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# Development of a Manual Threshold Immunoassay for *Bacillus anthracis* Spores

*Chun-Qiang Liu and Cindy Browning*

**Human Protection & Performance Division**
Defence Science and Technology Organisation

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## ABSTRACT

An immunoassay applicable to the Manual Threshold instrument was developed for the detection of *Bacillus anthracis* spores. The assay involved two antibodies raised against inactivated spores of *B. anthracis* Sterne strain 34F2, and they were labelled with biotin and fluorescein for capture and detection, respectively. The assay was linear over the range of 0 – $4x10^4$ spores/ml. The limit of detection was estimated to be approximately 500 spores/ml. The detection response for *B. anthracis* spores was at least 10-fold more sensitive than for the spores of *Bacillus cereus*, *Bacillus thuringiensis* and *Bacillus globigii*. The assay was also used to detect *B. anthracis* spores from aerosol samples.

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## Executive Summary

*Bacillus anthracis* is the causative agent of anthrax, and rapid detection of *B. anthracis* spores is critical in protecting the Australian Defence Force against the threat of anthrax. The Manual Threshold is an instrument that combines an antibody assay with a light-addressable potentiometric sensor, and is compact and field deployable. The aim of this project was to develop a Manual Threshold-based detection system for *B. anthracis* spores.

Two antibody preparations were raised in sheep and rabbits against inactivated spores of *B. anthracis* Sterne strain 34F2, of which one was labelled with biotin for capture and the other with fluorescein for detection. Different combinations and concentrations of the two antibodies were tested to maximise the detection for *B. anthracis* spores. The assay was linear over the range of 0 – $4x10^4$ spores/ml. The limit of detection was estimated to be approximately 500 spores/ml. The detection response for *B. anthracis* spores was at least 10-fold more sensitive than for the spores of *Bacillus cereus*, *Bacillus thuringiensis* and *Bacillus globigii*. The assay was also used to detect *B. anthracis* spores from aerosol samples.

# Authors


## Chun Qiang Liu
Human Protection & Performance


*Chun Liu graduated with a PhD (Molecular Microbiology) from the University of New South Wales (UNSW) in 1988. His PhD study was focussed on cloning and expression of genes involved in bioconversion of cellulosic materials. Prior to DSTO, he worked as Research Scientist at UNSW for eight years and then Senior Research Scientist at CSIRO for three years. While at UNSW he and his group were interested in lactic acid bacteria, in particular the construction of food-grade vectors, identification and characterisation of genes encoding metal resistance and bacteriophage resistance. He published extensively in scientific journals in this field. His work at CSRIO was focused primarily on the molecular biology of bacteria and archaea used in the bioleaching of metals from mineral sulphide ores, and the development of molecular methods for tracking and monitoring leaching organisms in the environment. He joined DSTO in 2003, and is currently working towards developing novel high affinity reagents for the rapid detection and identification of biological warfare agents.*


## Cindy Browning
Human Protection & Performance


*Cindy Browning graduated with BSc (Hons) from University of Queensland in 2002. Her research project was carried out in Queensland Institute of Medial Research on the development of new vaccine candidates for group A streptococcal infections. After her BSc (Hons), Cindy Browning joined DSTO and was involved in developing immunoassays for biological warfare agents. She left DSTO in early 2005.*

# Contents

# 1. Introduction

*Bacillus anthracis* is the causative agent of anthrax in humans, sheep, cattle, horses and other mammals. *B. anthracis* spores enter the body through skin abrasions, ingestion or inhalation, and then germinate at the site of entry and proliferate. This often results in the death of the host within several days (Mock & Fouet, 2001). Therefore, early detection of *B. anthracis* spores is critical in responding to the anthrax threat within the field of biological warfare (BW).

Most current assays for the identification of bacterial cells and spores are based on either PCR (polymerase chain reaction) or antibodies. PCR assays are highly specific and sensitive, theoretically capable of detecting as few as one copy of target DNA. Although they often involve a sample processing step there may be sufficient DNA on the outside of the spore to enable detection by PCR without sample preparation (Belgrader et al., 1999). Antibody assays target cells or spores directly but they are generally less specific than PCR. The two methods are independent, and when combined, can provide a high degree of confidence in the detection of target cells or spores. The Manual Threshold (MT) is an instrument that combines the antibody assay with a light-addressable potentiometric sensor (LAPS) (Lee et al., 1993), and is compact and field deployable. The MT immunoassay involves two antibodies, one labelled with biotin for capture and the other with fluorescein for detection (Figure 1). In this system the analyte, such as a bacterial spore, is sandwiched between the biotinylated and fluoresceinated antibodies. The antibody-analyte complex is filtered through a biotinylated membrane and captured onto the membrane through streptavidin. The captured fluorescein antibody then reacts with the anti-fluorescein antibody, which is conjugated to urease. The urease enzyme degrades urea to produce ammonium, resulting in a change in pH detected by the LAPS. The MT has been developed to detect several BW agents including ricin, *Brucella melitensis* (Lee et al., 2000), Venezuelan equine encephalitis virus (VEE) (Hu et al., 2004), *Yersinia pestis* and spores of *Bacillus globigii* (as simulant for *B. anthracis*) (Dill et al., 1997; Lee et al., 2000). The MT has also been used to detect *E. coli* O157:H7 cells (Gehring et al., 1998).

Here we report the development of an MT immunoassay for direct detection of *B. anthracis* spores.

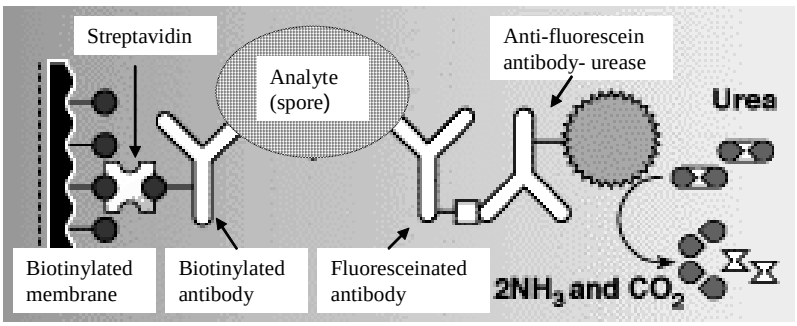


*Figure 1. Schematic representation of the Manual Threshold (MT) immunoassay system*

# 2. Materials and Methods

## 2.1 Bacterial antigens and preparations

The Sterne strain 34F2 of *Bacillus anthracis* (Colorado Serum Company, USA) was used in this study. *B. anthracis* 34F2 has reduced pathogenicity and is not pathogenic to humans because it lacks the pXO2 plasmid, which is required for the production of the poly-glutamate capsule that surrounds the vegetative cell (Boydston et al., 2005). This strain has been used effectively as a live veterinary vaccine against anthrax (Mock & Fouet, 2001; Turnbull, 1991). The spores of *B. anthracis* 34F2 used in this work were prepared from the live veterinary anthrax vaccine, which was supplied as a suspension in 50% glycerol with 0.1% saponin added as an adjuvant. Unless otherwise stated the spores were washed twice with water to remove glycerol and saponin before use. Other *Bacillus* species and strains used in the study were *Bacillus subtilus* var. *niger* strain ATCC9372 (*B. globigii*), *Bacillus cereus* MTL119 and *Bacillus thuringiensis* HD-1. Spores of *B. globigii* were prepared from liquid cultures grown for 4 days in Difco sporulation medium with shaking at 37°C (Cutting & Vander Horn, 1990). Spores were extensively washed in ice-cold water and purified through Percoll gradient centrifugation (Sue Pantelidis, personal communication). Spores of *B. cereus* and *B. thuringiensis* were prepared and inactivated by gamma irradiation at the Institute of Medical and Veterinary Science (IMVS), Australia. All spore samples were counted on a Double Neubauer Improved Counting Chamber SVZ2NIOU (ProSciTech, Australia).

*B. anthracis* 34F2 spores were inactivated by gamma irradiation and used for polyclonal antibody production in sheep and rabbits. Antibodies produced were purified by protein-G affinity chromatography, and conjugated to biotin or fluorescein using the Manual Threshold labelling kit (Molecular Devices, Sunnyvale, CA USA) according to the manufacturer's instruction. The protein concentrations of these reagents were determined spectrophotometrically with a BCA protein assay kit (Pierce Chemical Co., Rockford, IL, USA). Prior to use the reagents were stored at –20°C.

## 2.2 Instrument and reagents

The Manual Threshold (S/N T05075) with LAPS reader (S/N R006878) (Molecular Devices) was operated using reagents and sticks from the Manual Threshold Immuno-Ligand Assay kit (Molecular Devices). Assay buffer, wash solution, diluted capture reagent and diluted enzyme reagent were prepared according to the manufacturer's instructions.

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## 2.3 Standard assay procedure

One hundred microlitres of a spore suspension or assay buffer (negative control) were incubated with 100 µl of the antibody mix for 1 h at room temperature. After incubation, 1 ml of diluted capture reagent was added to the reaction mixture, and filtered through the

membrane sticks (mounted in filter blocks). The sticks were washed with 2 ml of wash solution, and 1ml of anti-fluorescein urease conjugate was then added to each well. After the solution was evacuated via the filter and the sticks washed again with 2 ml of wash solution, the membrane sticks were removed from the filter blocks, placed in wash solution and then inserted into the reader chamber containing urea in wash solution for measurement.

# 3. Results

## 3.1 Determination of antibody combination and concentration

Two types of antibodies were raised using inactivated spores of *B. anthracis* 34F2 as the immunogen in sheep and rabbits. These were labelled with biotin and fluorescein respectively, and mixed at equimolar concentrations. Different combinations of the antibody-biotin and antibody-fluorescein conjugates were tested against *B. anthracis* 34F2 spores using the standard assay procedure. Preliminary results showed that the highest MT signal was elicited with a combination of the sheep antibody-biotin conjugate and the rabbit antibody-fluorescein conjugate, and this combination of conjugates was chosen for subsequent assays.

The effect of conjugate concentrations on the assay was determined by mixing the two conjugates at equimolar concentrations (each ranging from 100 to 800 ng per test) and evaluating the different concentrations in assays to detect *B. anthracis* 34F2 spores ($2x10^4$ spores/ml) (Figure 2). An optimal conjugate concentration was defined as the lowest concentration that resulted in the highest net signal (test signal - background signal). The most intense signal was achieved when the conjugates were used at 500 to 800 ng/test. The 500 ng/test was chosen for further experiments because it gave rise to the lowest background signal.

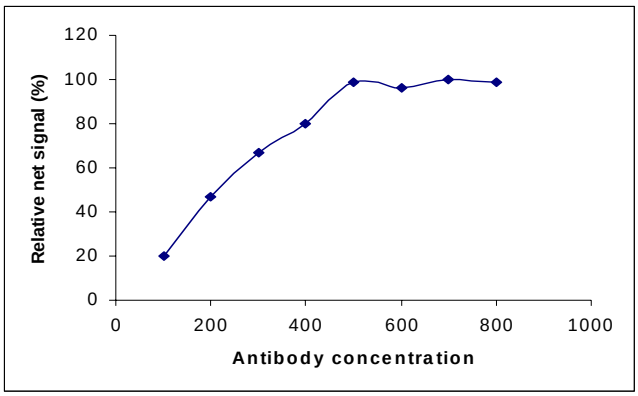


*Figure 2. MT signal response to antibody concentrations*

## 3.2 Determination of incubation time

The effect of incubation time on the assay was determined by mixing the conjugate pair with *B. anthracis* 34F2 spores ($2x10^4$ spores/ml) and incubating them for 0.5, 1, 2 or 4 h at room temperature before completing the standard assay. The highest MT response was obtained after two hours. However, approximately 82% of the signal was produced after one hour (Figure 3). In favour of a more rapid biodetection system, an incubation time of one hour at ambient temperature was chosen for further experiments.

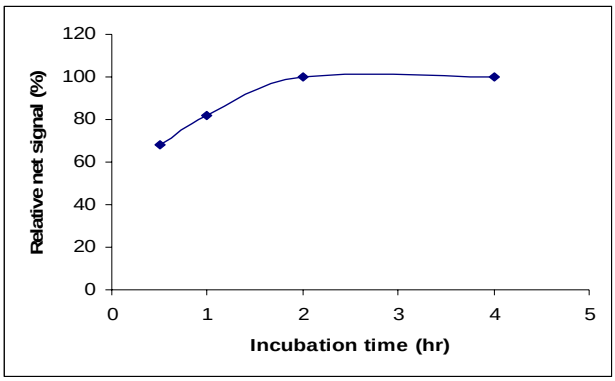


*Figure 3. MT signal response to incubation time*

## 3.3 Linearity and detection limit

To establish the linearity and detection limits of the assay, *B. anthracis* 43F2 spores were diluted serially and assayed in triplicate using the standard assay procedure. The assay for *B. anthracis* 34F2 spores was performed over a range of 0 – $6x10^4$ spores/ml. The MT response was linear over the range of 0 – $4x10^4$ spores/ml (Figure 4). A positive test was defined as having a signal greater than the background signal plus three standard deviations. The limit of detection for the assay was estimated to be approximately 50 spores/test (100 µl) or 500 spores/ml.

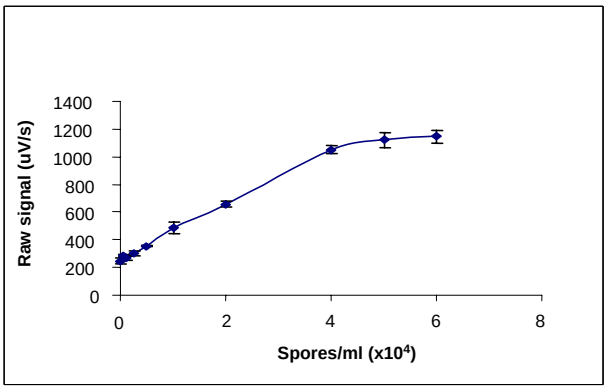


*Figure 4. MT signal response to B. anthracis spores over the range of 0 – $6x10^4$ spore/ml. The plot represents averaged MT responses of triplicate measurements ± standard deviation.*

## 3.4 Antigenicity of soluble fractions of *B. anthracis* 34F2 spores

The spores of *B. anthracis* 34F2 used were supplied as a suspension in glycerol with saponin added as an adjuvant. To evaluate the antigenicity of the soluble fraction, the supernatant was diluted 100 times and assayed using the standard procedure. The results showed that the MT signals were at least twofold higher for the soluble fraction than for the spores (Figure 5), indicating that the soluble fraction of the vaccine represents a substantial portion of the total antigenicity.

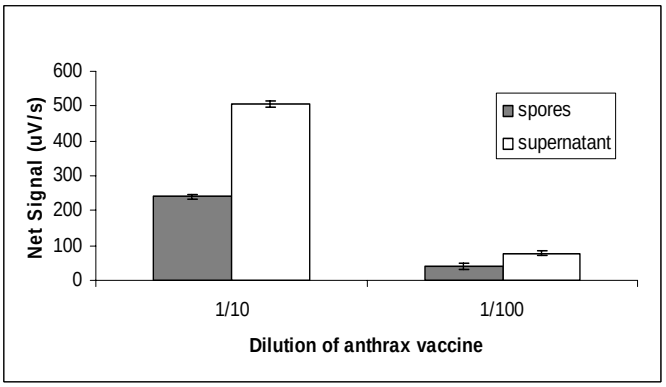


*Figure 5. Comparison of antigenicity between the supernatant and B. anthracis spores from the anthrax vaccine. There were approximately $10^4$ spores/ml at 1/10 dilution and $10^3$ spores/ml at 1/100 dilution.*

## 3.5 Assay specificity

The specificity of the assay for *B. anthracis* spores was evaluated using spores of *B. cereus*, *B. thuringiensis* and *B. subtilis* (Figure 6). Although some cross reactivities were observed with the three species, the activity for *B. anthracis* was approximately 10-fold higher than for the other species.

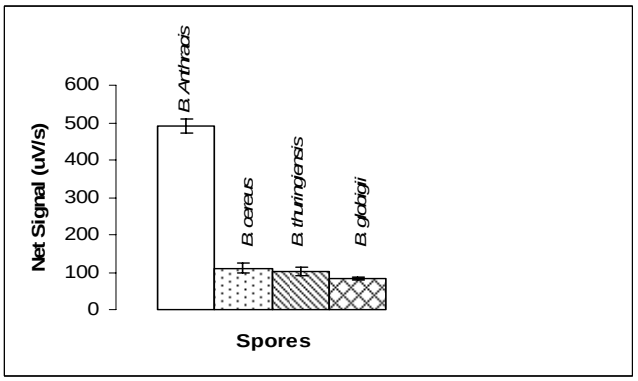


*Figure 6. Specificity of the assays using spores of B. anthracis ($2x10^4$ spores/ml), B. cereus ($4x10^4$ spores/ml), B. thuringiensis ($4x10^4$ spores/ml) and B. globigii ($4x10^4$ spores/ml).*

### 3.6 Assays for *B. anthracis* spores in an aerosol sample

An atmospheric aerosol sample was collected from a Horizontal Wetted-Well Cyclone (HWWC) positioned at Victoria Barracks for 12 days. The unprocessed aerosol sample was spiked with *B. anthracis* 34F2 spores prior to being assayed using the standard procedure. The sample exhibited a high background signal, and the MT signal reached its plateau at $3x10^4$ spores/ml (figure 7).

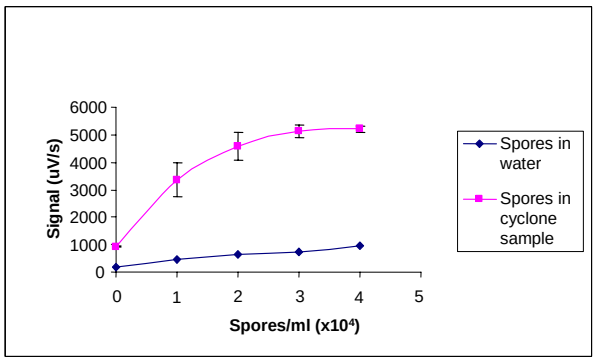


*Figure 7. Detection of B. anthracis spores in water and unprocessed cyclone sample*

# 4. Discussion

This paper describes a Manual Threshold (MT) immunoassay for live *B. anthracis* spores. The assay involved two antibodies that were raised against inactivated spores of *B. anthracis* Sterne strain 34F2, and they were labelled with biotin and fluorescein for capture and detection, respectively. Similar to observations made by Gehring *et al.* (1998), the overall detection efficiency was affected not only by the concentration but also by the combination of the two conjugates. The best result was achieved with the sheep antibody-biotin conjugate for capture, combined with the rabbit antibody-fluorescein conjugate for detection.

For spore suspensions with concentrations of up to $4x10^4$ spores/ml, the MT signal was directly proportional to the concentration. However, the signal reached a plateau with increasing spore concentration. This phenomenon commonly occurs in sandwich immunoassays when the antigen is present in high concentrations. Normally the antibodies are in great excess to the antigen causing a high probability of forming complete antigen-antibody complexes which, in this case, were captured on the membrane and detected by the LAPS. As the antigen concentration increases, the probability of forming the full antigen-antibody complex decreases, resulting in a decreased rate of signal production. Therefore, it is important that all samples should be diluted appropriately prior to assay.

The observation that a strong MT signal was detected with the supernatant meant that the soluble fraction represents a significant portion of the total antigenicity in the anthrax vaccine. A similar observation has been described for the *B. globigii* spore preparation (Lee et al., 2000). The supernatant most likely contained a mixture of surface and cellular proteins due to lysis of some spores, as well as those proteins bound loosely to the spores during storage, and these proteins were reactive with the antibodies that were raised against the whole *B. anthracis* 34F2 spore in the assay.

The MT assay was able to detect *B. anthracis* spores at concentrations as low as 50 spores per test, but still recognised, albeit weakly, related *Bacillus* species – *B. cereus*, *B. thuringiensis* and *B. globigii*. *B. anthracis* is closely related to *B. cereus* and *B. thuringiensis*, and they all produce a structurally similar exosporium (Steichen et al., 2003). Although *B. globigii* does not produce exosporium, it shares several similar coat proteins with *B. anthracis* (Lai et al., 2003). Neither of the two antibodies used had been purified by absorption with spores from related species. Further, similar titres were obtained with pre and test bleed samples from the *B. anthracis* spore immunised animals when tested against *B. globigii* spores (unpublished data), indicating that the animals had been immunised by *Bacillus* spores from the environment, including *B. globigii*. Taken together, these factors may have contributed to the cross reactivity exhibited.

The detection of aerosolised *B. anthracis* spores is important in monitoring anthrax outbreaks, including bioterrorist attacks. Our preliminary results demonstrate that the MT immunoassay can be used to monitor aerosol samples. However, given that the unprocessed cyclone samples required a prolonged filtration time (approx 30 min) and exhibited a high background MT signal, a prefiltration step is recommended.

In conclusion, we have demonstrated that the MT immunoassay can be used to detect live *B. anthracis* spores. Once appropriate reagents are acquired, this methodology can be readily adapted to the establishment of MT-based systems for other BW agents. In DSTO we are currently focusing on the development of new reagents including monoclonal antibodies and peptides with higher affinity and specificity for *B. anthracis* spores. These reagents will be incorporated into the MT system to allow the development of a more specific method for the detection of *B. anthracis* spores.

# 5. Bibliography

**Belgrader, P., Hansford, D., Kovacs, G. T. A., Venkateswaran, K., Mariella Jr., R., Milanovich, F., Nasarabadi, S. & Northrup, M. A. (1999).** A minisonicator to rapidly disrupt bacterial spores for DN Aanalysis. *Anal Chem* **71**, 4232-4236.

**Boydston, J. A., Chen, P., Steichen, C. T. & Turnbough, C. L., Jr. (2005).** Orientation within the exosporium and structural stability of the collagen-like glycoprotein BclA of *Bacillus anthracis*. *J Bacteriol* **187**, 5310-5317.

**Cutting, S. M. & Vander Horn, P. B. (1990).** Molecular methods for *Bacillus*. Chichester, United Kingdom: John Wiley & Sons Ltd.

**Dill, K., Song, J. H., Blomdahl, J. A. & Olson, J. D. (1997).** Rapid, sensitive and specific detection of whole cells and spores using the light-addressable potentiometric sensor. *J Biochem Biophys Methods* **34**, 161-166.

**Gehring, A. G., Patterson, D. L. & Tu, S. I. (1998).** Use of a light-addressable potentiometric sensor for the detection of *Escherichia coli* O157:H7. *Anal Biochem* **258**, 293-298.

**Hu, W. G., Thompson, H. G., Alvi, A. Z., Nagata, L. P., Suresh, M. R. & Fulton, R. E. (2004).** Development of immunofiltration assay by light addressable potentiometric sensor with genetically biotinylated recombinant antibody for rapid identification of Venezuelan equine encephalitis virus. *J Immunol Methods* **289**, 27-35.

**Lai, E. M., Phadke, N. D., Kachman, M. T., Giorno, R., Vazquez, S., Vazquez, J. A., Maddock, J. R. & Driks, A. (2003).** Proteomic analysis of the spore coats of *Bacillus subtilis* and *Bacillus anthracis*. *J Bacteriol* **185**, 1443-1454.

**Lee, W. E., Thompson, H. G., Hall, J. G., Fulton, R. E. & Wong, J. P. (1993).** Rapid immunofiltration assay of Newcastle disease virus using a silicon sensor. *J Immunol Methods* **166**, 123-131.

**Lee, W. E., Thompson, H. G., Hall, J. G. & Bader, D. E. (2000).** Rapid detection and identification of biological and chemical agents by immunoassay, gene probe assay and enzyme inhibition using a silicon-based biosensor. *Biosens Bioelectron* **14**, 795-804.

**Mock, M. & Fouet, A. (2001).** Anthrax. *Annu Rev Microbiol* **55**, 647-671.

**Steichen, C., Chen, P., Kearney, J. F. & Turnbough, C. L., Jr. (2003).** Identification of the immunodominant protein and other proteins of the *Bacillus anthracis* exosporium. *J Bacteriol* **185**, 1903-1910.

**Turnbull, P. C. (1991).** Anthrax vaccines: past, present and future. *Vaccine* **9**, 533-539.

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Immunoassay, Bacillus anthracis, Biological agent detection

19. ABSTRACT

An immunoassay applicable to the Manual Threshold instrument was developed for the detection of *Bacillus anthracis* spores. The assay involved two antibodies raised against inactivated spores of *B. anthracis* Sterne strain 34F2, and they were labelled with biotin and fluorescein for capture and detection, respectively. The assay was linear over the range of 0 – $4x10^4$ spores/ml. The limit of detection was estimated to be approximately 500 spores/ml. The detection response for B. anthracis spores was at least 10-fold more sensitive than for the spores of *Bacillus cereus*, *Bacillus thuringiensis* and *Bacillus globigii*. The assay was also used to detect *B. anthracis* spores from aerosol samples.