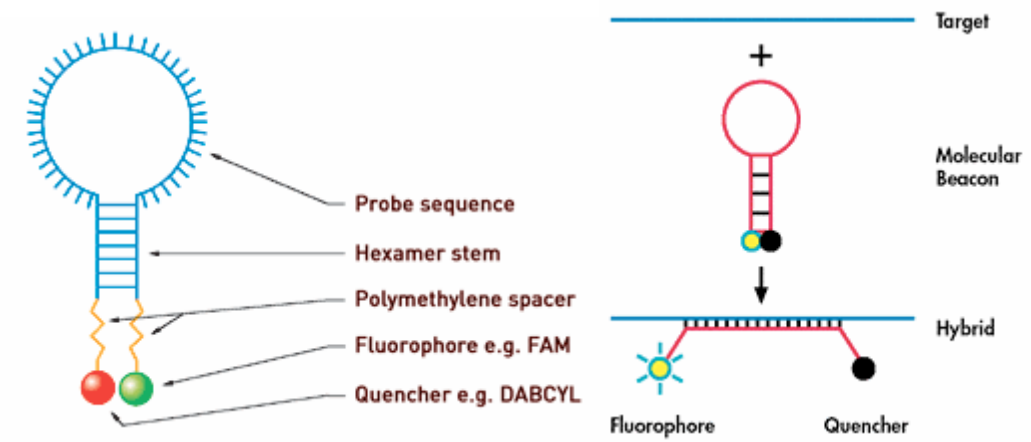

Figure 6: Molecular beacon probe mechanism
http://www.biocenter.helsinki.fi/bi/Programs/manual_files/image007.gif (a)
http://www.ebiotrade.com/buyf/productsf/qiagen/fig_molecular_beacons.gif (b)

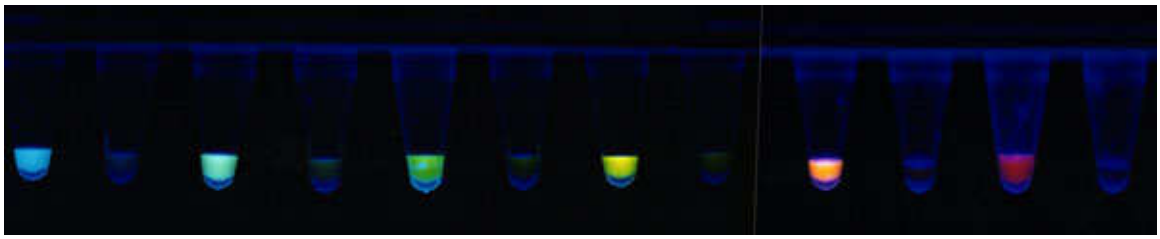

Figure 7: Molecular beacon reaction tubes with positive and negative samples (from http://www.molecular-beacons.org/Introduction.html)

4.3 Nucleic Acid Sequence-Based Amplification (NASBA)

NASBA is an isothermal technique that may be used for DNA or RNA amplification. RNA amplification is specific for viable cells and has been used to detect viable B. anthracis spores to a specificity of 10 spores/ml of sample (Baeumner 2004). A single strand copy of the region of interest is produced using a sequence specific primer that encodes the T7 RNA polymerase promoter at the 5□end and AMV reverse transcriptase. After cleavage with RNase H, a second primer is added to produce ds cDNA then T7 polymerase is added to amplify the target (http://www.ibi.cc/nasba.htm).

BioM□rieux□s NucliSENS EasyQ system (http://www.biomerieux-usa.com/clinical/nucleicacid/nasba.htm) uses NASBA combined with molecular beacon probes for real-time detection of targets (Figure 8). Results for 48 tests may be obtained in as little as 1.5 to 3 hours (depending on the assay).

NASBA may be used with DNA probe-impregnated membranes to capture RNA from lysed sample cells. A second set of DNA probes, bound to dye-containing liposomes, is applied to the membrane allowing visualisation of positive spots on the membrane. Dye intensity may also be measured with hand-held instrumentation. (Baeumner 2004).

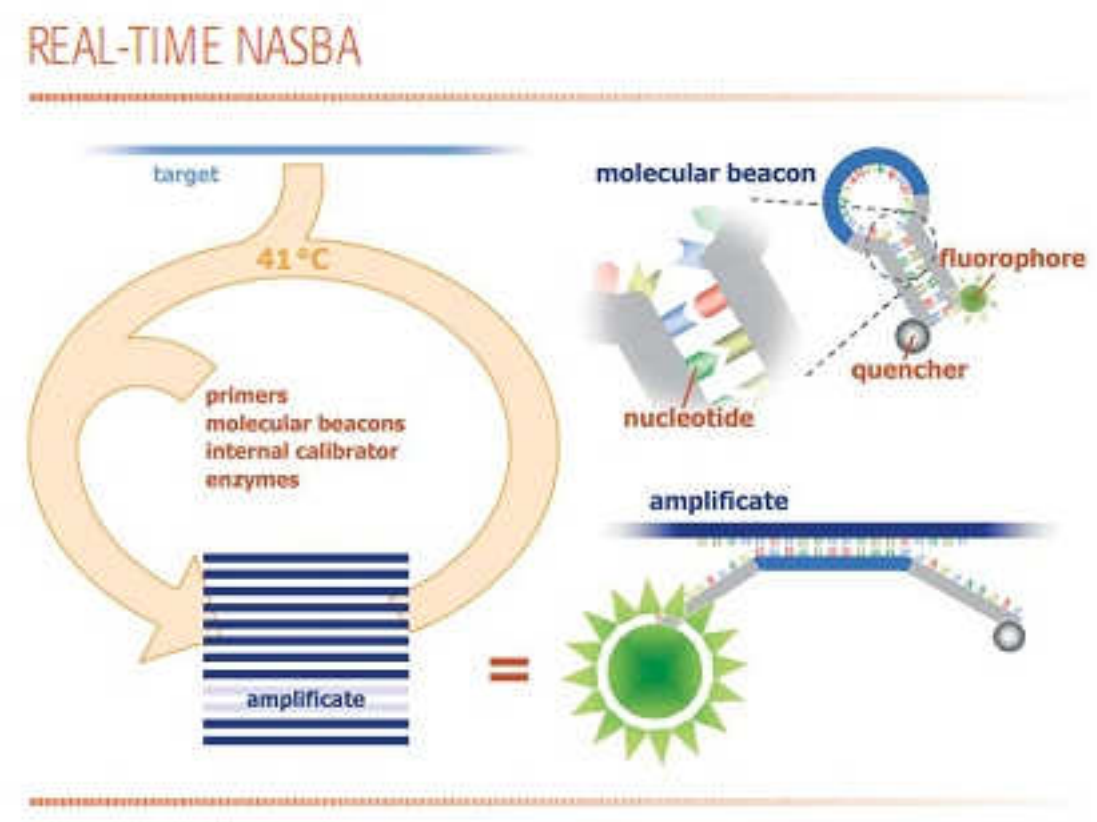

Figure 8: NucliSENS Easy Q Real-time NASBA procedure (from http://www.biomerieux-diagnostics.com/upload/real_time_nasba_thumb.jpg)

5. Fluorescence-Based Microfluidic Devices

Innovative Biotechnologies International Inc (IBI) has developed microfluidics devices to detect liposome-bound target sequences.. Liposome-bound sample is added to the sample channel where it may interact with magnetic beads in the serpentine regions. Any liposome:sample:bead complexes are drawn to the capture zone by the application of a magnetic field. Surfactant is added to lyse the liposomes thus releasing the fluorescent tags. The signal can then be detected under a fluorescent microscope.

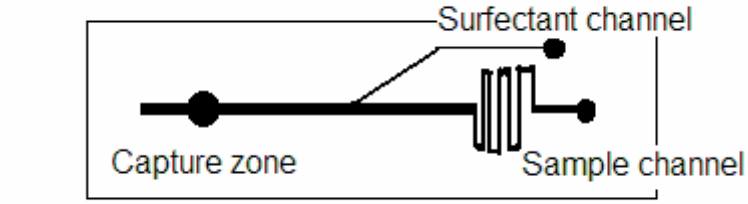

Figure 9: IBI microfluidics chip (derived from http://www.ibi.cc/microfluidics.htm)

5.1 Interdigitated Ultramicroelectrode Array (IDUA)

The use of electroactive compounds, instead of fluorescent dyes, within the liposomes is the basis of the IDUA biosensor. The liposome:sample complex is added to the IDUA chip then the liposomes are lysed. The "fingers" of the chip alternate between cathode and anode. The compounds released from the liposomes are reduced on the surface of the cathode fingers then diffuse to an anode finger where they are oxidized. This pattern (redox reaction) continues and the flow of electrons from these reactions (current) can be measured on a hand-held minipotentiostat. The current is directly proportional to the concentration of the target analyte.

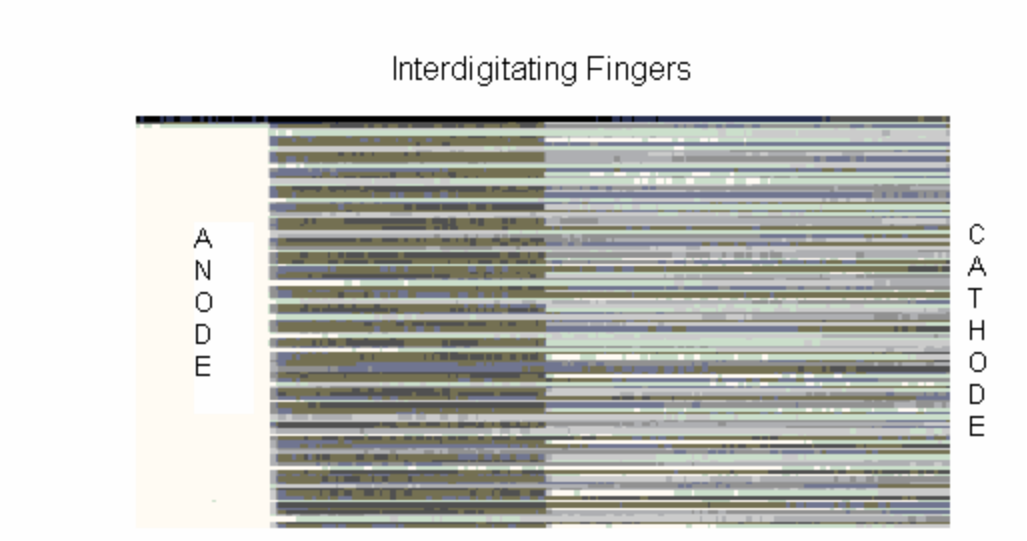

Figure 10: Close up of the IDUA redox chamber (from http://www.ibi.cc/interdigitated_array.htm)

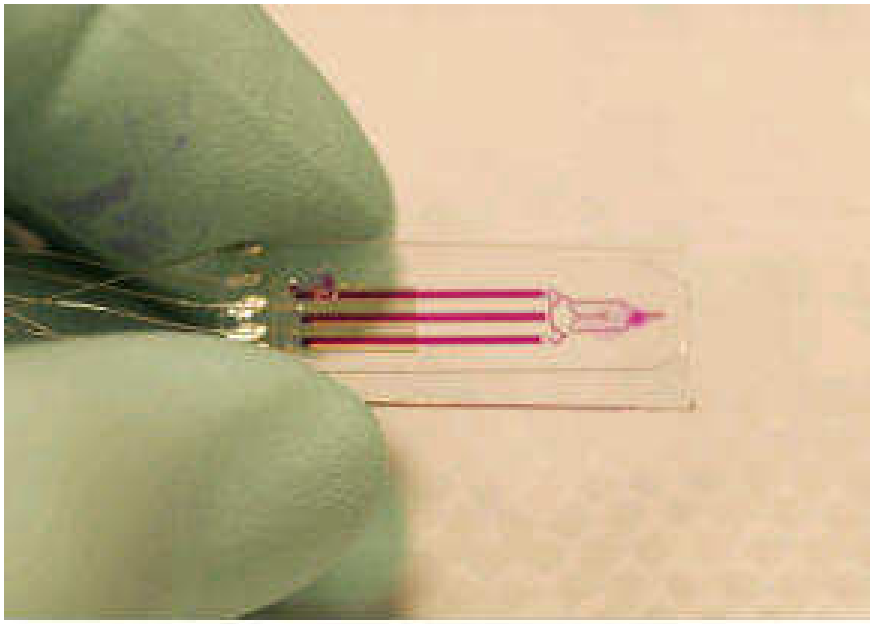

Figure 11: Three channel Interdigitated Ultramicroelectrode Microfluidics Biosensor (from http://www.ibi.cc/microfluidics.htm)

6. Antibody-based biodetectors

6.1 Fiber Optic Wave-Guide (FOWG)

A wave-guide is any material medium that confines and guides a propagating electromagnetic wave. In the FOWG biosensor, the wave-guide is an antibody-coated fiber optic probe, which will bind antigen from a solution containing a suspected agent. After washing the probes, a second solution containing fluorescently-labelled antibodies is introduced, which will also bind to the agent. Fluorescence is measured to confirm the presence/absence of the agent. An example of a biodetector using this technology is RAPTOR (Anderson et al, 2000).

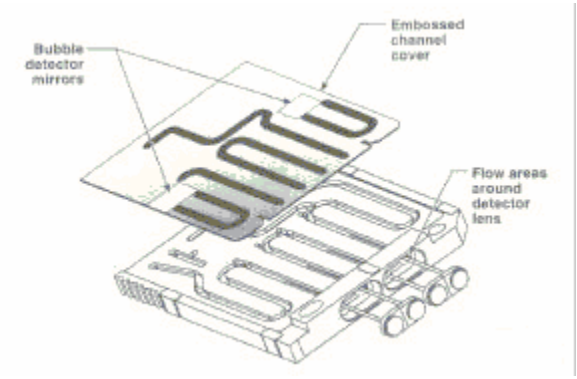

Figure 12: RAPTOR cassette with 4 fiber optic probes

6.2 Interferometry

The Bi-Diffractive Grating Coupler (BDG) is an optical transducer (Battelle Memorial Ins./Hoffman-LaRoche) that uses polarised light waves. Polarized light is divided into a transverse electric (TE) and transverse magnetic (TM) mode. The TM mode has an evanescent "tail" that moves with the light wave and above the medium (e.g. an

antibody-coated plastic wave-guide). The index of refraction of the wave-guide surface layer changes when a sample binds to the antibodies and can be measured using optical interferometry.

6.3 Surface Plasmon Resonance (SPR)

A surface plasmon (SP) is a thin film of metal on the surface of a wave-guide. In the presence of an incident light, a prism system (Kretschmann prism), fiber optic or planar wave-guide enables the coupling of some of the light to the SP film while the rest is reflected to a photodetector. If a sample binds to the SP, the refractive index of the surface changes and the difference in signal can be measured by the photodetector.

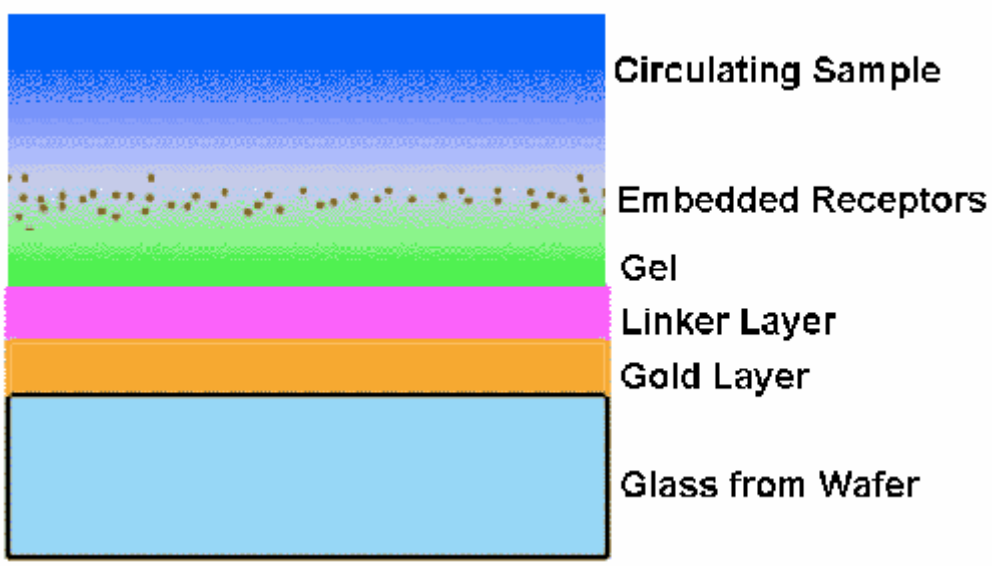

Figure 13: Typical sensor Chip (from http://www.mse.vt.edu/faculty/hendricks/mse4206/projects98/group07/prism.html)

6.4 Hand-Held Immunochromatographic Assays

Hand-Held Immunochromatographic Assays (HHAs) are simple, one-time-use devices that provide a yes/no response. For a skilled observer, the degree of colour change may be used to assess the amount of sample present. HHAs can be adapted to automated readers but their primary draw for field utility is their easy of use, speed and independence from the need for a power source.

The Biothreat Alert (BTA) Test Strips (Tetracore LLC/Alexeter Technologies, LLC) are an example of an HHA. The strips use lateral flow Immunochromatography, which is dependent on high specificity monoclonal antibodies. If the agent of interest is present in the sample above a certain concentration, a coloured band will appear in the target window. A control band is included to ensure the test runs properly.

Smart tickets (e.g. NDI Smart Ticket, from New Horizons Diagnostics Corporation and Instant Check from EY labs) are HHAs used for detecting and identifying multiple analytes. Antibodies are bound to gold beads and when bound to target molecules can be detected by immunofocusing onto small membranes, resulting in the appearance of a coloured line or dot. Positive results are obtained by measuring the light reflected by the gold beads bound to the antibody:sample complex.

6.5 Rapid Analyte Measurement Platform (RAMP)

RAMP uses antibodies labelled with fluorescent latex particles to detect targets of interest. The innovation introduced with RAMP is the use of a strip of known antibodies as an internal standard to improve test accuracy. Signal is measured with a

scanning fluorescence reader providing high sensitivity. RAMP may be adapted for virtually any immunologically active substance (Hoile et al., 2007).

6.6 Electronic Addressing (Nanochip) (www.nanogen.com)

One or more test sites on a chip are activated with positive charge. Negatively-charged biotinylated samples or probes are drawn to the positively-charged sites and are bound to a streptavidin permeation layer at these locations. The activated sites are turned off then red and green fluorescently-labelled probes or samples are hybridised to the bound complementary biotinylated strands. The NanoChip systems scan the chip and automatically analyse red and green fluorescent ratios to determine results.

7. Mass Spectrometry (MS)

MS allows the determination of the structure and molecular weight of an analyte using only a few ng of sample. The analyte is ionised then fragmented to produce a characteristic pattern (mass spectrum). Complex samples may be separated using gas chromatography (GC), high-performance liquid chromatography (HPLC) or capillary electrophoresis prior to MS analysis.

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Lim, D.V., Simpson, J.M., Kearns, E.A., Kramer, M.F. Current and Developing Technologies for Monitoring Agents of Bioterrorism and Biowarfare. Clin Microbiol Rev. (2005) 18(4): 583□607

Phizicky, E., Bastiaens, P.I.H., Zhu, H., Snyder, M. Fields, S. Protein analysis on a proteomic scale. Nature (2003) 422:208-215.

Annexes

Table 1: Category Classification

Category A	Category B		**Category C**
Bacillus anthracis	Brucella species	Japanese encephalitis virus	**Antimicrobial resistance, (engineered threats)**
Clostridium botulinum	Burkholderia mallei	Kyasanur forest virus	**Chikungunya virus**
Dengue fever	Burkholderia pseudomallei	LaCrosse virus	**Coccidioides immitis**
Ebola virus	Caliciviruses	Listeria monocytogenes	**Coccidioides posadasii**
Francisella tularensis	California encephalitis	Microsporidia	**Crimean-Congo Hemorrhagic fever virus**
Guanarito virus (arenavirus)	Campylobacter jejuni	Pathogenic Vibrios	**Emerging infectious diseases**
Hantaviruses (Bunyaviruses)	Chlamydia psittaci	Ricin toxin	**Influenza**
Junin virus (arenavirus)	Coxiella burnetii	Rickettsia prowazekii	**Multi-drug resistant Tuberculosis**
Lassa Fever (arenavirus)	Cryptosporidium parvum	Salmonella	**Other Rickettsias**
Lymphocytic choriomeningitis (arenavirus)	Epsilon toxin of Clostridium perfringens epsilon toxin	Shigella species	**Prions**
Machupo virus (arenavirus)	Cyclospora cayetanensis	Staphylococcus enterotoxin B	**Rabies**
Marburg (Flavirus)	Diarrheagenic E.coli	Toxoplasma	**SARS-associated coronavirus**
Rift Valley Fever (Bunyavirus)	Eastern Equine Encephalitis Virus	Venezuelan Equine Encephalitis Virus	**Tickborne encephalitis viruses**
Variola major and other pox viruses	Entamoeba histolytica	West Nile Virus	**Tickborne hemorrhagic fever viruses**
Yersinia pestis	Giardia lamblia	Western Equine Encephalitis Virus	**Yellow fever**

	Hepatitis A Virus	**Yersinia enterocolitica**	

Table 2: Biodetectors Selection

Test format	Test name	Manufacturer	Targets	Reference
Fatty acid methylester GC	Sherlock Bioterrorism Library	MIDI, Inc.	*B. anthracis, Y. pestis, Brucella spp., F. tularensis, B. mallei, B. pseudomallei*	Lim *et al*., 2005
Substrate utilization	MicroLog Dangerous Pathogen Database	BiOLOG	*B. anthracis, Y. pestis, Brucella spp., F. tularensis, B. mallei, B. pseudomallei*	Lim *et al*., 2005
Substrate utilization	Vitek II, API	bioM□rieux	Bacterial pathogens	Lim *et al*., 2005
Microsphere assay	xMap Technology	Luminex	Bacterial, viral and toxin agents	Lim *et al*., 2005
ELISA	QuickELISA *B. anthracis*-PA kit	Immunetics Inc.	*B. anthracis*	Lim *et al*., 2005
ELISA	BioThreat Alert	Tetracore	*B. anthracis, F. tularensis, Y. pestis, C. botulinum, Brucella spp., ricin, Staph.Enterotoxin B*	Lim *et al*., 2005
Protein microarray	Microwell, Nanowell	various	various	Phizicky *et al*., 2003
PCR	BioSeeq	Smiths Detection	Pathogens	Lim *et al*., 2005
PCR	*B. anthracis* PCR kit	Takara Mirus Bio	*B. anthracis*	Lim *et al*., 2005
PCR	O157:H7; StxI; StxII	Applied Biosystems Inc.	*E. coli* O157:H7	Lim *et al*., 2005
PCR	RealArt PCR kits	Artus	B. anthracis, Salmonella spp. dengue virus orthopox virus, other viruses	Lim *et al*., 2005
PCR	PathAlert detection system	Invitrogen	*B. anthracis*, *Y. pestis*, *F. tularensis*, smallpox	Lim *et al*., 2005
PCR	Certified Lux primer set	Invitrogen	*B. anthracis*, *Y. pestis*, *F. tularensis*, smallpox, *C. botulinum*	Lim *et al*., 2005
PCR	GeneXpert/Smart Cycler	Cepheid	*B. anthracis*	Lim *et al*., 2005

PCR	MiniPCR	Lawrence Livermore National Laboratory	various	Lim *et al*., 2005
PCR	LightCycler detection kit	Roche	*B. anthracis*	Lim *et al*., 2005
PCR	RAPID	Idaho Technology	*B. anthracis, F. tularensis, Y. pestis, C. botulinum, E. coli* O157:H7, *Salmonella* spp., *Brucella* spp., *Listeria* spp.	Lim *et al*., 2005
Hybridization	Nucleic acid sequence-based amplification (NASBA)		*viable B. anthracis spores, E.coli*, Dengue fever	Baeumner *et al*., 2004
Hybridization	Nucleic acid sequence-based amplification (NASBA)	bioM□rieux NucliSENS EasyQ	*viable B. anthracis spores, E.coli*, Dengue fever	Baeumner *et al*., 2004
Fluorescence	microfluidics chip	Innovative Biotechnologies Int.	various	Emanuel and Fruchey (2007)
Electrochemisty	Interdigitated Ultramicroelectrode Array	Innovative Biotechnologies Int.	various	Emanuel and Fruchey (2007)
Antibody-bound fiber optics	RAPTOR	Research International	*B. anthracis, F. tularensis, Y. pestis, Brucella spp., listeria spp., V. cholerae, ricin toxin, C. botulinum toxin, SEB, E. coli O157:H7, Salmonella spp. Giardia lamblia*	Anderson *et a*l., 2000
Electrochemilum inesce	Bi-Diffractive Grating Coupler	Battelle Memorial Ins./Hoffman-LaRoche	various	Emanuel and Fruchey (2007)
Antibody-based	Surface Plasmon Resonance		various	Emanuel and Fruchey (2007)

Antibody-based	BV Technology	BioVeris Corp.	*B. anthracis, E. coli* O157:H7, *Listeria* spp., *Salmonella* spp., *C. botulinum* toxins A, B, E, & F, ricin, Staph.Enterotoxin B	Lim *et al*., 2005
Antibody-based	Biothreat Alert hand-held assay	Tetracore	*B. anthracis*	Lim *et al*., 2005
Antibody-based	Sensitive Membrane Antigen Rapid Test (SMART)	New Horizons Diagnostic, Inc.	*B. anthracis, F. tularensis, Y. pestis, C. botulinum, V. cholerae, ricin, Staph. Enterotoxin B, E. coli O157:H7, Salmonella spp.*	Lim *et al*., 2005
Antibody-based	Instant Check Smart Ticket	EY Laboratories	Ricin	Lim *et al*., 2005
Antibody-based	Rapid Analyte Measurement Platform (RAMP)	Response Biomedical Corp.	*B. anthracis*, *C. botulinum*, *Y. pestis*, ricin, smallpox	Lim *et al*., 2005
Electronic addressing	NanoChip	Nanogen	Pathogens, Staph. Enterotoxin B, *V. cholerae* toxin B	Lim *et al*., 2005
Mass spectrometry	TEEMmate	JEOL	Spores	Lim *et al*., 2005

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With the current concerns over potential biowarfare incidents, attention has turned to improving technologies for the rapid detection and identification of biowarfare agents. These agents include viruses, protists, bacteria, fungi, parasites and toxins and may be classified into three levels. Category A agents have high rates of transmission and/or fatalities. Category B agents can spread with moderate ease and cause moderate rates of disease with low death rates. Category C agents could be modified for dissemination due to availability or easy production and spreading thus potentially leading to high infection and/or death rates. Due to the variety of agents of interest, a single approach for detection and identification is not feasible.

This report provides an overview of detection platforms that are currently available or being investigated for pathogen detection. Although the original objective of this contract was to provide DRDC Suffield with an independent analysis of the breadboards and prototypes delivered by IatroQuest Corporation through the development of applicable comprehensive immunoassays such could not be completed without the delivery of the necessary hardware. Therefore it is through this review of present detection and identification platforms that an understanding of the objectives of the work is shown.

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