

## BIOGUARDIAN® AIR SAMPLER PERFORMANCE TESTING

### BACKGROUND

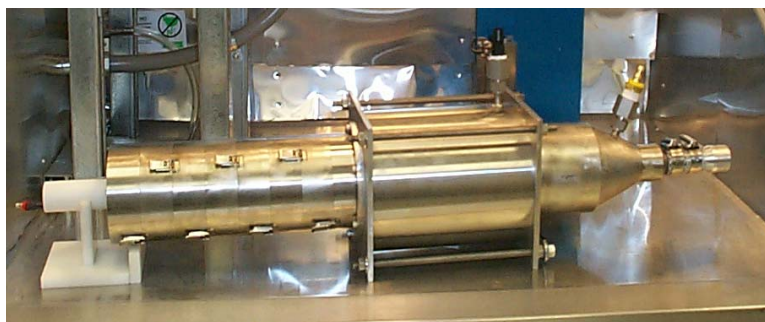
InnovaTek, Inc. has experience designing and testing wet-walled cyclones and electrostatic precipitators for a range of applications under both government and international commercial contracts. We have also developed high voltage plasma discharge technology for surface decontamination.

Since 1997 InnovaTek has been developing aerosol collectors for biological warfare agents under several government contracts. Work was completed in December 2002 under a contract from the U.S. Navy (Contract #N00178-01-C-3020) to develop an aerosol collector that collects particles in the 1-10  $\mu\text{m}$  size range and preseparates large interfering particles. Previously InnovaTek worked under subcontract on an Army SBIR project (Contract #DAAM01-97-C-0036) to develop aerosol collectors for biological warfare agents. Under this contract we investigated devices that used both cyclonic and electrostatic forces. InnovaTek was also funded by Eastern Washington University to develop an aerosol collector to be integrated with a photoacoustic detector they are developing for the Office of Naval Research. This detector is to be used as an early warning system for biological warfare agents. In addition, InnovaTek completed a Phase I SBIR project for U.S. Homeland Security Advanced Research Projects Agency to develop an electrostatic cyclone for biowarfare agents and is developing this technology further for both chemical and biological agents. These R&D contracts resulted in a commercial device, the BioGuardian® air sampler that is being produced under low-volume manufacturing (Fig. 1)



**Figure 1.** InnovaTek's BioGuardian® Air Sampler

InnovaTek successfully developed and tested a miniature electrostatic precipitator for separation of microfibers in an air stream for a Swiss Research and Development Company involved in toxicology studies (Figure 2). Their application is for a homogeneous supply of microfibers for controlled inhalation toxicology studies. The device was developed so that fibers of specific length could be prepared for controlled investigations with animals. The fiber classifier uses electrostatic forces to precipitate fibers with micrometer and submicrometer diameters. The fibers receive a charge using a corona discharge and are deposited according to size (length) along a continuum of the grounded collection section thereby generating categories of particles with specific sizes.



**Figure 2.** Coaxial tube/wire ESP device developed by InnovaTek for a Swiss company conducting toxicology studies.

## BIOGUARDIAN® TEST RESULTS

### Controlled Testing for Particle Collection Efficiency

System performance of the BioGuardian Air Sampler was measured on the basis of particle size in InnovaTek's laboratory using a gradient of sizes of mono-dispersed fluorescent polystyrene latex (PSL) spheres and microorganisms. A series of tests was conducted utilizing InnovaTek's test platform that produces an airstream with the desired particle sizes inside a Biosafety cabinet (Figure 3). A nebulizer is used to produce aerosolized particles in an airstream that is sent through a drying column to reduce hydration of the particles (which would increase aerodynamic particle size).

Particles, either PSL or *Bacillus subtilis* (single pure spores) are aspirated from a water suspension and delivered into the nebulizer (at a rate of approximately 0.2 ml/min). Pure argon is filtered by an MSA high pressure HEPA filter and delivered under a pressure of 175 psi and at a flow rate of 1 LPM into the gas inlet of the nebulizer. The nebulized particles are injected into a glass drying tube with an internal diameter of 25 mm (external diameter of the nebulizer is 6 mm) (Figure 4).

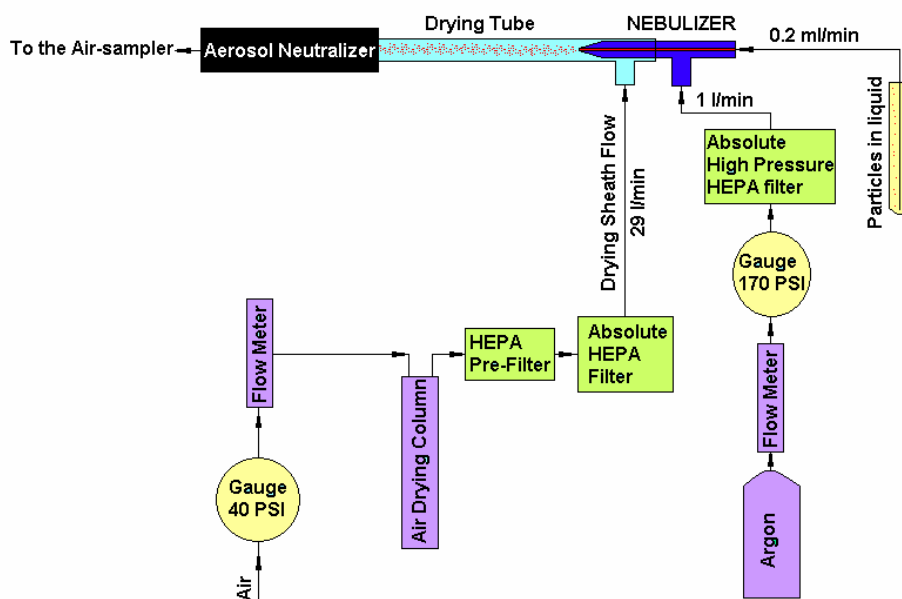


**Figure 3.** Photograph of InnovaTek's Aerosol Generation Test Platform

Sheath air flow (28 LPM) passes through the drying column ( $\text{CaSO}_4$ ) followed by double filtering with HEPA filters, forming the co-axial sheath flow, which is designed to dry the aerosol produced by the nebulizer and to prevent particle loss on the walls. The flow rate at the outlet of our drying system is approximately equal to the sampling flow rate (28.3 LPM) of the Particle Analyzer (Met One Laser Particle Counter, Model 2913).

The Particle Analyzer allows us to measure airborne particles using 8 programmable channels in ranges from 0.3  $\mu\text{m}$  up to 25  $\mu\text{m}$  and display the number of particles in each range as a cumulative count or differential count. It is interfaced with a PC to allow electronic data recording as well as system operation using computer commands. A temperature/humidity sensor is interfaced with the device and recorded during all tests. A data tape is also produced by the instrument. The number of viable organisms produced is determined by using a particle counter and culturing techniques.

At the outlet of the aerosol generation tube, the airstream passes through a charge neutralizer to remove any charge that may have been applied through nebulization and is then focused toward the inlet of our prototype to assess performance. A Turner Digital Fluorometer, Model 450 is used to quantify the fluorescent spheres captured in the liquid output. Testing is also conducted using microorganisms such as *Bacillus subtilis* var *niger*, an anthrax simulant, and *Staphylococcus epidermidis* using microbial culturing to verify collection and biological efficiency.



**Figure 4.** Schematic of InnovaTek's Aerosol Generation Test Platform

In previous studies our test platform was shown to produce results that were in agreement with those that were obtained in the aerosol testing chamber at the U.S. Army's Edgewood Chemical Biological Center. Figure 5 compares results obtained at InnovaTek facilities with those obtained at Edgewood facilities for our 1000 LPM BioGuardian air sampler.

We studied collection efficiency for spores of *Bacillus subtilis*, var. *niger* (an anthrax simulant). In repeated tests the average collection efficiency for pure 1  $\mu\text{m}$  *Bacillus* spores is about 70%. In a study to examine the effect of liquid output amount on collection efficiency, we observed that the BioGuardian based on eight tests with liquid output amounts greater than 12 mL (Figure 6). It is important to note that for our testing protocol, the collection efficiency is determined by what is collected and released in the collection liquid of the instrument.

### Concentration Factor Calculations

The concentration factor (CF) is calculated using the following procedure: CFU (colony-forming units) per ml of collection liquid divided by CFU per liter of air for a 1-minute collection period. Using our test results for the BioGuardian® Model 12.03-1000 with an air flow rate of 1000 LPM and 70% average collection efficiency that concentrates particles into an average 14 mL liquid, (assuming 1 CFU per liter air) the concentration factor is 50,000 per minute.

$$\text{Concentration Factor (CF)} = \frac{(1000 \text{ L/min}) (1 \text{ min}) (0.7)}{0.014 \text{ L}} = 50,000$$

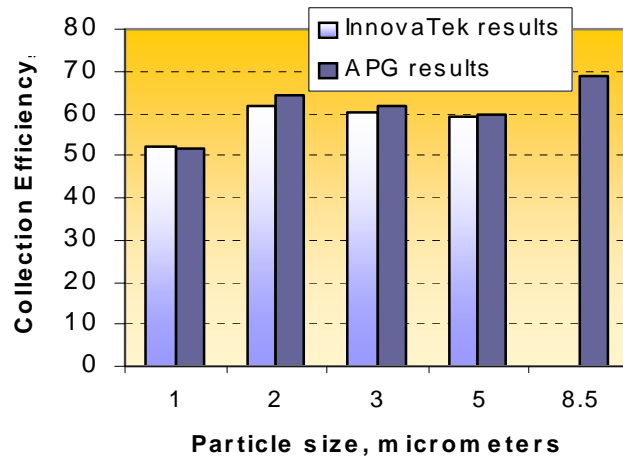


Figure 5. BioGuardian Collection Efficiency vs. Particle Size for Tests at InnovaTek and Aberdeen Proving Ground - Edgewood.

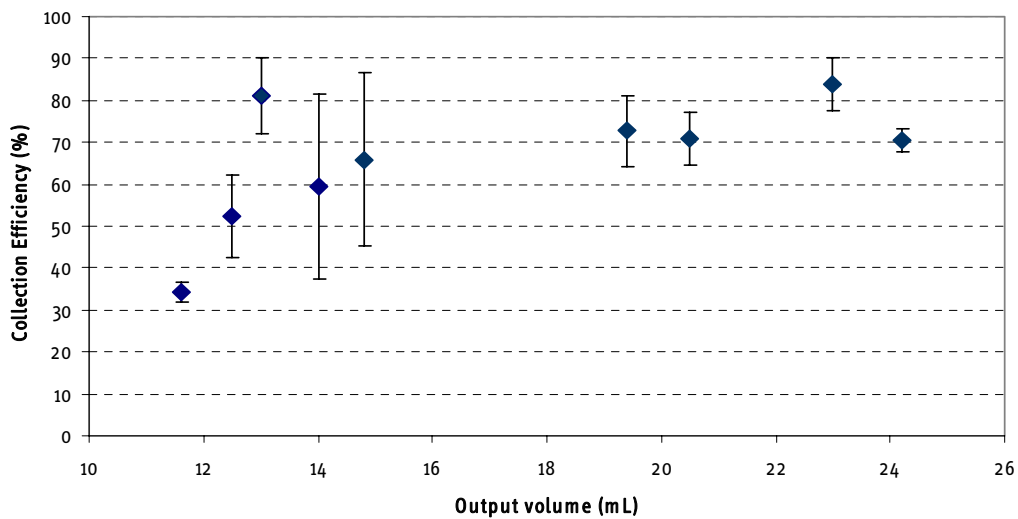


Figure 6. BioGuardian collection efficiency vs liquid amount for *Bacillus* spores

## Environmental Biological Collection Efficiency

Preliminary tests were performed at InnovaTek to obtain additional information on the impacts of certain modifications to the existing BioGuardian operating procedures to preserve viability of organisms collected. Since the product was originally designed to optimize collection efficiency for spores, there was not a great concern for high biological efficiency. However, because this may be an important feature for other applications, we examined some possible changes in operating procedures that might improve biological efficiency. Two sets of tests were conducted to 1) compare results using nutrient broth as the collection liquid with our customary triton+anti-foam, and 2) use a flow-through mode for the collection liquid instead of re-circulation.

### Nutrient Broth Collection Liquid

A standard nutrient broth solution was used as the collection liquid. A liquid bottle bag was filled with broth and kept cold (4°C) until just before testing was started. Environmental samples were obtained by operating two BioGuardians side-by-side according to standard procedures (i.e. instructions in the operators manual), for 2 minute collection periods in a location outside InnovaTek's facility, on the loading dock. Samples were plated immediately after the test. Results indicated that more than 4x the number of organisms were collected with the use of nutrient broth compared to our standard 0.01% triton plus 0.01% antifoam A (Table 1).

Note: When using nutrient broth as a collection medium, samples must be plated within 10 minutes of collection or else placed in a 4°C refrigerator for no more than 1 hour before plating.

**Table 1. Collection/Biological Efficiency based on Collection Liquid for BioGuardian Air Sampler**

BioGuardian Serial #	Test #	Collection Liquid	
		Total CFUs Recovered	
Day 1		Triton+antifoam	Nutrient Broth
	1	825	3926
	2	1085	3750
	3	1740	5765
Day 2			
	1	187	2424
	2	142	1971
	3	315	2990
<b>Average</b>		<b>716</b>	<b>3471</b>

### Flow-Through Mode for Collection Liquid

The liquid fluidics of the system can be revised to operate as a flow through system rather than recirculating. Recycling of liquid allows for concentration of the organisms being sampled over time. However, for short sampling times of a few minutes there is not a great advantage to recycling. This will eliminate the need for the large reservoir currently used in the system design where liquid swirls very rapidly and probably causes stress to the organisms. This will also significantly reduce the total length of tubing in the system, thus reducing "dead" volume and improve sterilization procedures.

The BioGuardian Air Sampler was modified so that the liquid fluidics system operates in a flow-through mode rather than a recirculation mode.

We conducted environmental sampling in recycling and flow-through mode using nutrient broth as the collection liquid. Because the existing BioGuardian was modified for these tests, the analysis gives a preliminary view of possible benefits. Greater benefits are possible through re-design to eliminate the large reservoir.

Results indicate that the flow-through mode is about 2x more efficient in collecting viable organisms than the recirculation mode (Table 2). Note that on Day 1 the first test was conducted for 2 minutes and the next two tests were for 1.5 minutes. Samples collected were plated in triplicate on nutrient agar and incubated for 2-3 days at 37°C, then counted.

**Table 2. Collection/Biological Efficiency for Flow-through versus Re-Circulation Modes for BioGuardian Air Sampler**

Test #	Day	Duration, min	Total CFUs Recovered	
			Flow-Through	Re-Circulation
1	1	2	839	708
2	1	1.5	816	505
3	1	1.5	758	149
4	2	1.5	225	100
5	2	1.5	225	40
6	2	1.5	120	170
<b>Ave.</b>			<b>499</b>	<b>277</b>

## CAUTION

Because of the low number of repetitions, these results should be considered preliminary. Also, organisms present in the environment in Richland WA, USA are certain to be different than those sampled in other regions, therefore tests results could be significantly different among sites.