

Biologic Air-Borne Particle Detection, Sampling, and Identification

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Introduction

The aim of this study was to verify the compatibility of the MesoSystems AirSentinel[®] BioAerosol Sensor and the MesoSystems BioCapture[®] 650 hand held air sampler with the RAZOR[™] system for the detection of weaponized, airborne biological particles simulating an actual bioterror release.. The study also shows the efficacy of doing DNA amplification with very simple sample preparation methods on the RAZOR instrument using PathFinder[™] freeze-dried agent specific reagents.

Methods and Materials

All the testing was carried out at MesoSystems' test facility in Albuquerque, NM on 14 March 2005. All bioparticle releases and sample collection were performed in the MesoSystems test chamber. Weaponized *Bacillus globigii* (Bg) spores (Dugway Proving Grounds) were used as the test biological particle and collected with three air samplers (two Mesosystems AirSentinels and one Mesosystems BioCapture 650). All subsequent testing, identification and quantification of BG spores was done by one or more of the following methods: particle counting, PCR, microscopy and plate enumeration.



Mesosystem's Air Sentinel



Mesosystem's BioCapture 650

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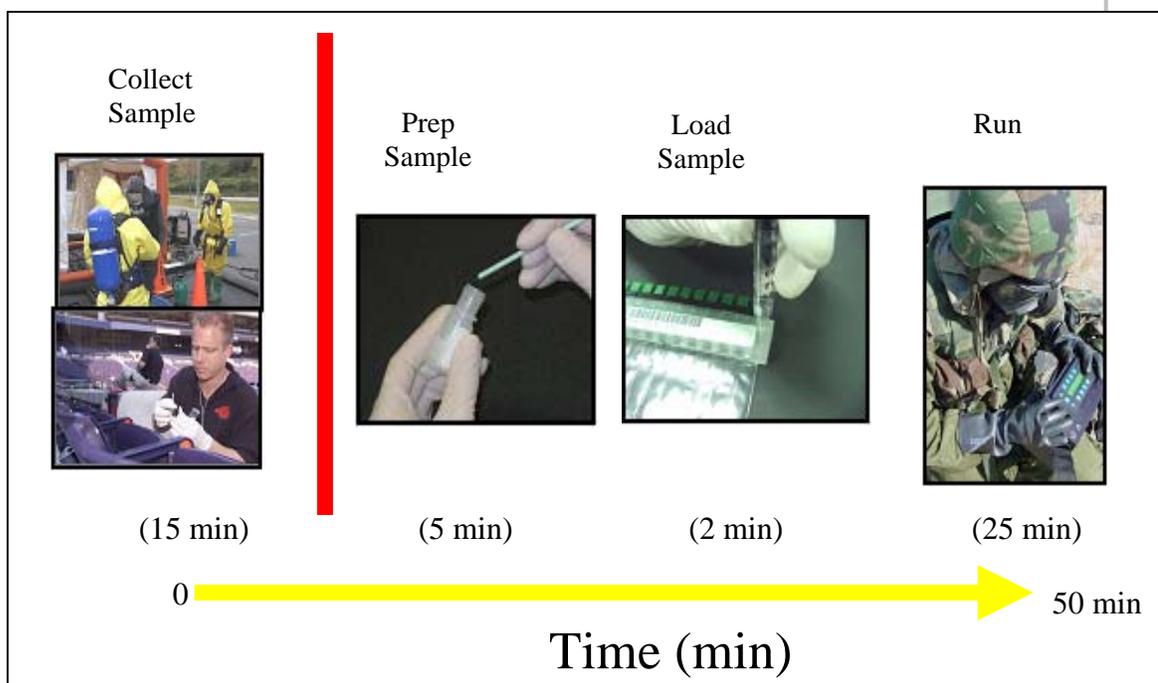
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Sampling and ID workflow

General workflow for the RAZOR is highlighted in the following diagram. All tests for this study were conducted in a laboratory setting.



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Testing

Three background tests were performed after the successive releases, two initial and one final. Four releases were performed in the test chamber ranging in concentration from 1 mg to 0.1 ug of weaponized Bg (1mg, 1mg, 100ug, 0.1ug). Positive samples were compared against background levels of Bg.

The AirSentinel is a trigger type air sampler. This means that the air sampler only collects a sample when it detects an elevated level of biological particles from the established background. It samples approximately 40 liters air/minute for 10 minutes onto a dry disposable disc that is processed after sampling to elute the collected material into a liquid. For these tests, the elution from the air collection mechanism was accomplished by gentle agitation in water for 30 minutes.

The BioCapture 650 is a battery powered, handheld, rotating impactor type air sampler. It pulls approximately 200 liters air/minute and collects for 10 minutes into a liquid media. Note: a 10 minute collection was used for this test to be compatible with the AirSentinel collection time but standard collection intervals for the BioCapture 650 are 5, 15 or 30 minutes.

The RAZOR system is a handheld battery powered real-time PCR instrument. PathFinder pouches containing freeze-dried reagents specific to *Bacillus globigii* were loaded with syringes containing samples obtained by the two AirSentinels (AS1 and AS2) and the BioCapture 650. Sample preparation for the RAZOR is based on simple dilution to reduce levels of potential inhibitory compounds to ensure adequate DNA amplification and fluorescent reading. The samples were run at three concentrations from the three MesoSystems air samplers – one without dilution, a 1:10 dilution, and a 1:100 dilution. This is based on the established sample preparation method for PathFinder preparation kits designed for liquid samples.

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For each test, a small amount of Bg stock material was weighed on an analytical scale prior to release in the chamber. When the limits of the scale were reached, a subsequent dilution of the dry sample was performed using CAB-O-SIL TS 530 powder. After dispersion of the material into the chamber, the chamber air was allowed to mix for a few minutes prior to sampling being initiated. A sampling cycle of ten minutes was run with the three air samplers. Samples were removed from the test chamber after the chamber air was pumped through HEPA filters. In addition to the test collectors, a gelatin filter was used as a reference to determine the Bg level in the chamber. Gelatin filters were dissolved, samples diluted and aliquots cultured to determine the cfu per liter of air in the chamber during testing. AirSentinel discs were prepared by gentle agitation in 10mls water with surfactant for 30 minutes. BioCapture 650 samples were taken directly from the sample vial and removed from the sampler. BioCapture and AirSentinel samples were not cultured, so concentrations of liquid samples provided to the RAZOR system are unknown.

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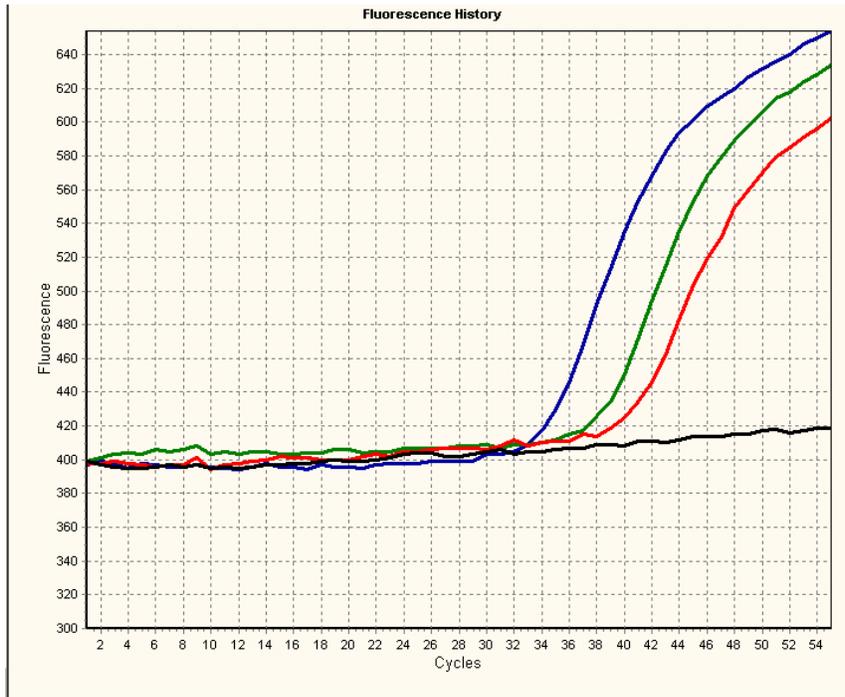


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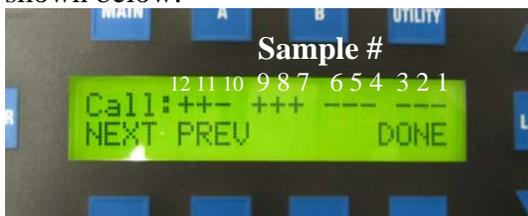
Results

Good PCR amplification was achieved on the RAZOR for the aerosolized Bg spores as seen in the following amplification traces from the RAZOR software.



Screen shot of dilution series from the RAZOR software. Samples were prepared from the AirSentinel collector disc post 1.0mg Bg release by agitation for 30 minutes in water and then loaded into the a 12 x 1 custom Pathfinder pouch specific for Bg (lot# P05031) at 1, 1:10 and 1:100 concentration (blue, green and red traces respectively).

The RAZOR does not require a PC for field applications. Analysis is done with the unit's internal software automatically with results displayed as shown below.



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RAZOR results screenTABLE 1.

Test	CFU/L (average)	RAZOR w/AS1	RAZOR w AS2	RAZOR w/ BC
High dry BG #1 (1 mg)	4.74E+06	Pos	Pos	Pos
High dry BG #2 (1mg)	8.17E+06	Pos	Pos	Pos
Medium dry BG #1 (100 ug)	4.55E+05	Pos	Pos	Pos
Low dry BG #1 (0.1 µg)	795	Neg	Pos	Pos

Note: all positive and negative controls gave appropriate results.

Discussion

The series of releases showed good correlation between the three air samplers and the RAZOR in terms of collection and detection efficiency of aerosolized *Bacillus globigii* spores (TABLE 1). All systems performed as expected.

Limitations of the testing facility prevented lower concentrations from being dispersed, so a lower limit of detection of the system was not determined in this study.

The field PCR only requires a fraction of the sample available from the BioCapture[®] or AirSentinel[®], allowing the ability to test for multiple agents in the field and leaving residual sample available for subsequent confirmatory testing.

Use of the AirSentinel system to automatically alarm and capture an air sample combined with the RAZOR's ability to determine presence of target agents is a powerful tool enabling constant monitoring of critical infrastructure. Similarly, use of the BioCapture with the RAZOR system, allows sampling of random or suspect cargoes entering a facility for presence of airborne agents. In either case, monitoring costs are minimized because system operation does not require highly trained, specialized personnel.

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