3rd National Conference on Environmental Sampling and Detection for Bio-Threat Agents

Sampling Tools for First Responders

High Efficiency Bio-Agent Recovery from Simulated Environmental Surfaces with Robust Liquid Rinse Vacuum Collection System

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Sampling Tools for First Responders

- In response to a biological incident/attack, First Responders must have sampling devices with the capabilities to efficiently and safely collect samples.
    1. Sampling is the initial step in determining the nature of the contamination
    2. There is a need for new sampling tools and technology

- The ultimate goal of any sampling device/method is to provide a representative sample of the bio-burden on a surface of interest and successfully deliver that sample to a detection device.

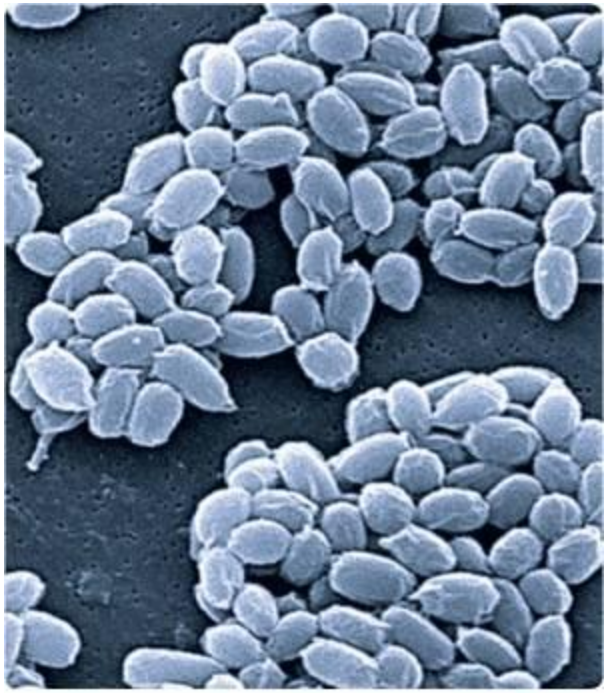
- This presentation will focus on a new sampling device: The M-Vac

Sampling Challenges for First Responders

- Bio-agents may be widely dispersed over a large area
- Samples must be taken off a variety of surfaces with a high degree of confidence
- Surface levels may be very low
- A number of potential bio-agents can cause disease at extremely low levels
    1. Q-Fever: 1 organism can cause disease in a susceptible person
    2. Tularemia: Approximately 10-50 cells can cause disease
    3. Anthrax: Estimated ≈10,000 cells. May be significantly less for susceptible people

List of High Priority Threat Bio-Agents:

- Anthrax
- *Burkholderia* bacteria
- *C. botulinum* toxins
- Plague
- Q-fever
- Smallpox
- Typhus
- Tularemia
- Various viral hemorrhagic fevers



***Bacillus anthracis* spores**

Source: Center for Disease Control and Prevention

Sampling Challenges: Determining Sampling Efficiency

Sampling efficiency: A measurement of a sampling device's ability to effectively sample a surface of interest. Involves 3 main steps:

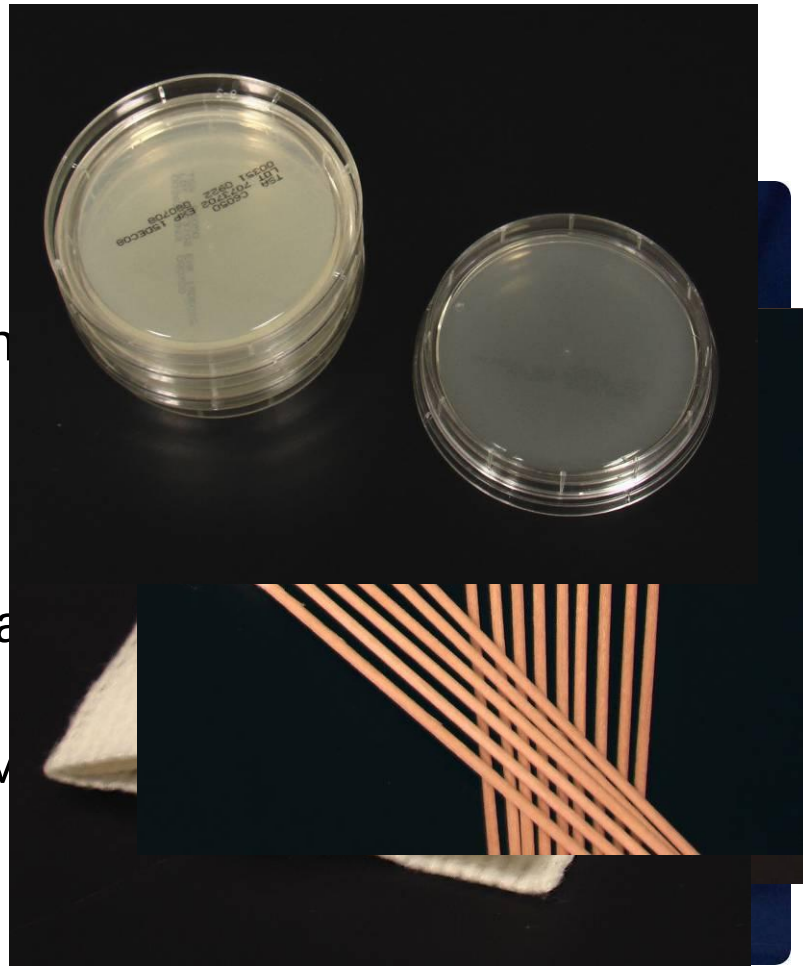
1. Location/discovery: Locating target organisms on the surface of interest.

2. Extraction: Extracting the target organism from the surface of interest with the sampling device.

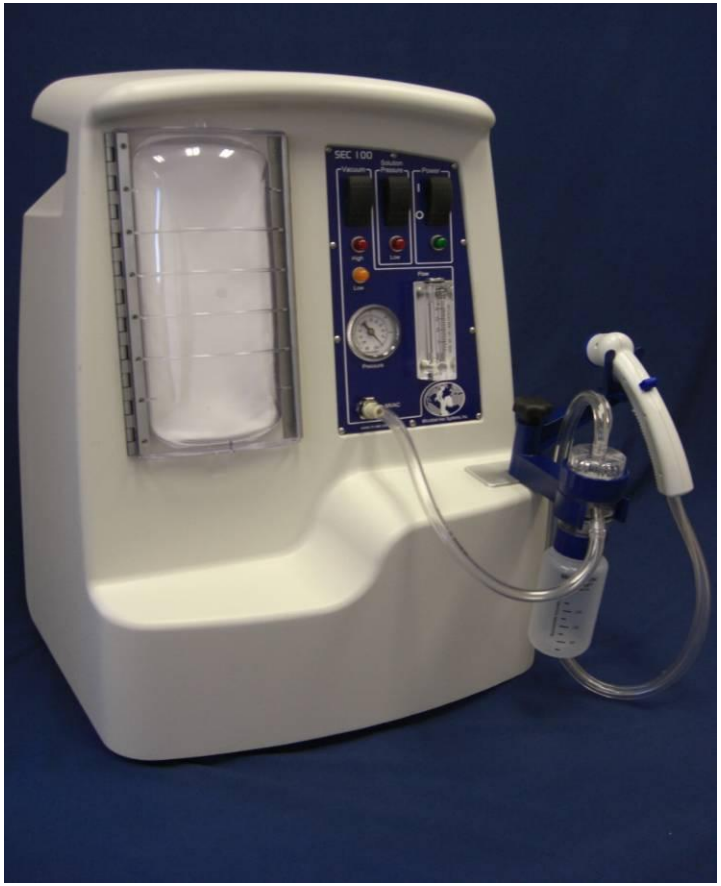
3. Elution: Removing the target organism from the sampling device into solution for detection.

Currently Utilized Sampling Devices

1. Swabs: cotton, rayon, polyester,
2. Sponges: cellulose based
3. Surface Wipes: commonly cotton
4. HEPA Vacuum Socks
5. Agar Contact: contact plates, aga
6. The Microbial-Vacuum System (M



The M-Vac System



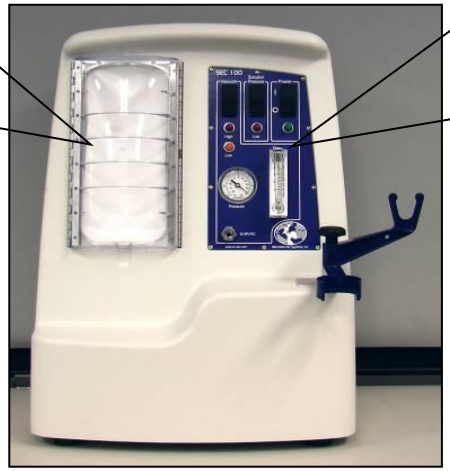
SEC with attached MS-Kit

- Non-destructive wet-vacuum surface sampling device
- Applies a sterile surface rinse solution (SRS) at a constant pressure
- Vacuum pressure capture of surface suspension at 15 in. Hg (-50 kPa)
- Collects up to 150 ml of surface suspension per sample bottle. Delivers 160 ml/min.
- Unique filter system ensures that the sample is isolated to the collection bottle.

The M-Vac System Overview

Solution Pressurization Chamber

Support Equipment Case (SEC)



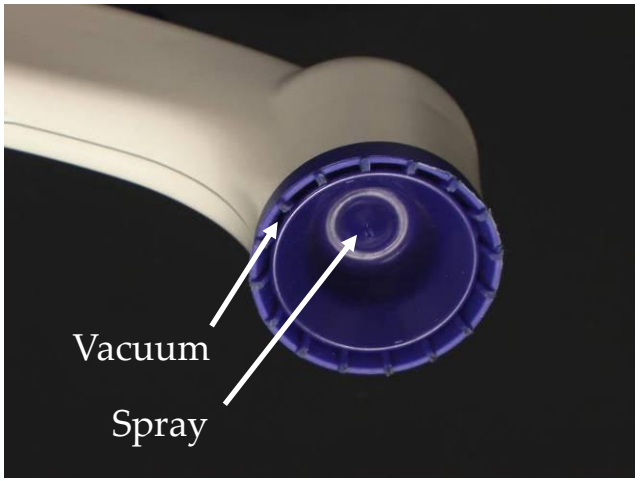
SEC Control Panel

•SEC footprint: 19”x16”x25” (48.3x40.6x63.5 cm), 58 lbs (26.3 kg), portable with “suitcase style” handle and wheels. 110 volts AC, 220 volt system also available

The M-Vac System Overview

SRS Bag

M-Vac & Sampling Head (MS Kit)



SRS: PBS, Neutralizing Buffer, Butterfield's Buffer, etc.

Sampling Efficiency

1. Location/discovery: Locating target organisms on the surface of interest.
    a. Location efficiency of sampling device is determined by:
        i. Concentration of bio-agent (average # bio-agent per surface area)
        ii. Distribution of bio-agent (uniform vs variable)
        iii. Surface area of each sample
        iv. Number of samples

2. Current Swab/Swipe Devices
    a. Small surface area per sample: typically 25 cm^2 for swabs, 100 cm^2 for sponges
    b. Large number of samples needed

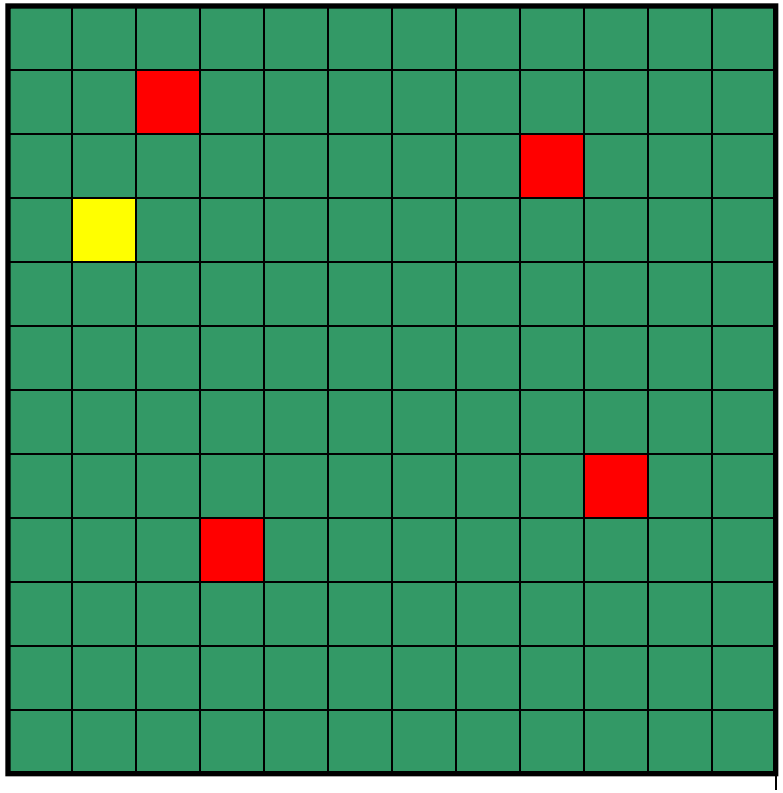
3. M-Vac
    a. Large surface area per sample: up to 1600 cm^2.
    b. Fewer samples needed

Location Efficiency

= Contamination

= Swab sample sites*

*Swab sample sites are 5x5 cm
~25 cm^2



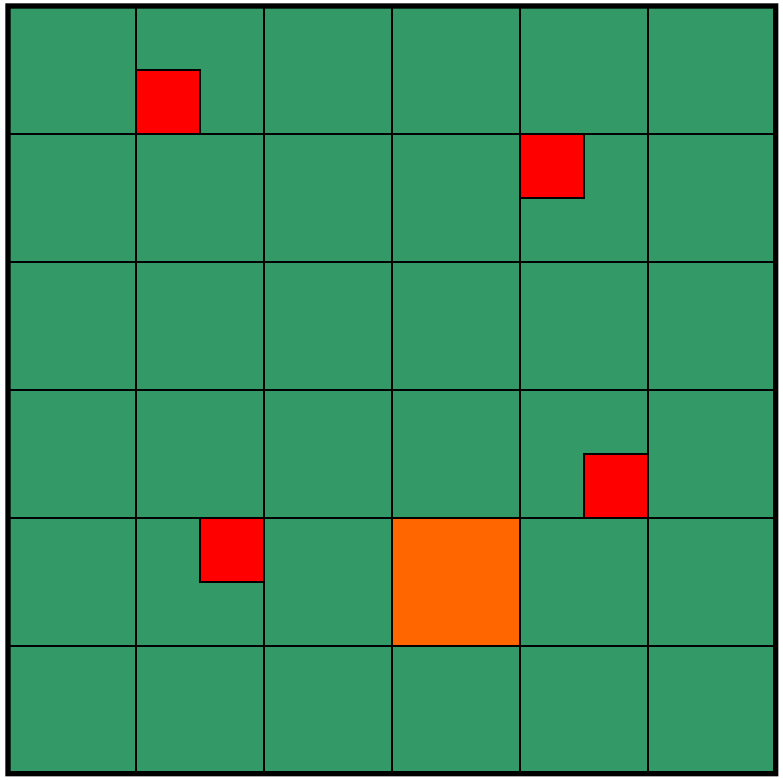
3600 sq cm surface with 5x5 cm grid and 4 isolated sites of contamination

Location Efficiency

= Contamination

= Sponge sample sites*

*Sponge sample sites are 10x10 cm
~ 100 sq cm



3600 sq cm surface with 10x10 cm grid and 4 isolated sites of contamination

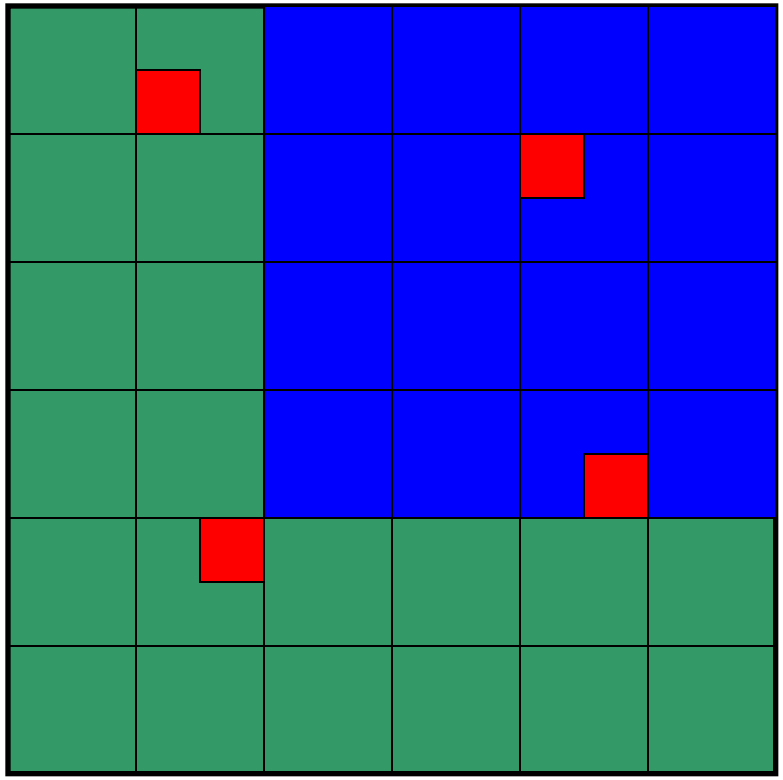
Location Efficiency

= Contamination

= M-Vac sample sites
1 M-Vac sample is 40x40 cm
~1600 cm^2

Method	# of samples	Total surface area covered (sq cm)	P*
Swab	64	1060	92%
Sponge	16	1600	92%
M-Vac	1	1600	91%

*Probability of finding at least 1 contamination site with a random sample within the given area. Assumes sampling a contaminated site would result in a positive detection result.



3600 sq cm Surface with 10x10 cm grid and 4 isolated sites of contamination

Recovery Efficiency

1. Recovery Efficiency: Number of organisms recovered from a surface by a sampling device from a known population of organisms. Includes extraction and elution steps.

2. Current Swab/Swipe Devices
   a. Historically low rates of recovery*
   b. Difficulty in sampling rough or porous surfaces**
   c. Retain organisms within device**

3. M-Vac
   a. Experimentally high rates of recovery
   b. Can sample rough or porous surfaces more efficiently
   c. No elution step

*Rose L, Jensen B, Peterson A, Banerjee SN, Arduino MJ. Swab materials and *Bacillus anthracis* spore recovery from nonporous surfaces. Emerg Infect Dis [serial on the Internet]. 2004 June [11/14/08]. Available from: http://www.cdc.gov/ncidod/EID/vol10no6/03-0716.htm

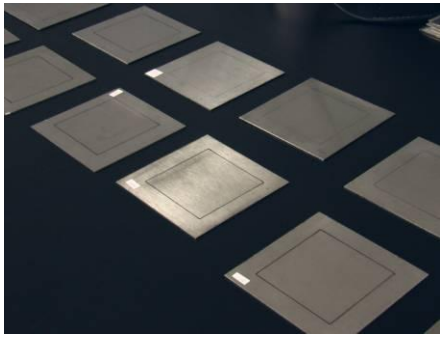
**Sanderson WT, Hein MJ, Taylor L, Curwin BD, Kinnes GM, Seitz TA, et al. Surface sampling methods for Bacillus anthracis spore contamination. Emerg Infect Dis [serial online] 2002 Oct [11/14/08];8. Available from: URL: http://www.cdc.gov/ncidod/EID/vol8no10/02-0382.htm

Comparison of Recovery Efficiency

Outline of Experimental Procedures

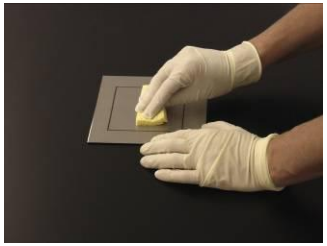
1. Inoculation with *B. subtilis* spores

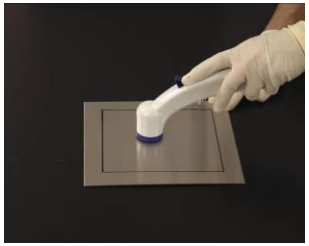
- a. Approx. 1.5 x 10^2 spores per coupon, very low level
- b. Spot inoculate onto sterile environmental surfaces
  - i. Stainless steel, HDPE, ceramic tile, carpet, polypropylene
  - ii. Sampling area of 100 cm^2 on all coupons
- c. Inoculum spread evenly, allowed to dry



2. Surface Sampling

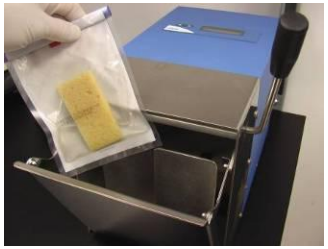
- a. Sample devices were used per manufacturer's instructions

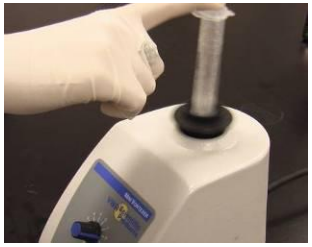




3. Sample Processing

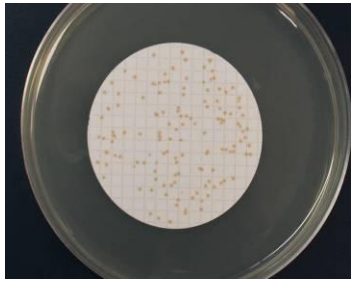
- a. Elution of spores from sampling device
  - i. Stomaching – sponges and gauze
  - ii. Vortexing – swabs



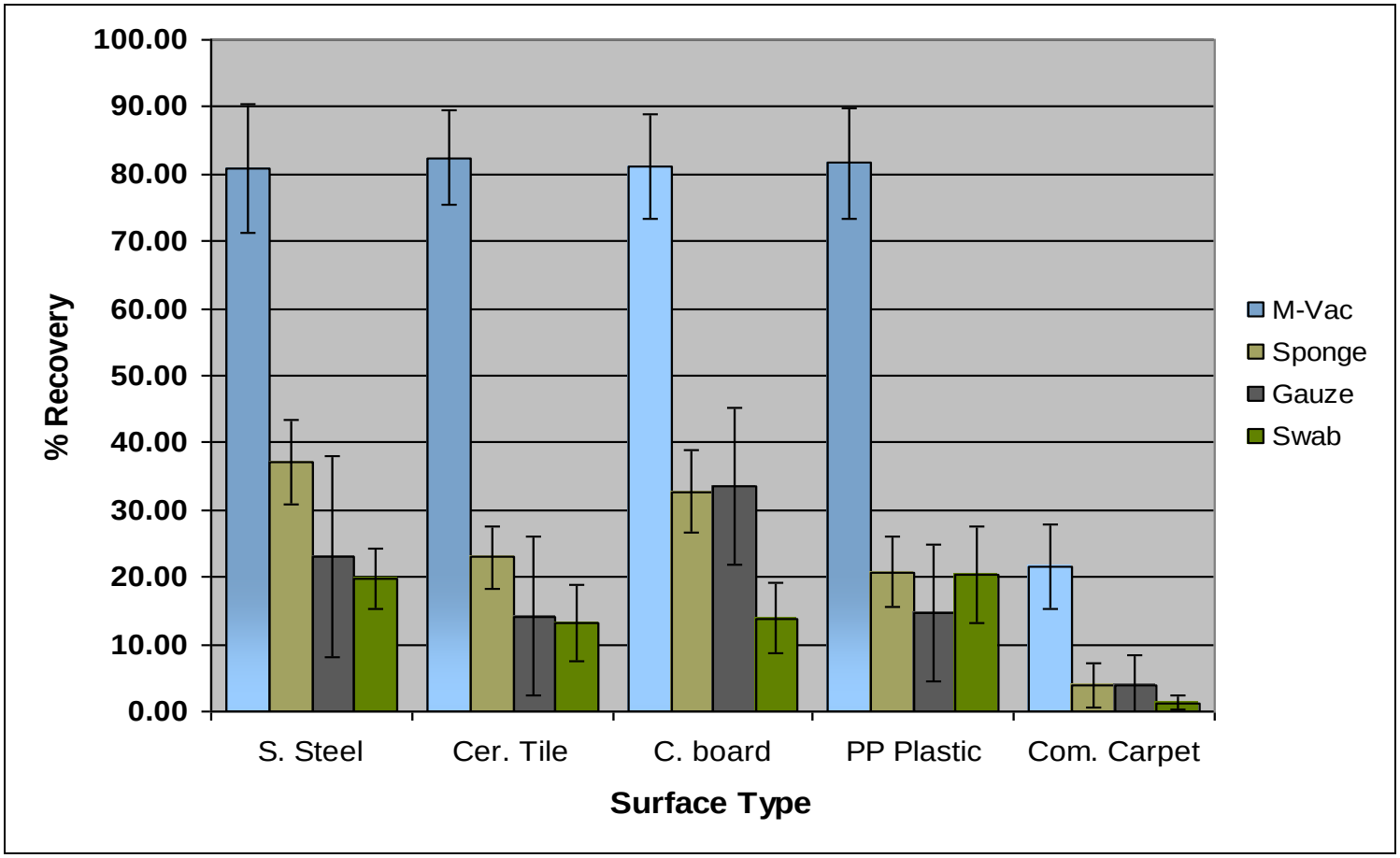


4. Quantitation

- a. Membrane filtration
- b. Counts following 24h incubation



Recovery of *B. subtilis* Spores from Simulated Environmental Surfaces



n=25 for all treatments

Summary

Advantages of the M-Vac

- Larger surface area per sample
- Reduced number of samples
- No elution step
- Higher rate of recovery from a variety of surfaces

Contributors:

Jared Maughan
Kelly Black
Dr. Bruce Bradley

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Questions?