

# Development of an integrated surface extraction and concentration system

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## Abstract

Rapid and effective surface monitoring is an important tool for first responders and for determining levels of pathogens on surfaces in food preparation facilities, hospitals, operating rooms, and clean rooms. Surface samplers generally collect into volumes ranging from 1 mL to 250 mL or more. A portion of the sample is transferred directly to a detector for analysis; and while great strides have been made in recent years in detector technology making them faster and more accurate than ever, they are only capable of analyzing volumes from 0.005 mL to 0.1 mL of liquid at a time. A great improvement in surface monitoring can be gained by concentrating the hydrosol from the surface sampler into a volume matched to the detector analysis volume. The hydrosol concentrator thereby functions as a macro-to-micro interface between the surface sampler and the detector. AlburtyLab, Inc. of Drexel, Missouri, obtained funding through the LWI and the Army Research Laboratory to provide initial proof-of-concept data for a small, battery-powered unit that is capable of concentrating biological agents from surfaces into small analysis-compatible volumes thereby increasing the sensitivity of advanced detection systems. The system is based on a novel "wet foam elution" method developed jointly by AlburtyLab and Page Applied Research LLC of Kansas City. A surface-to-liquid module was developed and coupled with an InnovaPrep liquid-to-liquid concentrator. The system was tested using "New Dugway" *Bacillus globigii* (Bg) spiked onto flat white-board test coupons. The system proved to be rapid, reliable, and efficient for recovery and concentration of surface deposited Bg.

## Materials and methods

Prototype extraction wands were tested using 1.0 µm fluorescent polystyrene microspheres and the best performing unit was chosen for the Bg testing.

Figure 1. Removal of fluorescent polystyrene microspheres using the surface extraction wand is made more apparent with the use of a UV light.



Test coupons were 4 in x 32 in sections of melamine resin-coated masonite. The tickets were spiked with 250 µL of a Bg suspension by dispensing a line of fluid down the center length of the test coupon. The fluid was then spread using a cell spreader in a 24 in<sup>2</sup> area of each coupon. The coupons were dry in approximately one hour and were extracted approximately 40 hours after spiking. The surface extraction wand is able to extract an approximate width of 2.5 inches so this allowed each test run to be performed with a single pass followed by using suction alone to remove any remaining foam and liquid. Low level spikes were 3.36 x 10<sup>2</sup> CFU per coupon and high level spikes were 6.09 x 10<sup>6</sup> CFU per coupon.



Figure 2. The prototype surface extraction system designed and built at AlburtyLab was one method used for surface sampling. One of the spiked coupons is visible in the foreground.

For comparison, three coupons were extracted using a commercially available Biological Sampling Kit (BiSKit, QuickSilver Analytics, Abingdon, MD). The surface extracts from both sampling systems were concentrated using a research model InnovaPrep system. A commercially available hollow fiber unit from Spectrum Laboratories, Inc. was used for all liquid-to-liquid concentration cycles.

Figure 3. The InnovaPrep hydrosol concentrator used to concentrate surface sample extracts collected during this testing.



## Results

The spiking material as well as the recovered and concentrated material were analyzed by plating and a portion by quantitative PCR. A portion of the samples were also analyzed for Bg with lateral flow immunoassay strips (LFI) from the Critical Reagent Program in order to verify that the concentrated material could be detected using these tests. Because the extract was to be analyzed by several methods for comparison, relatively large (~1.5 mL) extraction volumes were used. Only the plating results are presented here. The average volume of the surface extracts collected using the surface extraction wand was 18.8 mL. Data for the high level spike test runs conducted using the surface extraction wand is presented in Table 1.

Table 1. The surface extraction wand was used to extract samples from four test coupons with high level Bg spikes. After removing an aliquot for plating, the extract was concentrated using the InnovaPrep system.

	High Level Spikes				Average	SD
	Run 31	Run 32	Run 33	Run 34		
<b>Spike</b>						
Bg Spiked, CFU	6.09x10 <sup>6</sup>	6.09x10 <sup>6</sup>	6.09x10 <sup>6</sup>	6.09x10 <sup>6</sup>		
<b>Surface Extract</b>						
Recovery Efficiency by CFU	119.4%	87.6%	88.3%	103.4%	99.6%	15.0%
<b>Concentrate of Surface Extract</b>						
Volume, mL	1.2	1.15	1.18	1.2	1.18	0.02
InnovaPrep Efficiency by CFU	70.4%	102.4%	92.7%	55.8%	80.3%	21.2%
Concentration Factor	11.8	14.8	12.7	8.13	11.8	2.8
Total System Efficiency by CFU	84.0%	89.7%	81.9%	57.7%	78.3%	14.1%

During the low level test runs, two passes were performed with the surface extraction wand on each coupon. The fluid was then concentrated using the InnovaPrep system. Results of these tests are contained in Table 2.

Table 2. The surface extraction wand was used to extract samples from three test coupons with low level Bg spikes. The titer of the surface extract sample was below the detection limit of plating and therefore was not determined. The extract was concentrated using the InnovaPrep system and a total system efficiency was determined.

	Low Level Spikes			Average	SD
	Run 40	Run 41	Run 42		
<b>Spike</b>					
Bg Spiked, CFU	3.36x10 <sup>2</sup>	3.36x10 <sup>2</sup>	3.36x10 <sup>2</sup>		
<b>Surface Extract</b>					
Volume, mL	24.97	32.48	35.91	31.12	5.59
<b>Concentrate of Surface Extract</b>					
Volume, mL	1.64	1.32	1.26	1.41	0.2
Number in Concentrate, CFU	2.78x10 <sup>2</sup>	3.48x10 <sup>2</sup>	2.19x10 <sup>2</sup>		
Total System Efficiency by CFU	82.7%	103.5%	65.1%	83.7%	19.2%

The average volume of the surface extracts collected using the BiSKit was 9.8 mL. Data for the high level spike test runs conducted using the BiSKit is presented in Table 3. The average recovery efficiency of 36.4% was higher than the 11.3% previously reported (Buttner, 2004). This can be attributed to the smaller surface area that was extracted and to the smooth surface of the coupon.

Table 3. Surface samples were recovered from three test coupons with high level Bg spikes using BiSKits. After removing an aliquot for plating, the extract was concentrated using the InnovaPrep system.

	High Level Spikes			Average	SD
	Run 35	Run 36	Run 37		
<b>Spike</b>					
Bg Spiked, CFU	6.09x10 <sup>6</sup>	6.09x10 <sup>6</sup>	6.09x10 <sup>6</sup>		
<b>BiSKit Surface Sample</b>					
Recovery Efficiency	33.3%	47.3%	28.5%	36.4%	9.8%
<b>Concentrate of Surface Sample</b>					
Volume, mL	1.34	1.26	1.18	1.26	0.08
InnovaPrep Efficiency by CFU	129.1%	91.2%	115.1%	111.8%	19.1%
Concentration Factor	8.98	6.34	7.66	7.66	1.32
Total System Efficiency by CFU	42.9%	43.1%	32.9%	39.6%	5.9%

A comparison of the recovery efficiencies determined for each type of tests is presented in Figure 3.

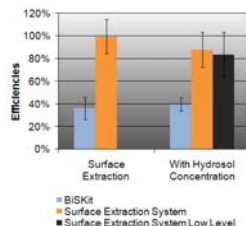


Figure 3. High recovery efficiencies were determined for both the surface extraction wand and the InnovaPrep hydrosol concentrator. The recovery efficiency for the surface extraction wand could not be determined for the low level testing since the titer of the extract was below the detection limit for plating. This highlights the advantage of using the InnovaPrep system for the detection of low level surface contamination.

## Conclusions

AlburtyLab was able to demonstrate recovery and concentration of Bg from surfaces using a wet foam elution method to extract particles from flat surfaces and concentrate the subsequent liquid with a wet-foam elution hollow fiber concentrator system. Demonstrated efficiencies for the system were high and processing was rapid.

Using the wet foam elution surface extraction system a one meter square surface can be extracted in less than 5 minutes. Liquid samples can be processed at a rate of approximately 20 mL/min plus an additional 30 seconds for recovery. Surface extraction of a one square meter area will produce a volume of approximately 600 mL. While this volume of fluid is manageable the system will require fairly large volumes of extraction buffer and significant time periods will be required for concentration of the fluid. Based on the current configuration, the time required for concentration of the output fluid from a one square meter surface extraction will be approximately 37 minutes. Future enhancements to the system will work towards reducing the fluid usage.

The system developed by AlburtyLab and tested during this project will be useful to military and civilian first responders during investigations of possible biological weapons attacks. Food preparation and health care facilities, as well as researchers can benefit greatly from the ability to monitor levels of pathogens on surfaces at levels where they were previously undetectable.

## Literature cited

Buttner, M.P., Cruz, P., Stetzenbach, L.D., Klima-Comba, A.K., Stevens, V.L., and Emanuel, P.A. 2004. Evaluation of the Biological Sampling Kit (BiSKit™) for Large-Area Surface Sampling. Applied and Environmental Microbiology. 70(12):7040-7045.

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