DEVELOPMENT AND TESTING OF A NOVEL STANDARD PARTICLE FOR PERFORMANCE VERIFICATION OF BIODEFENSE/BIOTERRORISM DETECTION SYSTEMS

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ABSTRACT

This paper describes a novel simulant test particle that allows for the calibration of biodetection systems without causing safety concerns. Specifically, this test particle is a polystyrene sphere, of an aerodynamic diameter similar to that of *Bacillus anthracis*, with *Bacillus globigii* genomic DNA attached to the surface. The DNA attachment is strong enough to withstand areosolization and collection by a SpinCon sampler, and yet weak enough to detach during the GeneXpert analysis. Tests performed at MRI have shown that the tagged beads can be collected in the SpinCon and then successfully analyzed in the GeneXpert without modifying currently established analysis protocols.

THE PROBLEM

In recent years, biodefense and bioterrorism detection systems have become more widely available as new needs and new technologies have evolved. The sophistication of these systems has increased substantially as new methods and new technologies have been implemented. One aspect of this rapid development that has kept biodetection

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
19 NOV 2003	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Development And Testing Of A Novel Standard Particle For Performance Verification Of Biodefense/Bioterrorism Detection Systems	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Midwest Research Institute, 425 Volker Blvd. Kansas City, MO 64110**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
See also ADM001851, Proceedings of the 2003 Joint Service Scientific Conference on Chemical & Biological Defense Research, 17-20 November 2003. , The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**7**	

systems from reaching the level of reliability of other analytical systems is the lack of test particles for verifying system performance on a regular and frequent basis.

Within the scientific, medical, and commercial communities the frequent use of tests and controls to verify analytical system performance is essential. Quality assurance (QA) standards are set to allow comparison of performance to objectives, and tests are run both prior and subsequent to sample sets to verify proper system performance during analysis. End-to-end testing that verifies performance of the entire system is considered ideal.

Currently, all of the possibilities for checking of bioaerosol sampling and detection systems involve functional biological organisms. These possibilities are:

- Actual pathogens
- Surrogate organisms (if available)
- Vaccine/attenuated strains
- Irradiated organisms

The inherent danger associated with biowarfare agents rules out the use of live agents for biosampling and detection system checks. Testing of these devices with non-pathogenic biological surrogate organisms is currently a common approach for testing these devices. However, truly representative surrogates are not available for every organism of interest. Furthermore, it is possible for surrogate organisms to cause disease, particularly in immunocompromised individuals. These health concerns are also associated with the use of vaccine and attenuated strains for system testing. The use of irradiated microorganisms could result in interference with detection systems over time. Finally, there is a "comfort factor" that becomes a concern within this context. People may be uncomfortable with periodic disseminations of biological organisms to testing these systems, particularly if these individuals must work close to the bioaerosol sampling and detection devices on a consistent basis. This is particularly true for non-scientific personnel.

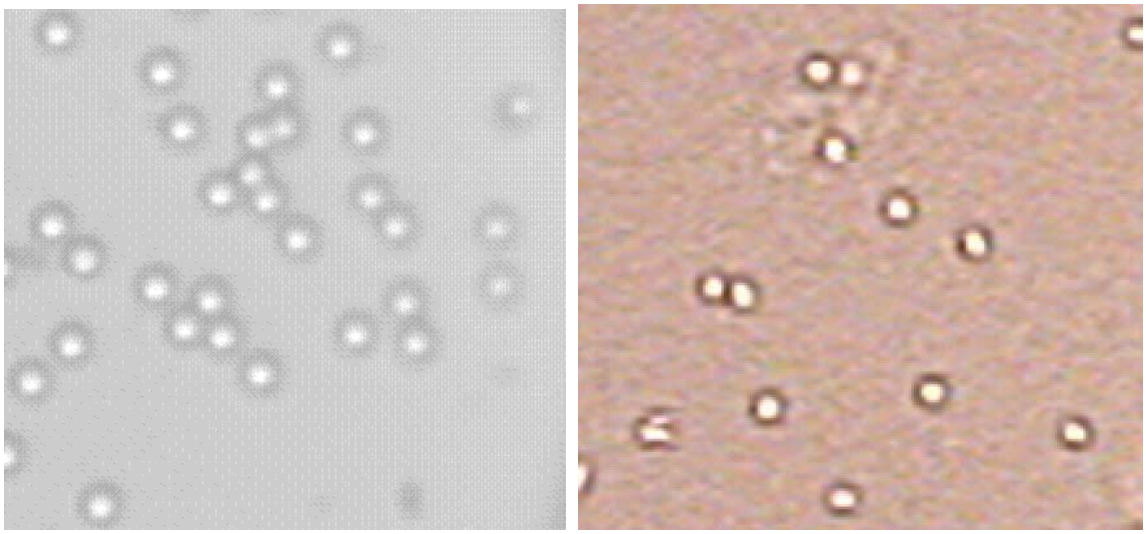
Because no acceptable test particles have been available for verification of biosampling and detection system performance, most systems have limited, or even no, performance assessments. What would be considered unacceptable with other analytical systems has become the norm with biosampling and detection systems. Until now, biodetection systems have faced a unique and daunting challenge because the introduction of microorganisms for quality checks posed an unacceptable contamination risk or health concern. No test particles are available for verifying the performance of bioaerosol sampling and detection systems on site, and on a regular basis.

THE SOLUTION

BioSim particles have been conceived as a solution to this pervasive problem in the area of biosampling and detection. BioSim particles are generated in the laboratory, and are custom-designed for end-to-end evaluation of the system of interest. These particles contain only the biological features necessary for complete end-to-end testing of the system, and contain no extraneous features. These particles are non-viable and safe. The BioSim particles are disseminated into the test system, collected by the system of interest and then analyzed by the method of interest; thus providing the desired end-to-end evaluation.

A specific example of a BioSim particle, BioSim-Bg, has been designed, prepared, and evaluated as a proof of concept. BioSim-Bg particles are made using 0.95 micron polystyrene microspheres (Bang's Laboratories, Fishers, IN) which were chosen to closely mimic the aerodynamic diameter of *Bacillus anthracis* endospores. Total genomic DNA from *Bacillus globigii* (Bg) is attached to the surface of the polystyrene microspheres using biotin/streptavidin linkages.[1, 2] The microspheres were purchased pre-coated with streptavidin (ProActive Microspheres, Bang's Laboratories). Genomic DNA was biotinylated and then attached to the microspheres using protocols provided by two manufacturers (Bang's Laboratories and Pierce Laboratories). The resulting BioSim-Bg particles were then subjected to dissemination, collection, and analysis using fluorescence PCR-based methods.

Figure 1 shows a side-by-side visual comparison of BioSim Beads with *Bacillus anthracis* endospores, as viewed by light microscopy.



BioSim Beads ***Bacillus anthracis*** **Endospores**

Figure 1. Side-by-side comparison of BioSim Beads with endospores from *Bacillus anthracis,* viewed using light microscopy. As shown in these photographs, the BioSim Beads and *B. anthracis* endospores have the same approximate shape.

DISPENSING BIOSIM-BG

In this study, BioSim-Bg was dispensed using metered-dose inhalers (MDIs) containing a mixture of suitable propellants. The materials used in the BioSim-Bg delivery system must be physically, chemically, and biologically compatible with the BioSim particles. Specifically, this means that the microspheres, the DNA, and the streptavidin/biotin linkages must not be adversely affected by the components in the propellant formulation. Furthermore, the density of the propellant formulation must be similar to the BioSim-Bg particles, to ensure homogenous distribution of the beads throughout the solution. The vapor pressure must be adequate for proper aerosolization when the MDI is actuated[3].

From the beginning, a safe alternative to living pathogens and surrogate organisms was the main objective for the BioSim particles. Therefore, propellant blend itself should be non-flammable and non-toxic, and should not contain ozone-depleting compounds. These safe formulation selection guidelines were used and proven to provide the DNA-tagged BioSims with a compatible propellant that is safe to use.

Potential propellants were evaluated simultaneously for safe use, dispensing system compatibility, and biodetection system compatibility. Some candidate propellant blend components, such as vinyl chloride, were eliminated from consideration due to health risks (VC is a known IARC-listed carcinogen). Others, such as chlorofluorocarbons (CFCs, i.e., Freons®), were not acceptable because they are harmful to the environment. CFCs are still conditionally approved by EPA and FDA for use in metered-dose inhalers, but are becoming costly and hard to obtain, and are known to harm the earth's ozone layer. Table 3 lists some examples of candidate propellant blend candidates.

Table 3. Non-CFC MDI Propellants

Propellant	General Information	Vapor Pressure (psia@77° F)	Density (Liquid) (g/ml@77°F)
1,1,1,2-tetrafluoroethane	HFA 134a – Used in pharmaceutical inhalers	96.6	1.206
1,1,1,2,3,3,3-heptafluoropropane	HFA 227ea – Used in pharmaceutical inhalers Boiling point (2.5° F)	66.0	1.39
1,1,1,3,3,3-hexafluoropropane	HFA 236fa – New Dupont Dymel for pharmaceutical inhalers Higher boiling point (29.4° F)	39.5	1.36
1,1-difluoroethane	HFA 152a – Not used for pharmaceutical inhalers, is used for personal products Boiling point (-13° F)	63 (psig@70°F)	0.908
Isobutane	Flammable at high concentrations	35	0.56
Acetone	Degrades DNA	4	0.79
Ethanol	Disrupts DNA bond to microsphere	0.5	0.79

Some of the important technical considerations are: even mixing, adequate vapor pressure for aerosolization, and compatibility with all components of the MDI and the beads. How the formulation might need to be different for different biodetection systems must also be considered[4], as well as cost and availability of components.

TESTING

The BioSim-Bg was tested for its utility as an end-to-end test particle for a bioaerosol collection and detection system. As shown in Figure 2, BioSim-Bg was disseminated from an MDI as a dry aerosol into a flow tube, concentrated into a liquid by the bioaerosol sampler (SpinCon PAS-450-10, Sceptor Industries, Kansas City, MO), and detected by using a polymerase chain reaction (PCR) assay for *B. globigii* (GeneXpert, Cepheid Corporation, Sunnyvale, CA). There were two considerations that were of particular importance for the design of the BioSim beads for this detection system. The first consideration is that the attachment of the DNA to the polystyrene microsphere must be durable enough to withstand the collection vortex of the SpinCon collector. The second consideration is associated with the specifics of the GeneXpert system. The GeneXpert system is a combination of an automated extraction system coupled with a fluorescence-based PCR detection system. In the initial stages of the automated GeneXpert extraction process, the sample is subjected to a sonication regimen. A critical aspect of the design of the BioSim beads for this application is that the attachment of the DNA to the bead must be disrupted by this sonication so that the DNA is made available for analysis by PCR.

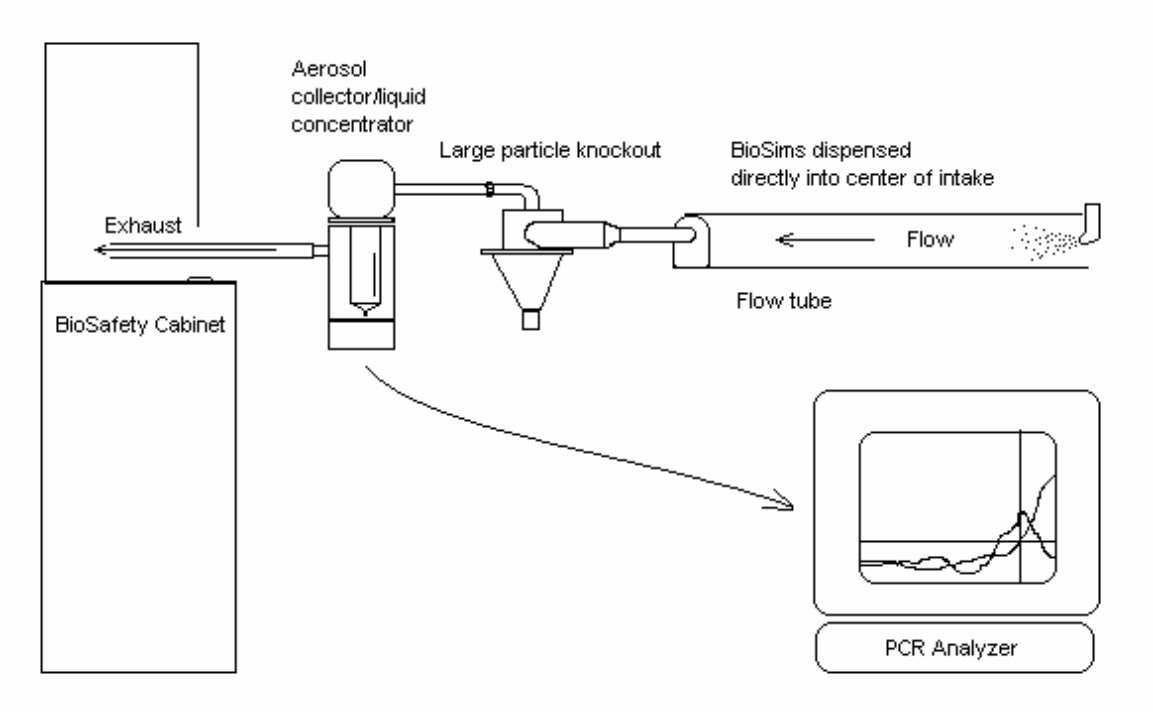


Figure 2. Collection and analysis of BioSim-Bg particles. The use of BioSim-Bg as an end-to-end test particle for a bioaerosol collection and detection system was evaluated using this set up.

Figure 3 shows a typical GeneXpert positive result for detection of Bg DNA originating from the disseminated BioSim-Bg beads.

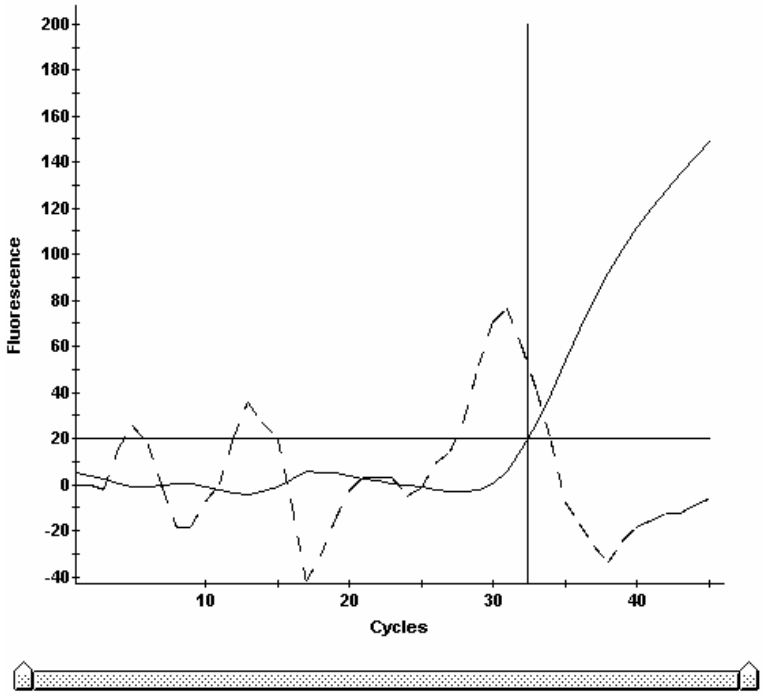


Figure 3. Typical result for BioSim-Bg beads collected by SpinCon and analyzed by GeneXpert. The line indicating geometrically increasing fluorescence as a function of number of cycles indicates the presence of Bg DNA (originating from the disseminated BioSim Beads) in the reaction.

CONCLUSIONS

The proof-of-concept studies reported here indicate that synthetic test particles, BioSims, can be constructed in the laboratory, and that these particles can be used for an end-to-end assessment of the performance of bioaerosol collection and detection devices. These BioSims particles can be designed to very precisely meet specific bioaerosol collection and detection system requirements. In order for the BioSim-Bg particles described in this study to successfully evaluate the test system, two important experimental criteria needed to be met: the attachment of the DNA to the polystyrene bead needed to be robust enough to withstand the aggressive SpinCon collection vortex, and yet be labile to the sonication of the GeneXpert system. These experiments indicated the promise of BioSims for use as non-biological surrogates for dissemination-type testing.

THE FUTURE OF BIOSIMS

The basic concept of the BioSim test particle can be extended to encompass different particle variations. For example, BioSims can be prepared with genomic DNA or DNA fragments from any organism of choice. Multiplex BioSims can be prepared by placing DNA from several organisms simultaneously on the beads. Proteins and other cellular constituents may also be attached to beads to generate test particles.

There is a wide array of potential uses for BioSim particles. In addition to testing bioaerosol collection and detection systems, BioSims could be used in large-scale open air releases, training for cleaning up contamination events, mapping building or subway flow diagnostics, as well as many other applications. In short, any testing or training event which would normally use viable surrogate organisms is a potential application for the safer BioSim test particle.

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