BIOSENSOR™ 2200R TECHNOLOGY

MSA
The Safety Company

Portable, handheld biohazard detection instrument for first responders

On-site portable analysis offering:

- Rapid detection
- Ease of use
- Excellent sensitivity
- 1 in 1,000,000 false positive

BIOSENSOR 2200R Biological Agent Detector features & benefits:

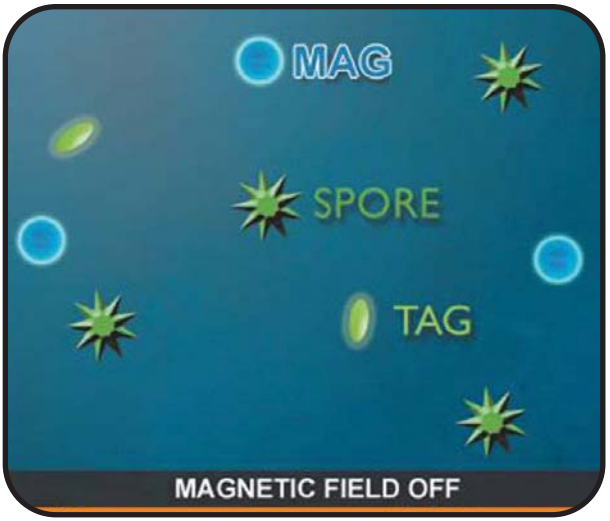
- Five minute time-to-answer
- Extremely easy to use after one training hour
- IP67-rated; fully deconable with lid open
- Visual and audible alarms provide clear status indication
- Non-destructive test; sample is retained
- Battery-operated, can run 50+ tests on a single charge
- Integrated RFID *(radio frequency identification)* for automatic cartridge recognition
- Data storage for 50 tests including results, target type and cartridge serial number

Title: Detection – QTL Biosensor	
Effective Date: Revised 6/15/06	**CALEA:** N/A
Review Date: DRAFT	**Distribution:** HMRT

Operational theory

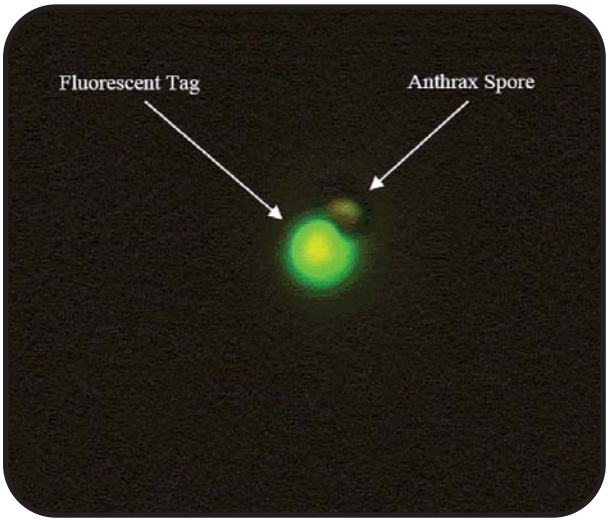
The Biosensor 2200R Detector assay resembles a standard immunomagnetic sandwich assay. The sample is added to the Biosensor Detector cartridge, which contains the sensing reagents. Sensing reagents are composed of two materials: a magnetic component and a fluorescent component. Receptors for the biological agent(s) of interest are contained in both sensing reagents. Upon mixing of the sample, both the magnetic and fluorescent components will bind to the biological agent for which they are specific. A magnetic field is then applied. This process separates all magnetic materials (including any complexes containing the biological agent) from the solution, which contains any excess fluorophore. A wash is then performed to remove all materials from the solution. The magnetic pellet is then excited and if the pellet is a fluorescent, the presence of the biological agent is indicated by the detector.

1 | MIX



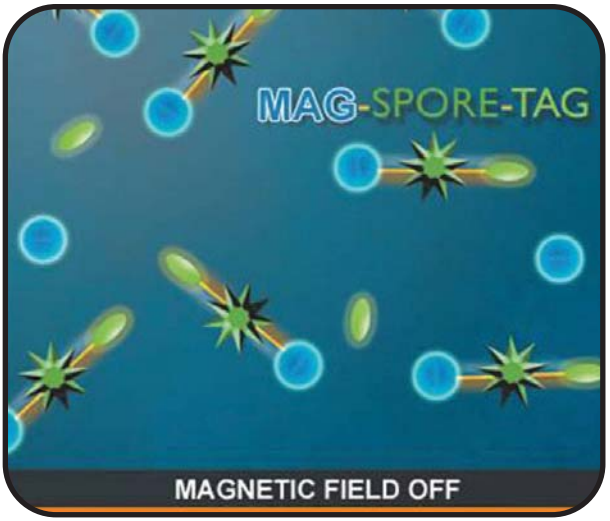
Sample is mixed with BIOSENSOR Detector Sensing Solution

The bioassay incorporates highly fluorescent particles displaying superior brightness relative to many commercially available materials.



A single tagged Anthrax spore

2 | BIND



Sensing materials bind to target during incubation

Several bioassays incorporate proprietary antibodies that show high binding along with superior specificity relative to their commercially available counterparts. In addition, the BIOSENSOR Detector detects a protein on an anthrax spore's outside coating. Other immunoassay tests detect a secondary marker that is highly unlikely to be present in a purified anthrax spore sample.

U.S. Environmental Protection Agency

Battelle
The Business of Innovation

ETV Joint Verification Statement

TECHNOLOGY TYPE: **IMMUNOASSAY TEST KITS**

APPLICATION: **DETECTING ANTHRAX**

BIOSENSOR 2200R Biological Agent Detector

Anthrax Concentration *(spores/mL)*	**Positive Results** *(out of 3 replicates)*
5,000,000	3 of 3
1,000,000	3 of 3
500,000	3 of 3
100,000	1 of 3

BIOSENSOR Detector assays were performed using a 0.1 mL sample. The actual number of spores in the tested samples above were 500,000; 100,000; 50,000 and 10,000.

Competitor A

Anthrax Concentration *(spores/mL)*	**Positive Results** *(out of 3 replicates)*
800,000,000	3 of 3
80,000,000	0 of 3
8,000,000	0 of 3
800,000	0 of 3

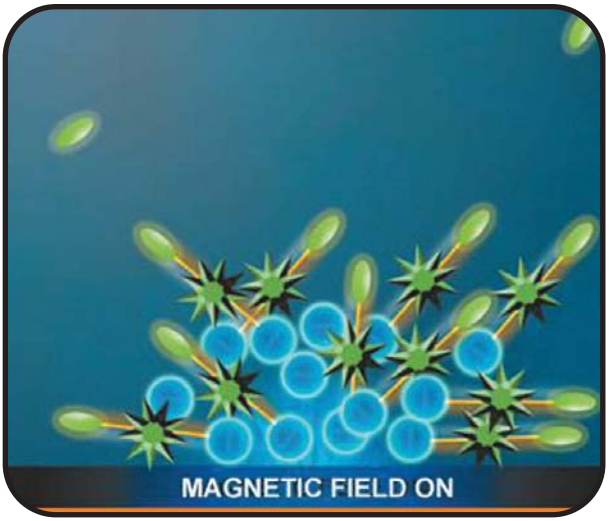
Competitor B

Anthrax Concentration *(spores/mL)*	**Positive Results** *(out of 3 replicates)*
80,000,000	3 of 3
40,000,000	2 of 3
8,000,000	0 of 3
800,000	0 of 3

Competitor C

Anthrax Concentration *(spores/mL)*	**Positive Results** *(out of 3 replicates)*
800,000,000	3 of 3
80,000,000	3 of 3
8,000,000	3 of 3
800,000	0 of 3

3 | MAGNETIZE



All bound and unbound magnetic material is pulled to surface.

This step effectively concentrates the sample for subsequent reading, resulting in an extremely low level of detection.

R E S E A R C H R E P O R T

Evaluation Report on the QTL Biosystems *Bacillus anthracis* Cartridges and ß-unit Reader
By: Biodefense Laboratory, Wadsworth Center

II - Sensitivity

Table 4. Serial dilutions of *B. anthracis* strains to determine limit of detection. A positive result was defined as response (mV) greater than 100 as per manufacturer's instructions. Red numbers indicate a positive result. ND = not done.

Avirulent *B. anthracis* Sterne (pX02-) strain

# of spores ➤	1,000,000	100,000	10,000	1,000	500
Test #1	2896	3031	1967	228	219
Test #2	2861	2898	1385	209	139

Avirulent *B. anthracis* Cohn (pX02-) strain

# of spores ➤	1,000,000	100,000	10,000	1,000	500
Test #1	2656	2971	2488	214	115
Test #2	2533	3020	ND	142	125

Avirulent *B. anthracis* Pasteur (pX01-) strain

# of spores ➤	1,000,000	100,000	10,000	5,000	2,500
Test #1	2976	1932	749	289	102
Test #2	899	2506	620	488	344

Virulent *B. anthracis* Ames strain (Brentwood)

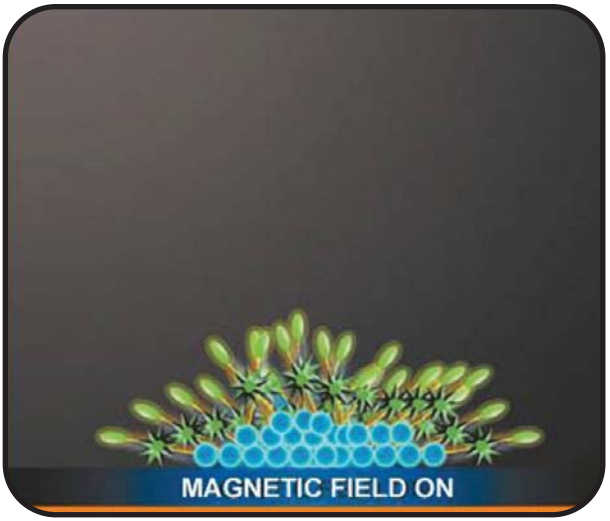
# of spores ➤	1,000,000	100,000	10,000	5,000	2,500
Test #1	2673	1505	672	351	161
Test #2	1680	1387	574	511	70

Conclusions and Recommendations

All strains of *B anthracis* tested were detected consistently above 5,000 spores, below the reported lethal dose of 7000 spores for this organism. Strain or spore preparation differences may account for 10 fold better sensitivity (LOD of 500 spores) seen in the Cohn and Sterne strain spore preparations. Interestly, both of these strains lack the pX02 plasmid. Recommend establishing consistent LOD of 5000 organism, but indicate in materials that detection below 1000 spores is common.

For full NYDIH report, please visit www.msanet.com

4 | WASH



All remaining sensing and non-target material is washed away

False positives and false negatives are virtually eliminated by removing potential interferants from the sample solutions.

R E S E A R C H R E P O R T

Evaluation Report on the QTL Biosystems *Bacillus anthracis* Cartridges and ß-unit Reader
By: Biodefense Laboratory, Wadsworth Center

Table 5. Matrix effects on *B. anthracis* detection. 0.1 mg/mL of the indicated powders were mixed with 5000 Pasteur spores and analyzed on the *B. anthracis* specific cartridges. A positive result was defined as Response (mV) greater than 100 as per manufacturer's instructions. Average Response from at least duplicate tests is shown. Red numbers indicate a positive result. The range of responses from different Pasteur strain analyses are shown as metric for response variability above LOD.

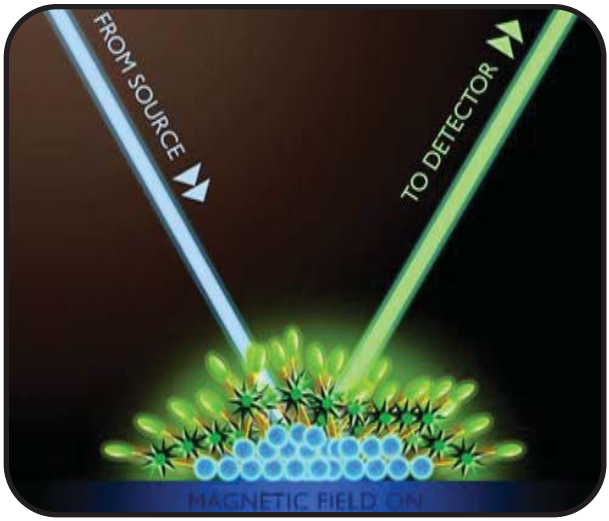
Added Powder (0.1mg/mL)	Response (mV)	Result
None *(neat Pasteur spike)*	312 - 573	true positive
Spackling powder	179	true positive
Baking soda *(Arm & Hammer)*	934	true positive
Instant non-fat dried milk *(Nestle Carnation)*	652	true positive
Talcum powder *(Equate)*	453	true positive
Flour *(Gold Medal)*	499	true positive
Salt *(Morton)*	550	true positive
Yeast *(Red Star)*	609	true positive
Powdered sugar *(Confectioners)*	294	true positive
Dipel™ *(Abbot Laboratories)*	238	true positive
Chalk *(Bison)*	272	true positive
Foot powder *(Total Body)*	522	true positive
Ajax™ with bleach *(Colgate-Palmolive)*	213	true positive
Non-fat dairy creamer *(Great Value)*	520	true positive
Kaolin *(Fisher-Scientific)*	528	true positive
Bentonite *(Aldrich)*	356	true positive
Aerosil R812S™ *(Degussa)*	485	true positive

Conclusions and Recommendations

All strains of *B anthracis* tested were detected consistently at 5,000 spores, regardless of matrix material spiked. Baking soda and milk proteins increased response readings at LOD and might indicate paths to improving reader Response for *B. anthracis* detection.

For full NYDIH report, please visit www.msanet.com

5 | READ

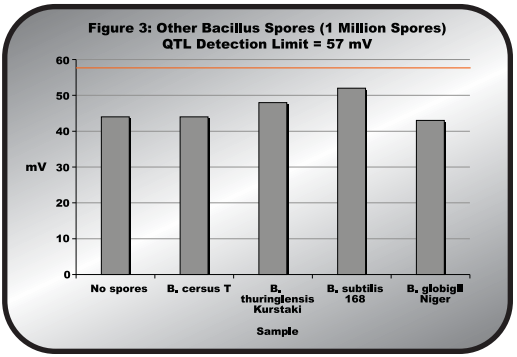


Concentrated sample (pellet) is illuminated and emits a signal if target is present

The BIOSENSOR Detector's dynamic surface generation technology effectively binds, concentrates and isolates the sample before detection, resulting in exceptional specificity, superior sensitivity, and greater reliability than other immunoassays.

Other bacillus spores (1 million spores)

The assay was then performed using four "nearest neighbor" bacillus spores at a level of a million spores per assay *(100-fold excess over the detection threshold for B.a., see Figure 3)*. None of these four spores tested showed a response significantly different from the background.



Bacillus Anthracis (Sterne) results

The detection limit for B.a. (Sterne) is less than 5,000 spores.

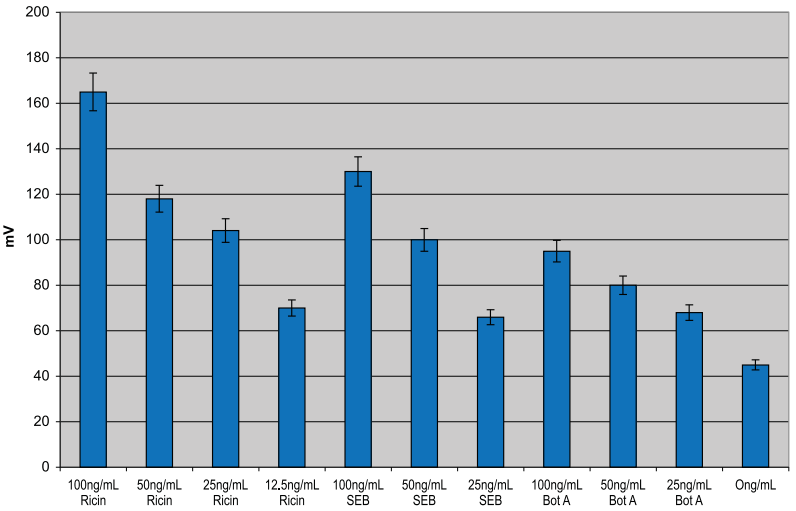
Bacillus Anthracis (Ames) limit of detection

Very good sensitivity was observed for the Δ Ames spores; the detection limit is approximately 12,000 spores with a statistically significant false positive rate of one in a million. With a false positive rate of one in a thousand, the detection limit is below 10,000 spores.

For full UAB report, please visit www.msanet.com

Key competitive advantages of the Biosensor Detector's assay process:

- Immunoassays can detect toxins while tests using PCR *(polymerase chain reaction)* cannot.
- Toxins do not contain DNA, necessary for PCR testing. Ricin, botulinum and SEB *(staph infection)* are toxins.
- PCR testing is more expensive, takes longer *(20+ minutes)* and requires more training and experience than immunoassays.



Triple Toxin Assay Data

- Biosensor anthrax antibodies bind to a protein on the outer spore coating of bacillus anthracis. Other immunoassays detect secondary markers unlikely to be present in a purified B. anthracis spore sample. A high probability exists that weaponized anthrax will not contain these secondary markers.

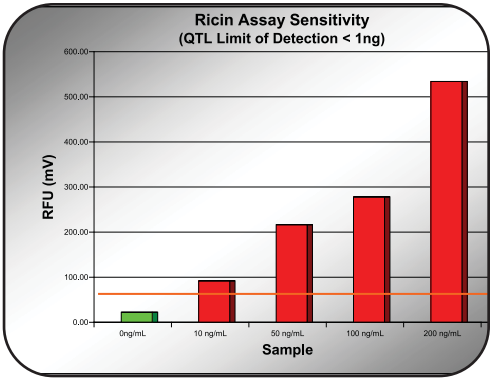
JOURNAL OF BACTERIOLOGY, Jan. 2004, p. 566–569 Vol. 186, No. 2
0021-9193/04/$08.00+0 DOI: 10.1128/JB.186.2.566–569.2004

NOTES

Surface Layer Protein EA1 Is Not a Component of *Bacillus anthracis* Spores but Is a Persistent Contaminant in Spore Preparations

David D. Williams and Charles L. Turnbough, Jr.*
Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294

- The MAG-TARGET-TAG sandwich is formed in a free solution, resulting in an extremely low level of detection *(the Biosensor Detector can detect nanograms of some targets)*.



< 1 ng detected within 5 minutes

- The wash step eliminates interferants that, when mixed with bioterrorist agents, can cause false positives and false negatives in other immunoassays.

B.a. Sterne spores in interfering powders

Finally, the assay has been tested for background and signal for 100,000 B.a. (Sterne) spores in the presence of potential interferants *(or suspect powders)*. As shown in **Figure 4**, none of these substances produce changes in either the background (blank) or samples containing spores.

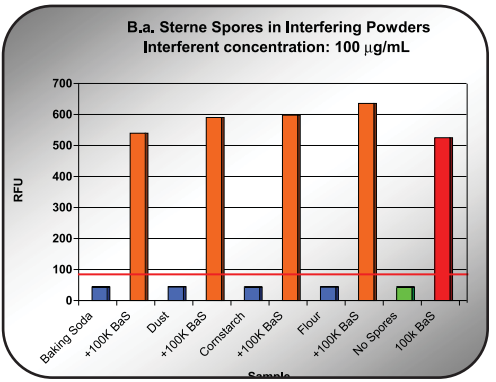
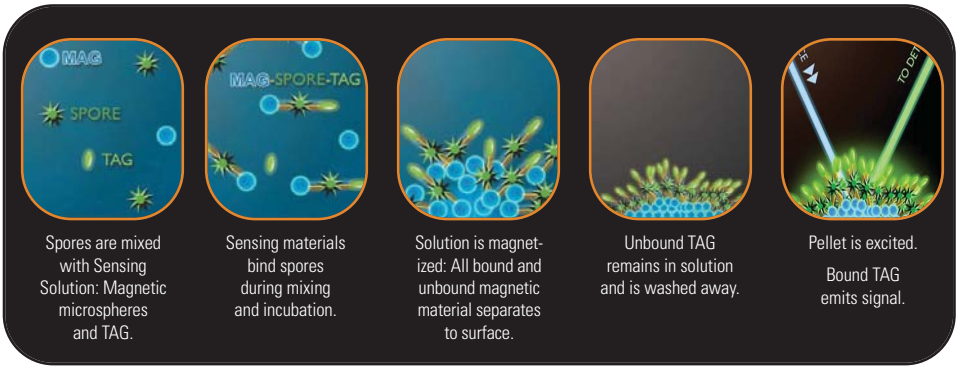


Figure 4

MSA BIOSENSOR™ Technology



Spores are mixed with Sensing Solution: Magnetic microspheres and TAG.

Sensing materials bind spores during mixing and incubation.

Solution is magnetized: All bound and unbound magnetic material separates to surface.

Unbound TAG remains in solution and is washed away.

Pellet is excited. Bound TAG emits signal.

The MSA BIOSENSOR 2200R Biological Agent Detector has an established track record in real-world applications and has been subject to successful third-party testing.

Wadsworth Center
New York State Department of Health

UAB

The Biosensor Detector has been tested and used by the U.S. Army, U.S. Marines, U.S. Navy, U.S. Air Force, the German Army, and local, state, and federal HazMat teams.

ID 0815-41-MC / May 2008

Corporate Headquarters
P.O. Box 426, Pittsburgh, PA 15230 USA
Phone 412-967-3000
www.MSAnet.com

U.S. Customer Service Center
Phone 1-800-MSA-2222
Fax 1-800-967-0398

MSA Canada
Phone 1-800-672-2222
Fax 1-800-967-0398

MSA Mexico
Phone (52) 55 2122 5770
Fax (52) 55 5359 4330

MSA International
Phone 412-967-3354
FAX 412-967-3451

Offices and representatives worldwide
For further information:

MSA
The Safety Company